AN ABSTRACT OF THE DISSERTATION OF

Paul M. Severns for the degree of Doctor of Philosophy in Botany and Plant Pathology, presented on May 4, 2009.
Title: Conservation Genetics of Kincaid's Lupine: A Threatened Plant of Western Oregon and Southwest Washington Grasslands

Abstract approved:

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Kincaid's lupine (*Lupinus oreganus* Heller) is a federally listed threatened species native to remnant grassland of western Oregon and southwestern Washington, and is the primary larval host plant of a once thought extinct butterfly, *Plebejus icarioides fenderi* Macy. Past studies concerning Kincaid's lupine reproduction suggested that populations may suffer reductions in fitness and progeny vigor due to inbreeding depression, but no direct investigation into range-wide patterns of genetic variation has been undertaken. I used nuclear DNA and chloroplast DNA simple sequence repeat (SSR) markers to determine genet size and patterns of non-adventitious rhizomatous lupine spread, to estimate the number of genets within Kincaid's lupine populations, and to assess whether seed transfer for the purpose of genetic rescue is an appropriate genetics management strategy for Kincaid's lupine.

Patterns of allelic diversity at nDNA SSR loci within study patches revealed that non-adventitious spread of rhizomes can extend to at least 27 m and may dominate a portion of a lupine patch or small population. However, genet spread and arrangement in study patches were sufficiently integrated such that interplantlet *Bombus* foraging flights exceeding 2 m had > 90% probability of occurring between different genets. Within-lupine patch genetic diversity was

well-undersampled, refuting the supposition that Kincaid's lupine populations suffer from inbreeding depression due to small effective population sizes. Estimation of Kincaid's lupine abundance through leaf cover and inflorescence number was tightly correlated with plantlet number, a unit of vegetative and sexual growth, within lupine patches but the relationship was not consistent between patches within populations or between populations. We used genet to plantlet ratios (determined through genotyping) and plantlet density to estimate genet population size in Kincaid's lupine patches. Because of the strong correlation between cover and plantlet density, historically collected lupine abundance data could be used to estimate genet population size provided that plantlet density is calibrated to patch-specific cover measurements.

Within patches and populations across the range of Kincaid's lupine there was little DNA evidence suggesting severe inbreeding. Only one of 24 populations and five study patches had strong statistical evidence of a recent genetic bottleneck despite the range-wide fragmentation of lupine populations and habitat. Mean population fixation index values for nearly half of the populations were near Hardy-Weinberg equilibrium expectations and only one small lupine population had a F-value > 0.20, suggestive of high inbreeding levels. Half of the populations actually had an excess of heterozygotes, suggesting that genetic diversity is not being lost. Chloroplast DNA coincides well with the observation that genetic diversity is not being lost through inbreeding or genetic bottlenecking in Kincaid's lupine. The mean number of cpDNA haplotypes per population was approximately 4 maternal lineages, which is very high for an animal pollinated plant with heavy seeds that have limited dispersal. Even relatively small populations of Kincaid's lupine had 2 or more cpDNA haplotypes, indicating that populations are not severely inbred. Both nuclear and chloroplast DNA SSR genetic marker diversity suggests that Kincaid's lupine does not require genetic rescue for effective conservation. Due to the longevity of Kincaid's lupine and the apparently large amount of within population genetic diversity, the encouragement of natural recruitment from vegetation management that improves

habitat conditions is likely to maintain the relatively large amount of genetic diversity within Kincaid's lupine populations.

Conservation Genetics of Kincaid's Lupine: A Threatened Plant of Western Oregon and Southwest Washington Grasslands

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Paul M. Severns, Author

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Chapter 1: Introduction to the conservation genetic issues surrounding Kincaid's lupine

Paul M. Severns

Kincaid's lupine (*Lupinus oreganus* = *Lupinus sulphureus* ssp. *kincaidii*) and Fender's blue butterfly (*Plebejus icarioides fenderi* = *Icaricia icarioides fenderi*) are evolutionarily and ecologically intertwined. Kincaid's lupine is the primary larval host plant for Fender's blue butterfly and both species are largely restricted to the confines of remnant Willamette Valley upland prairies (Wilson et al. 2003), suggesting a biogreographically coevolved relationship. Kincaid's lupine populations also occur to the north and south of the Willamette Valley, in southwestern Washington and Douglas, Co., Oregon, respectively (Fig. 1.1). The species are also linked in how they became the poster children of Willamette Valley conservation. Fender's blue butterfly was rediscovered in the late 1980s, about 60 years after the last butterfly specimen was collected. *Plebejus icarioides* are restricted to *Lupinus* as larval host plants (Warren 2005), so the Oregon State University herbarium was searched for records of rare Willamette Valley *Lupinus* species, of which Kincaid's lupine was one. From Kincaid's lupine herbarium localities and amateur lepidopterists more Fender's blue butterfly populations were found, scattered up and down the Willamette Valley. This intensive focus on butterfly rediscovery was well-documented in local, regional, and national media, which ultimately resulted in the focus on Willamette Valley grasslands, both upland and wetland types. Fender's blue butterfly and Kincaid's lupine were simultaneously listed as endangered and threatened, respectively, by the U.S. Fish and Wildlife Service in 2000.

It did not take long after the rediscovery of Fender's blue butterfly for biologists to realize the precarious situation of Willamette Valley grasslands, most of which was substantially reduced in area over the last 150 years from intensive agricultural development, urbanization, degradation, and habitat fragmentation

(Noss et al. 1995, Alverson 2005). Since both butterfly and plant share the same habitat, the degradation, loss, and fragmentation of upland prairies affect both species. For example, the invasion of tall stature, exotic grass species, that outcompete native bunchgrasses, appears to inhibit Kincaid's lupine seed germination (Severns 2008a), suppress lupine leaf production (Severns 2008b), and interferes with Fender's blue butterfly oviposition and other fitness related behaviors (Severns 2008b). In addition to the negative effects of habitat degradation on plant reproduction, habitat fragmentation has subdivided lupine patches that would likely have shared the same prairie into smaller, more isolated islands of habitat surrounded by non-grasslands (Severns 2003a, Wilson et al. 2003).

Current conservation and management of Kincaid's lupine must account for Fender's blue butterfly as well as the plant. In many remnant prairie parcels, vegetation management is key to controlling the invasion of exotic grasses and woody plants (*e.g*. Wilson and Clark 2001), and is often attempted by a combination of active and dormant season mowing. Because many of the current Kincaid's lupine populations appear relatively small and Fender's blue butterfly populations appear to be more stable the larger they are (Schultz and Hammond 2003), a common strategy has been to increase population sizes through direct seeding and transplants (Kaye and Cramer 2003). This approach addresses two issues central to draft Endangered Species Act recovery plans: 1) supplemental planting of lupines increases the potential size that a butterfly population can attain by providing more larval resources, and 2) they increase the population size to buffer against the chance of stochastic-based plant population extinction. The more individuals within a population, the less likely an unpredictable and unforeseen occurrence is to lead to population extinction (Lande 1995). A draft recovery plan for Fender's blue and Kincaid's lupine requires population size estimates to calculate population growth rates for the butterfly and minimum acceptable population size for the plant, which determines downlisting and recovery (U.S. Fish and Wildlife Service 2008). One of the problems with

determining Kincaid's lupine population size is that the plant can spread through non-adventitious rhizomes at least up to 10 m (Wilson et al. 2003). A simple count of Kincaid's lupine plants is unlikely to reveal the genet population size because multiple plants can be connected through underground rhizomes. While excavation would establish the extent of rhizome spread, it is not justifiable given its rarity and larval host relationship to Fender's blue butterfly. We applied a DNA fingerprinting approach with simple sequence repeat DNA loci to determine the expanse of Kincaid's lupine rhizome spread and how patterns of rhizome spread may impact inbreeding (Chapter 2). We use the same SSRs to estimate genet population size by calibrating genet to plantlet ratios against commonly employed measurements of Kincaid's lupine abundance, such as leaf cover and raceme number (Chapter 3). We also compare the variation within vegetative measurements to determine whether historically collected lupine abundance data should be used to calculate genet population sizes given vegetative relationships within and between different lupine populations (Chapter 3).

Seemingly small population size, potentially extensive rhizomatous spread, and habitat fragmentation suggest that the effective population size in many Kincaid's lupine populations is small, leading to inbred populations. Low natural seed set in populations may be a sign of inbreeding depression (Ågren 1996, Kéry et al. 2000) or pollinator limitation (Jennerston 1988). Kincaid's lupine populations are often observed to have low natural seed set (Severns 2003a, 2008, Wilson et al. 2003), suggesting that they may be inbred. In one small, isolated population hand crosses between populations more than doubled seed set when compared to within-population hand crosses, suggesting that North Green Oaks suffered a penalty in seed production from inbreeding (Severns 2003a). Kaye and Kuykendall (2001) found that only 55% of seeds from a relatively small population germinated under controlled laboratory conditions while nearly 95% of seeds from a relatively larger population germinated under the same controlled conditions, suggesting population level inbreeding. Although Kincaid's lupine requires pollinator visitation for seed set (Kaye 1999), it is genetically self-

compatible and about 80% of mean total *Bombus* (bumblebee) foraging flight time was spent on geitonogamous flights (Severns and Lewis 2007), and this may be an underestimate depending upon patterns of rhizome spread. It is possible that Kincaid's lupine populations are comprised of one or several large individuals due to extensive rhizome spread and that this may account for the evidence of inbreeding depression (addressed in Chapter 2). Alternatively, there may be spatial genetic architecture that develops within populations and that mixing seed even from within populations may be enough to produce an outcrossing-like effect in progeny vigor (Severns 2003b) (addressed in Chapter 4).

Interpopulation seed transfer may genetically rescue small populations that suffer from inbreeding depression (Richards 2000, Newman and Tallmon 2001) but it can also buffer against chance extinction through an increase in population size. However, genetic rescue can only occur if the populations being rescued are inbred and harbor genetic load. An allozyme genetic diversity study by Liston et al. (1995) spanned the range of Kincaid's lupine and sampled a representative portion of the larger populations known at the time. They did not find much evidence for severe inbreeding depression in allozyme markers, but other population properties, such as seed set, germination rate, and differential population performance in a common garden (Severns 2003) suggest otherwise. While controlled crossing experiments and progeny grow-outs from known crosses would give accurate estimates of population level inbreeding, controlled crossing and progeny tests for vigor are time consuming, especially in a long-lived perennial like Kincaid's lupine. Molecular markers can be used to detect processes such as inbreeding and genetic bottlenecking, which is useful for answering questions about the severity of inbreeding and patterns of genetic diversity across the range of Kincaid's lupine. I used simple sequence repeat (SSR) nuclear and chloroplast DNA markers to investigate levels of inbreeding across the range of Kincaid's lupine to assess whether remnant populations are in need of genetic rescue (Chapter 4).

Literature Cited

Ågren, J., 1996. Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. Ecology 77:1779–1790.

Alverson E. 2005. Preserving prairies and savannas in a sea of forest: a conservation challenge in the Pacific Northwest. Plant Talk 40: 23-27.

Jennerston, O., 1988. Pollination in *Dianthus deltoides* (Caryophyllaceae): effects of habitat fragmentation on visitation and seed set. Conservation Biology 2:359–366.

Kaye, T. N. 1999. Obligate insect pollination of a rare plant: Lupinus sulphureus ssp. kincaidii. Northwest Science 73:50-52.

Kaye, T. N. and J. Cramer. 2003. Direct seeding or transplanting: the cost of restoring populations of Kincaid's lupine. Ecological Restoration 21:224-225.

Kaye, T.N. and K. Kuykendall. 2001. Effects of scarification, cold stratification, and source population on germination of *Lupinus sulphureus* ssp. *kincaidii*. Seed Science and Technology 29:663-668.

Kéry, M., Matthies, D., Spillmann, H.H., 2000. Reduced fecundity and offspring performance in small populations of the declining grassland plants *Primula veris* and *Gentiana lutea*. Journal of Ecology 88:17–30.

Lande, R. 1995. Mutation and conservation. Conservation Biology 9: 783–791.

Liston, A., K. St. Hilaire, and M.V. Wilson. 1995. Genetic diversity in populations of Kincaid's lupine, host plant of fender's blue butterfly. Madroño 42:309-322.

Newman, D. and D. A. Tallmon. 2001. Experimental evidence for the beneficial fitness effects of gene flow in recently isolated populations. Conservation Biology 15:1054–1063.

Noss, R. F., LaRoe, E. T. & J. M. Scott. 1995. Endangered ecosystems of the United States: a preliminary assessment of loss and degradation. National Biological Service, Biological Report 28, Washington D.C.

Richards, C. M. 2000. Inbreeding depression and genetic rescue in a plant metapopulation. The American Naturalist 155:383–394.

Schultz, C.B. and P.C. Hammond. 2003. Using population viability analysis to develop recovery criteria for endangered insects: case study of Fender's blue butterfly. Conservation Biology 17:1372-1385.

Severns, P., 2003a. Inbreeding and small population size reduce seed set in a threatened and fragmented plant species, *Lupinus sulphureus* ssp. *kincaidii* (Fabaceae). Biological Conservation 110, 221-229.

Severns, P.M., 2003b. Propagation of a long-lived and threatened prairie plant, *Lupinus sulphureus* ssp. *kincaidii*. Restoration Ecology 11, 334-342.

Severns, P.M. and B. Lewis. 2007. Is it appropriate to rely on seed set to assess candidate plant populations for genetic rescue? A case study with a threatened species. Natural Areas Journal 27:313-319.

Severns, P.M. 2008a. Seedling population size and microhabitat association in *Lupinus oreganus* A. Heller var. *kincaidii* C.P. Sm. (Fabaceae), a threatened plant of western Oregon grasslands. Native Plants Journal 9:358-365.

Severns, P. M. 2008b. Exotic grass invasion impacts fitness of an endangered prairie butterfly, *Icaricia icarioides fenderi*. Journal of Insect Conservation 12:651-661

United States Fish and Wildlife Service. 2008. Draft recovery plan for the prairie species of western Oregon and southwestern Washington. U.S. Fish and Wildlife Service, Portland, Oregon. 212pp.

Warren, A. D. 2005. Lepidoptera of North America 6. Butterflies of Oregon their taxonomy, distribution, and biology. Contributions of the C.P. Gillette Museum of Arthropod Diversity, Colorado State University, Fort Collins, 408 pp.

Wilson, M.V. and D. L. Clark. 2001. Controlling invasive *Arrhenatherum elatius* and promoting native prairie grasses through mowing. Applied Vegetation Science 4:129–138.

Wilson MV, Erhart T, Hammond PC, Kaye TN, Kuykendall K, Liston A, Robinson AF Jr, Schultz CB, Severns PM. 2003. Biology of Kincaid's lupine (*Lupinus sulphureus* ssp. *kincaidii* [Smith] Phillips), a threatened species of western Oregon native prairies. Natural Areas Journal 23:72-83.

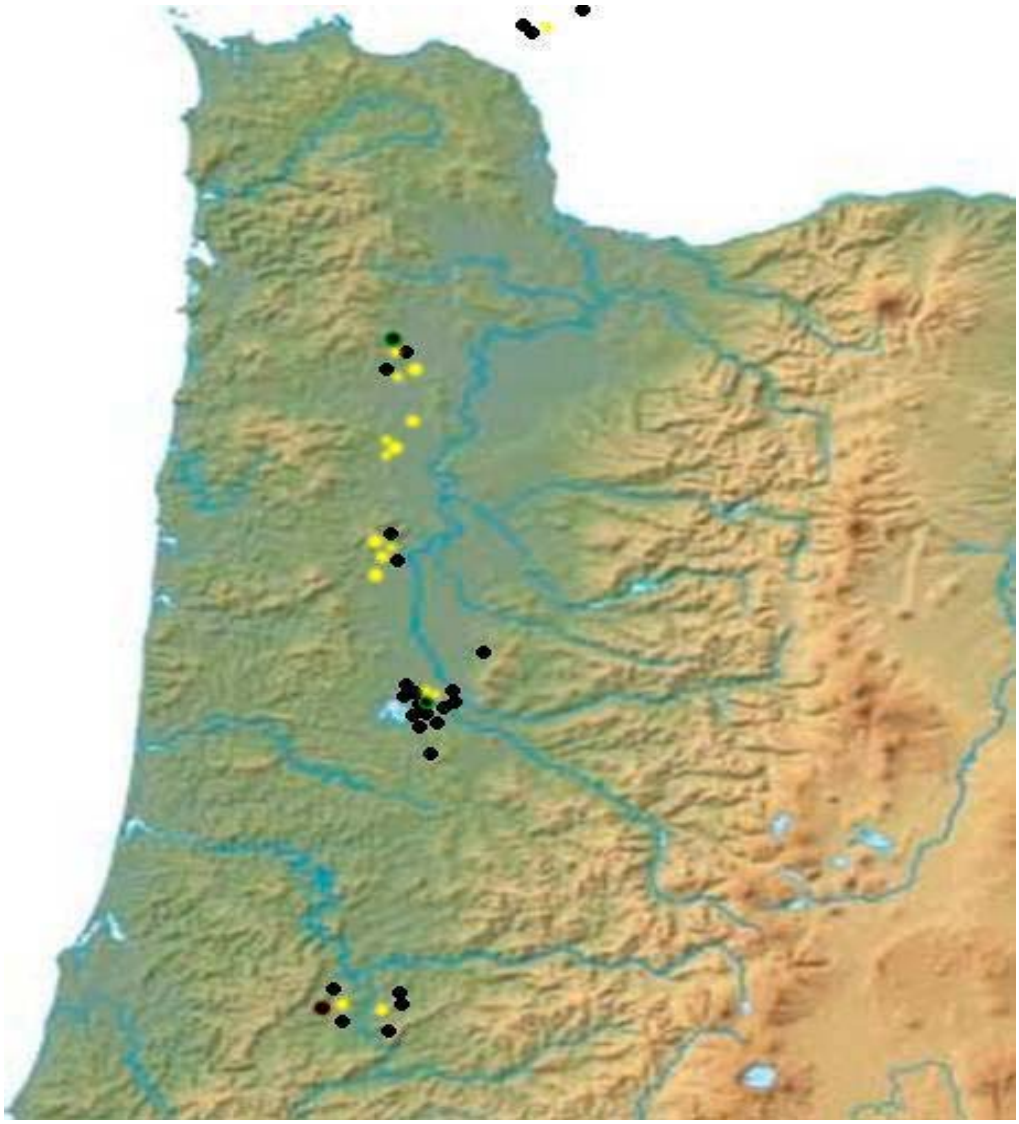

Figure 1.1. Relative location of Kincaid's lupine populations genotyped in this thesis (black circles) and relative location of Kincaid's lupine populations not genotyped (yellow circles).

Chapter 2: Implications of extensive non-adventitious rhizome spread on Kincaid's lupine reproduction, inbreeding, and conservation

Paul M. Severns, Aaron Liston, and Mark V. Wilson

Abstract. Kincaid's lupine has a unique trait not shared by any other known *Lupinus* species, it produces non-adventitious rhizomes that appear capable of spreading to at least 10 m. This threatened species is largely restricted to remnant grasslands in western Oregon, USA, and conservation of the endangered Fender's blue butterfly will depend upon the persistence of Kincaid's lupine larval host plant populations. Prior studies on relatively, small fragmented Kincaid's lupine sites suggest that populations may experience low seed set and diminished progeny vigor from inbreeding depression due to small population size. We used nuclear simple sequence repeat (SSR) DNA markers to determine the extent of rhizome spread, examine the possibility of small population sizes, and model the potential effect of *Bombus* spp (bumblebee) foraging flights on geitonogamy in six lupine patches from three different populations. Genotyping revealed one genet that spread to at least 27 m and this individual appeared to dominate a region of the lupine patch it was sampled from. However, the large genet appeared to be an exception as the median maximum distance of rhizome spread in 15 other genets was approximately 5.5 m and genets appeared to be well intermingled. Study patches did not seem to be small enough to engender concerns for severe inbreeding. When averaged over all study patches, a new genotype was detected from every two lupines genotyped, indicating that genetic diversity was undersampled. Genets appeared to be so well intermixed within lupine patches that bumblebee flights between lupines of approximately 1 m had > 80% chance of occurring between different genets. While Kincaid's lupine population size may be perceived as alarmingly small, patches and populations appear larger than originally thought and pollinator foraging movements may be sufficient to produce outcrossed progeny. While we cannot rule out the possibility of locally inbred

demes within lupine patches, greater than expected genetic diversity, the lack of genets frequently dominating entire lupine patches, larger than hypothesized population size, and that the longevity of Kincaid's lupine is likely to help maintain genetic diversity, population extinction from severe inbreeding depression seems unlikely to occur in the immediate future.

Introduction

Interactions between life history and evolutionary ecology are not routinely integrated into rare plant population conservation. Identifying mechanisms that limit or encourage population growth rates operating over multigenerational time spans that impact ecological processes may greatly enhance conservation effectiveness (Ashley et al. 2003, Stockwell et al. 2003, Hairston et al. 2005). Effective conservation requires an understanding of both short-term ecological causes of changes in population growth rates, such as prescribed fires (Pendergrass et al. 1999), safe sites (Enright and Lamont 1989, Lamont et al. 1993, Maron and Gardner 2000), herbivory (Pfab and Witkowski 2000, Kéry et al. 2001, Gómez-Aparicio 2005), seed predation (Auld and Denham 2001, Severns 2008b), as well as multigenerational evolutionary processes such as inbreeding and outbreeding depression (Ellstrand and Elam 1993, Fischer and Matthies 1997, Schemske et al. 1994, Montalvo and Ellstrand 2001, Newman and Tallmon 2001, Tallmon et al. 2004). Inbreeding depression, the accrual of deleterious alleles that reduce mean population fitness (genetic load) can limit the persistence of rare plant populations and is a common concern for those managing rare plant species. Plants from populations that harbor sufficiently high levels of genetic load commonly experience reductions in fruit and seed production (Ågren 1996, Kéry et al. 2000), and progeny vigor and fitness (Karoly 1994, Husband and Schemske 1996, Buza et al. 2000, Sheridan and Karrowe 2000). But negative inbreeding depression effects can be mitigated by the introduction of genotypes into inbred populations that lead

to heterosis upon outcrossing (Brown and Kodric-Brown 1977, Richards 2000, Newman and Tallmon 2001, Tallmon et al. 2004).

Distinguishing between a high incidence of selfing among individuals in populations lacking genetic load and populations that are truly inbred is important information for effective conservation. Severe inbreeding in plant populations can be directly assessed by performing a series of hand pollination experiments or indirectly evaluated with molecular markers (Edmands 2007). The fixation or inbreeding coefficient (*F*) inferred through DNA and protein markers, is argued as an index for inbreeding depression risk because larger than expected levels of homozygosity across marker loci are hallmarks of inbred populations (Reed and Frankham 2003, Leimu et al. 2006). Estimates of population reproductive effort, progeny vigor, and progeny fitness are typically drawn from a subsample of individuals under unmanipulated field conditions and may be used to infer genetic status of differently sized populations (*e.g.* Kéry et al. 2000). However, the conditions under which the subsampled plants occur may explain the observed reductions in seed set and progeny performance that is not due to inbreeding depression. For example, reduced fecundity and lower relative progeny vigor due to selfing can occur among individuals in populations without genetic load if between genet distances exceeds pollen and seed dispersal (Kunin 1997, Bosch and Waser 2001, Mustajärvi et al. 2001). In this case, the reduction in parental generation fitness and progeny vigor is due to selfing, not because the population is so severely inbred that crossing between different individuals within the population yield the same seed set and progeny fitness as selfing. A management strategy for the persistence of a rare plant population with genetic load should differ from one that is genetically diverse but where individuals frequently self.

Lupinus oreganus Heller = *Lupinus sulphureus* ssp. *kincaidii* (Smith) Phillips (hereafter Kincaid's lupine) is a long-lived, threatened legume of western Oregon and larval host plant to the endangered Fender's blue butterfly, once thought to be extinct (Wilson et al. 2003). Kincaid's lupine populations exhibit symptoms of inbreeding depression such as low seed set (Kaye and Kuykendall

1993, Severns 2003a), decreased germination (Kaye and Kuykendall 2001), and differences in plant vigor among a group of locally fragmented populations grown in a common garden (Severns 2003b). However, Kincaid's lupine was only recently determined to have a mixed breeding system and shown to be self-compatible (Severns and Lewis 2007), calling into question the interpretation of population seed set measurements as a reliable indicator for population inbreeding depression. From the current knowledge of Kincaid's lupine life history traits, there are several likely mechanisms that could yield low population seed set. One possibility is that Kincaid's lupine populations are comprised of genets that are all closely related to each other (Severns 2003a), yielding near relative matings resulting in the familiar inverse relationship between genetic diversity and inbreeding depression. However, Kincaid's lupine appears capable of spreading through non-adventitious roots exceeding 10 m (Wilson et al. 2003, Chapter 3), suggesting that mechanisms involving genet size, number, and pollinator behavior may come into play. Kincaid's lupine populations could also be composed of one or a few large genets resulting in a small effective population size and severe population level inbreeding (Liston et al. 1995). Another possible scenario is that selfing may be common due to a combination of pollinator behavior and Kincaid's lupine genet size when the population is not a collection of inbred lineages nor restricted to a few large genets. *Bombus* spp., the most frequent and conspicuous pollinator of Kincaid's lupine appears to have short between lupine plant flight distances (median ≈1.2 m), and > 75% of an individual's median total foraging flight time is spent on geitonogamy (Severns and Lewis 2007), suggesting that selfing could be very common depending upon lupine genet distribution. We used nuclear DNA simple sequence repeats (SSRs) to investigate the expanse of non-adventitious rhizome spread, genetic diversity, and small population size on Kincaid's lupine reproduction.

Methods

Study species. Kincaid's lupine is largely restricted to remnant grasslands in the Willamette Valley of western Oregon, U.S.A., with outlying populations to north in southwestern Washington State and about 100 km south of the Willamette Valley in Douglas County, Oregon (Wilson et al. 2003). Like many other endemic western Oregon species (Oregon Natural Heritage Information Center 2007), the rarity of Kincaid's lupine is primarily due to loss and degradation of native grasslands, that now constitutes only ≈ 1% of the former 100,000 ha of Willamette Valley grasslands circa 150 years ago. Many of the remnant Kincaid's lupine populations are considered small, relatively isolated (Wilson et al. 2003), and are a common focus for habitat restoration projects because the lupine is the primary larval host plant for an endangered butterfly, *Plebejus icarioides fender* Macy.

Kincaid's lupine shares some life history common among perennial *Lupinus*; heavy seeds that are ballistically dispersed relatively short distances (e.g. Schaal 1980a, b) and a piston-keel mechanism of pollen presentation (Juncosa and Webster 1989) that appears to enhance outcrossing pollinator flights (Haynes and Mesler 1984, Harder 1990). But, Kincaid's lupine also has life history traits that are either uncommon or unique among the 200 to 300 delimited *Lupinus* taxa (Lewis et al. 2005). Kincaid's lupine appears to be long-lived, up to hundreds of years, and may spread to distances exceeding 10 m through non-adventitious rhizome growth (Wilson et al., 2003). Although herbaceous plant longevity of centuries is not rare (Stehlik and Holderegger 2000, Ehrlén and Lehtilä 2002, García et al. 2008), lupines are not known to spread rhizomatously and if rhizomes are produced they are well under a meter in length (Dunn and Gillett 1966, Hickman 1996, Antos and Halpern 1997, Bishop 2002). Even among Andean lupines, the most rapidly speciating group of plants known (Hughes and Eastwood 2006), no plants appear to spread rhizomatously (C. Hughes Oxford University pers. com. 2007), although small trees are a rare growth form (Hughes and Eastwood 2006). Even with its potentially extensive rhizome spread, Kincaid's

lupine does not appear to adventitiously root (Wilson et al. 2003, Severns pers. obs.), a rare trait among herbaceous plants with conspicuous rhizome spread (Tomlinson 1973, Silvertown 2008). The putative genetic advantage of clonal reproduction with extensive rhizome spread (Silvertown 2008) is not realized by Kincaid's lupine because clusters of leaves and flowers cannot persist if physiologically separated from the original, taprooted plant. Kincaid's lupine has a tree-like growth form where the flowering branch terminals are aboveground and the trunk and branches buried.

Kincaid's lupine produces multiple, unbranched racemes with ≈ 30 to 150 flowers per inflorescence and they arise from a cluster of long-petioled leaves ranging from several centimeters to several decimeters in length. Leaves often arise from distinct point on the ground and typically form a dome shaped crown (Severns 2003b) however clusters of leaves may also be broadly linear, extending for several decimeters. Small increments of vegetative spread (< 30 cm) were observed by the end of three growing seasons from seedlings (Severns 2003b, Severns personal observation), suggesting that vegetative spread may be slow. Kincaid's lupine requires pollinator service for fruit and seed set (Kaye 1999) and there appears to be a substantial penalty for inbreeding in reduced seed set. The inbreeding coefficient, $\delta = 1 - (\omega_{self} / \omega_{outcross})$, where ω = seed set from self pollination and outcrossing (Husband and Schemske 1996), ranges from 0.50 to 0.65 in Kincaid's lupine (calculated from data presented in Severns 2003a, Severns and Lewis 2007).

Study sites and tissue sampling. We selected three study sites, Royal, North Eaton, and South Eaton, which have been the subjects of several past studies involving Kincaid's lupine (Severns 2003a, b, Severns 2008a, b), in which specific site conditions were described. For this study of estimating genet size and patterns of vegetative spread, three patches of lupine plants in North Eaton, two patches in South Eaton, and one large patch at Royal were selected for investigation. We defined a lupine "patch" as a group of lupines separated from another group of

lupines by at least 10 m (Severns 2003a). The North Eaton and South Eaton sites are adjacent to each other and share similar degraded upland prairie habitats but are separated by a dense deciduous forest of native and non-native trees. The lupines at Royal grow in a different grassland habitat, a seasonally flooded wetland prairie (see Severns 2006 for a more detailed habitat description), but they grow on slightly elevated mounded areas just above the high water table in the wet season. In the wetland prairies, the flooded soils are likely anaerobic once covered by water while mound soils are saturated but short-term standing water drains off into the slightly lower flooded areas.

Because a cluster of aboveground leaves may represent a part of a genet or an entire genet, tissue sampling needed to be collected by a clearly delimitable aboveground unit that is related to vegetative spread. We considered a lupine "plantlet" to be a group of leaves separated from another group of leaves from the margins of where petioles arise from the ground by at least 10 cm. From observations of Kincaid's lupine plants started from seed that did not flower, rhizome spread produced plantlets spaced > 10 cm from the parent plant (Severns 2003b, Severns pers. obs.), suggesting that the distance threshold to separate plantlets has biological relevance. To select plantlets for tissue sampling, the boundaries of the lupine patches were identified and a coordinate from a rectangular grid was randomly selected as a starting point. From this starting point a random compass bearing and distance between 0.5 and 10 m was selected for each plantlet. Depending upon the size of the lupine patch and sampling effort, there were from one to several random starting points selected. The position of each plantlet sampled was recorded with a Leica GS20 handheld GPS unit to within ±0.3 m after data correction. Young leaf tissue was collected from each plant, placed on ice, and then transported to a -20°C freezer where the tissue was stored until DNA was extracted. Lupine plantlet density ranged from 2.2 to 8.4 plants per m^2 depending on the sample patch (49 m^2 to 530 m^2) and generally < 5% of the plants per patch were sampled (Chapter 3).

Molecular markers. We used five variable nuclear simple sequence repeats (SSRs) to identify genets and describe the spatial patterns of vegetative spread in three study populations of Kincaid's lupine. Two of the five SSR loci are considered neutral (*AG55-26-16*, *CAC60-13-16*), with no known relationship to a coding region (Drummond and Hamilton 2005). The locus *AG-81* is immediately downstream of a seryl-tRNA synthetase gene in *Glycine*, and has been treated as neutral (Peakall et al. 1998). The last two SSR loci are two linked, exonic, trinucleotide repeats from a *CYCLOIDEA* paralog, *LEGCYC1B* (Citerne et al. 2003, Citerne 2005). *LEGCYC1B* contains a glycine (GGT) repeat near the 5' end of the exon and an asparagine (AAC) repeat towards the 3' end of the exon (Ree et al. 2004). The trinucleotide repeats are separated by approximately 800 bp (Ree et al. 2004). The CYCLOIDEA family of proteins is known to function in zygomorphic flower symmetry (Citerne et al. 2000, 2003, Ree et al. 2004, Howarth and Donoghue 2006) and, in papilinoid legumes, appears to be involved in banner petal development (Feng et al. 2006).

Genomic DNA from 50-100 mg of frozen leaf tissue per individual was extracted with the FastDNA® kit and FastPrep® instrument according to manufacturer recommended protocols (QBiogene, Inc., CA). Nuclear SSR loci were amplified from 0.5-1.2 μL (10 – 30 ng) of genomic DNA, 1.2 μL of 10X Thermopol Buffer (New England Biolabs), 0.6 μL of 100X BSA, 1.0 μL (0.25mM of each dNTP), 0.5 μL of each forward and reverse primer (5 pM), 0.5 to 1.0 units of *Taq* polymerase (New England Biolabs) and 7.0 μL of ddH_20 for an approximate 11μL reaction volume. SSR loci were amplified with a BIO-RAD MyCycler™ thermal cycler at 94 °C for 4 minutes, 36 cycles of 94 °C for 1 min, 54 °C for 1 min, 72 °C for 1 min and 30 sec, and 72 °C for 7 minutes.

SSR primers were fluorescently labeled and the amplicon lengths were recorded using an ABI3100 sequencer with GS500Rox as an internal standard. The ABI3100 output data was visualized and SSR lengths were scored using the program Genographer 1.6 (Benham 2001 http://hordeum.oscs.montana.edu/genographer). Since the two *LEGCYC1B* amino acid repeats are separated by ≈

800 nucleotides we created internal standards from a library of known homozygous individuals that varied in total exon length (including the two SSRs) to determine phase between "haplotypes" that were heterozygous at both loci. The double heterozygous individuals were multiplexed with the LEGCYC homozygous standards and rerun on the ABI3100. Since the total *LEGCYC1B* standard length was approximately 1200 bp and this length exceeds the fragment size limit Mapmaker (internal size standard) and the ABI3100 software could reliably size, we only scored mismatches within a single capillary tube. If the double heterozygous individuals shared the same number of repeats but had different phase then linkage was recorded and accounted for in the statistical assignment of genets.

Analysis. Within a lupine patch, plantlets with identical shared multilocus genotypes (hereafter MLG) could belong to the same genet due to rhizome spread. A shared multilocus genotype among a group of plantlets could also arise independently from different sexual reproduction events. We used the estimator P_{sex} (Arnaud-Haond et al. 2007) as a filter to separate groups of two or more plantlets with identical multiple locus genotypes into two categories: plantlets with identical genotypes due to rhizome spread and plantlets with identical genotypes resulting from different sexual reproduction events. *Psex* gives an estimate of the probability of obtaining *n* samples with a shared multilocus genotype from the pool of genotyped samples through sexual reproduction under the assumption of random mating. (We assumed the absence of somatic mutations at SSR loci.) The *Psex* estimator also accounts for the influence of inbreeding on the occurrence of identical multiple locus genotypes through independent sexual reproduction events (Arnaud-Haond et al. 2007). We performed the calculations with the program Genclone 2.0 (Arnaud-Haond and Belkhir 2007) and selected a 5% cutoff probability for lupine plantlets. Two or more lupine plantlets within a patch sharing identical genotypes with $P_{sex} > 0.05$ we considered as having arose from independent sexual reproduction events, and groups of two or more lupine

plantlets within a patch sharing identical genotypes with $P_{sex} \leq 0.05$ we identified as belonging to a single vegetatively spreading genet.

To provide an estimate of how thoroughly Kincaid's lupine genets are interspersed within study patches, we used the aggregation index, A_c, proposed by Arnaud-Haond et al. (2007). The A_c index ranges from 1 to 0; a value of one represents a situation when all nearest neighbors sampled share the same MLG that have P_{sex} values < 0.05 and a zero indexes a situation where all nearest sample neighbors belong to different genets. The aggregation index, A_c, was assessed for statistical significance with 1,000 Monte Carlo randomization permutations in Genclone 2.0.

To determine how completely we sampled the pool of genetic diversity within each study patch we calculated the number of MLGs against the number of individuals sampled using the subsampling procedure (1,000 Monte Carlo permutations) provided in Genclone 2.0. The slope at the top of the accumulation curve indicates how thoroughly described the population of MLGs was in each study patch. A well-characterized study patch would have an accumulation curve that appears to asymptote, whereas undersampling the abundance of MLGs would generate a curve with no apparent asymptote.

Vegetative spread interactions with bumblebee foraging flights. We investigated the interaction between genet vegetative spread and outcrossing probability by comparing *Bombus* interplantlet flight distances (recorded in a prior study Severns and Lewis 2007) with the probability that a lupine plantlet will belong to a different genet with increasing distance in each study patch. We fitted a logistic regression model where the likelihood of encountering a different genet depended upon the distance between lupine plantlets. We used the predicted probability of the logistic regression model to estimate the probability of outcrossing flights made by each bumblebee as if they foraged in the lupine patches genotyped in the current study.

Results

A total of 243 Kincaid's lupine plantlets were genotyped among the six study patches from three different lupine populations. There were at least two alleles at each of the five marker loci within each study population and a total of 35 different alleles were amplified from the three populations (Table 2.1). The genotypes of 140 plantlets were not matched by other plantlets occurring within the respective source patch. There were 16 genets that had 2 or more plantlets representing a genet among the plantlets with identical MLGs from the six study patches. Another 12 identical MLGs were shared among 26 plantlets but the genotype could have occurred from sexual reproduction ($P_{sex} > 0.05$) so we treated these plantlets as individual genets (Table 2.2). Accumulation of MLGs with increasing sample size in each patch at the three study sites did not asymptote (Fig. 2.1), indicating that we were far from representing the MLG diversity within lupine study patches given the number of plantlets genotyped.

The median maximum distance between plantlets with identical shared genotypes when combined, regardless of P_{sex} was ≈ 5 m (Table 2.3). Plantlets with identical shared MLGs were more or less evenly distributed into maximum interplantlet size classes, ranging from < 3m to > 10m (Fig. 2.2). The largest genet genotyped spanned a 27 m x 13 m area at Royal and contained 29 plantlets ($P_{sex} = 1.8 \times 10^{-21}$), but spreading genets in the remaining five study patches were not as large and did not appear to spatially dominate the lupine patch (Fig. 2.3). The aggregation index (A_c) for study patches ranged from 0.077 to 0.364 (Table 2.4), indicating that genets of the genotyped plantlets were well intermingled at the spatial scale that the tissue sampling occurred (from 0.5 m to 47 m).

Other than when Genclone analysis suggested that plantlets within a study patch shared a multilocus genotype by chance through reproductive events ($P_{sex} > 0.05$), we found four instances where a multilocus genotype was shared among plantlets in two different lupine patches when $P_{sex} < 0.05$. In two cases, one genotype at North Eaton and one genotype at South Eaton were shared between

different lupine patches occurring within the same site and two other genotypes were shared among plantlets occurring in one patch at North Eaton and Royal (≈ 1.5 km apart). The P_{sex} calculation appeared to misidentify the genet membership of plantlets when non-adventitious rhizome growth restricts a genet's ability to asexually spread between patches or populations.

Bumblebee outcrossing flight probability. Except for the South Eaton bike path patch, there was a statistically significant logistic regression model ($\alpha < 0.05$) for interplant distance and the probability that plantlets genotyped within the study patch belonged to a different genet. As the distance between genotyped lupine plantlets increased, the probability that the plantlets were from the same genet rapidly decreased with distance, except at Royal, where there was a large, dominant genet that occupied about one third of the lupine patch (Fig. 2.4). For four of the five study patches, if the distance separating two plantlets was > 1m, there was > 85% probability that the two plantlets would be from different genets and by 2 m the probability exceeded 90% (Fig. 2.4). The median maximum interplantlet bumblebee foraging flight recorded in a different set of lupine patches was about 1.2 m (Severns and Lewis 2007). If the median bumblebee maximum interplantlet flight distance of 1.2 m recorded by Severns and Lewis (2007) were to occur in any of the genotyped lupine study patches (except Royal), there would be > 85% probability that the maximum interplantlet flight would occur between plantlets from different lupine genets (Figs. 2.4 & 2.5). At Royal, a pollinator would need to fly approximately 17 m between lupine plantlets to have an 85% probability of the flight occurring between different lupine genets. The mean probability of a bumblebee foraging bout (data from Severns and Lewis 2007) occurring at least once between different genets in genotyped patches exceeded 90% for all patches except Royal, which was approximately 37% (Table 2.5).

Discussion

We proposed that extensive spread of non-adventitious rhizome growth in Kincaid's lupine may lead to a high incidence of inbreeding and inbreeding depression due to small population size if a lupine patch is dominated by one or a few expansive genets (Liston et al. 1995). Although low observed population seed set appears to be a common phenomenon (Severns 2003a, 2009, Wilson et al. 2003), we found only one expansive, dominant genet at Royal (Fig 2.2) and Liston et al. (1995) suggested a genet of approximately 10 m in diameter in a hybrid population of Kincaid's lupine and *L. arbustus*. In the other 5 lupine study patches, genotyped plantlets did not appear to belong to comparably large genets. While we cannot provide a reliable estimate for the frequency of large, continuous dominant genets in lupine populations from our six study patches, large genets appear to be an unlikely explanation for low observed seed set in Kincaid's lupine populations.

A scenario commonly linked to reductions in seed set and progeny vigor due inbreeding is small population size (Ellstrand and Elam 1993, Keller and Waller 2002, Willi et al. 2005, Pickup and Young 2008). In a group of fragmented Kincaid's lupine populations, the number of lupine patches within a population was positively correlated with population seed set, suggesting that small population size is responsible for reduced seed production in populations of Kincaid's lupine (Severns 2003a). Populations with multiple patches should have a limited number of genets in each patch if a decreasing number of patches results in an increase in inbreeding. In our molecular marker study, accumulation curves for the within patch multiple locus genotype number linearly increased with sample size in all study patches (Fig. 2.1) suggesting that the genotyping effort was far short of accounting for the genets within lupine study patches. After we had completed genotyping plantlets, study patches were still rapidly accumulating genotypes (Fig. 2.1), with a new genotype detected from about every two plantlets

sampled (Table 2.4). Low reliability of MLG number estimates from linearly increasing accumulation curves prevents us from projecting upwards to a genet number ceiling. However, estimates of genet population size from a combination of genet to plantlet ratios, mean plantlet density per m^2, and lupine patch area yielded genet number estimates ranging from 114 genets in the smallest patch (South Eaton bike path) to nearly 1,500 genets in one of the largest patches (South Eaton main) (Chapter 3). The rapidly accumulating MLG abundance curves and genet population estimates calculated from vegetation measurements suggest that Kincaid's lupine populations are likely much larger than originally thought. While small lupine patches with fewer than several hundred genets may be at risk for inbreeding depression, larger lupine patches with > 1,000 genets are unlikely to incur such a large risk (Lande 1994, 1995). Since we sampled and analyzed our genetic date at the patch level and patches appear to be genetically diverse, it seems reasonable to propose that low population seed set is due to a mechanism other than small population size, such as pollinator behavior.

Commonly observed pollinators of Kincaid's lupine, *Bombus* spp., appear to spend almost 80% of an entire foraging bout on geitonogamous flights and median maximum movements between plantlets are limited to about 1.2 m (Severns and Lewis 2007). Short, interplantlet pollinator flights might result in a high frequency of selfing if plantlets from genets are exclusively clustered so that rhizome spread commonly exceeds interplant pollinator movements. The aggregation index (*Ac*) values among the six study patches of Kincaid's lupine suggested that genets are fairly well intermingled and that above ground tissues from spreading genets do not appear to commonly dominate areas within a patch of plants (Table 2.3). Even in the Royal population, where there was one large, continuous genet, the *Ac*-value was comparable to other study patches where large, continuous genets were not detected (Table 2.3). Although, the low *Ac* value at Royal could be explained, in part, by the position of the large genet along the population edge (Fig. 2.2). If genets grew with short internodes from a central point in all directions, with a mat-like growth form, then the maximum geographic

distances between plants of the same genet should have been shorter in the logistic regression model (Fig. 2.1) and *Ac* values should have been greater. Our data indicate that Kincaid's lupine rhizome spread occurs over distances > 10 m, but distances between plantlets from a spreading genet do not appear to be short enough to commonly form an aggregate mat of plantlets. If *Bombus* interplant flight distances presented by Severns and Lewis (2007) are representative of the *Bombus* behavior in the genotyped study patches, the likelihood of the median interplant maximum flight distance would yield > 80% of an outcrossing flight in all patches but Royal (Fig. 2.4). When *Bombus* foraging behavior is overlaid on the spatial patterns of Kincaid's lupine rhizome spread, the isolation of genets through rhizome structure is an unlikely explanation for the low observed seed set in Kincaid's lupine populations. In all patches but Royal, the average *Bombus* foraging bout had > 90% probability of at least one flight occurring between plantlets from a different genet (Table 2.4), despite that > 75% of the time spent on a typical foraging bout was geintonogamous.

Although Kincaid's lupine population size, rhizome spread, and genet size does not appear to be a likely explanation for depressed seed set in Kincaid's lupine, locally inbred demes within patches is a plausible explanation. While outcrossing seems a likely possibility from *Bombus* foraging behavior, a large portion of their foraging flights were geitonogamous and the maximum median interplantlet flight distance were relatively short (Fig. 2.5). Pollen distribution is typically leptokurtically distributed from the source plant (Waser 1988, Waser and Price 1994) and the majority of pollen is commonly transferred to local neighbors by pollinators (Alder and Erwin 2006), except for rare instances where long-distance pollen transfer occurs (*e.g*. Schulke and Waser 2001). While pollinator movements suggest that the average maximum average pollen movement within lupine patches may be only 1 m to 2 m, seed dispersal may be more restricted. *Lupinus texensis*, an annual legume with height, fruit length, and seed weight similar to Kincaid's lupine *(L. texensis* 0.033 g/seed (Schaal 1980a) and Kincaid's lupine 0.029 g/seed (Severns unpublished data) had a mean genetic neighborhood

of about 0.5 m from ballistic seed dispersal (Schaal 1980b). Assuming the ballistic force during dispersal is comparable between *L. texensis* and Kincaid's lupine, it seems likely that within patch genetic architecture could develop between limited seed dispersal distance and pollinator foraging behavior. However, the relatively long distance spread of non-adventitious rhizomes may allow Kincaid's lupine genets to extend their genetic neighborhood, affecting outcrossing rates and breaking up locally inbred demes (*sensu* Cook 1983). Non-adventitious rhizome spread by Kincaid's lupine may be particularly effective when there are numerous spreading genets intermingled throughout different patch areas. However, this phenomenon remains to be empirically tested.

When considering how to plan for non-adventitious rhizome spread in the long-term conservation of Kincaid's lupine populations it is appropriate to conceptualize the aboveground leaves and flowers as the crown of a flowering tree and consider the longevity of a lupine genet. Typically, animal pollinated plants with large floral displays, like the crown of a tree, experience a high incidence of selfing through geitonogamy despite the attractiveness of large displays to pollinators (Barrett and Harder 1996, Eckert 2000, Galloway et al. 2002, Williams 2007). A high frequency of selfing in any one or even over several consecutive seasons that causes reductions in seed production is unlikely to place populations of a long-lived perennial plant, such as Kincaid's lupine which likely lives for centuries, at risk of extinction because longevity increases the chances of successful outcrossing over the life of the plant (Burd 1994, Morgan et al. 1997). Land managers of Kincaid's lupine populations should not be alarmed by small seed cohorts because it may be natural for this plant to produce a small number of seeds each year. In cases where a large, continuous genet is suspected, planting lupines within an expansive genet will likely lead to more frequent but local outcrossing in the short-term. Seed collection for seed banking, restoration, and long-term storage should incorporate the potential for rhizome spread and represent all regions within patches to maximize genetic diversity.

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Literature Cited

Ågren, J. 1996. Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. Ecology 77:1779–1790.

Alder, L. S. and R. E. Irwin. 2006. Comparison of pollen transfer dynamics by multiple floral visitors: experiments with pollen and fluorescent dye. Annals of Botany 97:141-150.

Antos, J. A. and C. B. Halpern. 1997. Root system differences among species: implications for early successional changes in forests of western Oregon. American Midland Naturalist 138:97-108.

Arnaud-Haond, S. and K. Belkhir. 2007. GENCLONE 1.0: a new program to analyse genetics data on clonal organisms. Molecular Ecology Notes 7:15-17.

Arnaud-Haond, S., Duarte, C. M., Alberto, F., and E. A. Serrão. 2007. Standardizing methods to address clonality in population studies. Molecular Ecology 16:5115-5139.

Ashley, M. V., Willson, M. F., Pergams, O. R. W., O'Dowd, D. J., Gende, S. M., and J. S. Brown. 2003. Evolutionarily enlightened management. Biological Conservation 111:115-123.

Auld, T. D. and A. J. Denham. 2001. The impact of seed predation by mammals on post-fire seed accumulation in the endangered shrub *Grevillea caleyi* (Proteaceae). Biological Conservation 97:377-385.

Barrett, S. C. H. and L. D. Harder. 1996. Ecology and evolution of plant mating. Trends in Ecology and Evolution 11:73-79.

Bishop, J. G. 2002. Early primary succession on Mount St. Helens: impact of insect herbivores on colonizing lupines. Ecology 83:191-202.

Bosch, M. and N. M. Waser. 2001. Experimental manipulation of plant density and its effect on pollinators and reproduction of two confamilial montane herbs. Oecologia 126:76-83.

Brown, J. H. and A. Kodric-Brown. 1977. Turnover rates in insular biogeography: effect of immigration and extinction. Ecology 58:445-449.

Buza, L., Young, A., and P. Thrall. 2000. Genetic erosion, inbreeding and reduced fitness in fragmented populations of the tetraploid pea *Swainsona recta*. Biological Conservation 93:177-186.

Citerne, H. L., Möller, M., and Q. C. B. Cronk. 2000. Diversity of *cycloidea*-like genes in Gesnerianaceae in relation to floral symmetry. Annals of Botany 86:167-176.

Citerne, H. L., Luo, D., Pennington, R. T., Coen, E., and Q. C. B. Cronk. 2003. A phylogenomic investigation of *CYCLOIDEA* like TCP genes in the Leguminosae. Plant Physiology 131:1042-1053.

Citerne, H. L. 2005. A primer set for specific amplification of two CYCLOIDEA-like genes in the genistoid clade of Leguminosae subfam. Papilionideae. Edingburgh Journal of Botany 62:119-126.

Cook, R. E. 1983. Clonal plant populations. American Scientist 71:244-253.

Dunn, D. B. and J. M. Gillett. 1966. The lupines of Canada and Alaska. Canada Department of Agriculture, Monograph No. 2, Ottawa, Canada.

Drummond, C. S. and M. B. Hamilton. 2005. Isolation and characterization of nuclear microsatellite loci in *Lupinus* group *Microcarpi* (Leguminosae). Molecular Ecology Notes 5:510-513.

Eckert, C. G. 2000. Contributions of autogamy and geitonogamy to self-fertilization in a mass-flowering, clonal plant. Ecology 81:532-542.

Edmands, S. 2007. Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. Molecular Ecology 16**:**463-475.

Ehrlén, J. and K. Lehtilä. 2002. How perennial are perennial plants? Oikos 98:308-322.

Ellstrand, N. C. and D. R. Elam. 1993. Population genetic consequences of small population size: implications for plant conservation. Annual Review of Ecology and Systematics 24:217-242.

Enright, N. J. and B. B. Lamont. 1989. Seed banks, fire season, safe sites and seedling recruitment in five co-occurring *Banksia* species. Journal of Ecology 77:1111-1122.

Feng, X., Zhao, Z., Tian Z., Xu, S., Luo, Y., Cai, Z., Wang, Y., *et al.* 2006. Control of petal shape and zygomorphy in *Lotus japonicus*. Proceedings of the National Academy of Sciences 103:4970-4975.

Fischer, M. and D. Matthies. 1997. Mating structure and inbreeding and outbreeding depression in the rare plant *Gentianella germanica* (Gentianaceae). American Journal of Botany 84:1685–1692.

Galloway, L. F., Cirigliano, T., and K. Gremski. 2002. The contribution of display size and dichogamy to potential geitonogamy in Campanula *americana*. International Journal of Plant Science 163:133-139.

García, M. B., Picó, F. X. and J. Ehrlén. 2008. Life span correlates with population dynamics in perennial herbaceous plants. American Journal of Botany 95:258-262.

Gómez-Aparicio, Zamora, R., and J. M. Gómez. 2005. The regeneration status of the endangered *Acer opalus* subsp. *granatense* throughout its geographical distribution in the Iberian Peninsula. Biological Conservation 121:195-206.

Hairston, N. G. Jr., Ellner, S. P., Geber, M. A., Takehito, Y., and J. A. Fox. 2005. Rapid evolution and the convergence of ecological and evolutionary time. Ecology Letters 8:1114-1127.

Harder, L. D. 1990. Behavioral responses by bumble bees to variation in pollen availability. Oecologia 85:41-47.

Haynes, J. and M. Mesler. 1984. Pollen foraging by bumble bees: foraging patterns and efficiency on *Lupinus polyphyllus*. Oecologia 61:249-253.

Hickman, J. C. (ed) 1993. The Jepson manual: higher plants of California. University of California Press, Berkeley, California, USA.

Howarth, D. G. and M. J. Donoghue. 2006. Phylogenetic analysis of the "ECE" (*CYC/TB1*) clade reveals duplications preceding the core eudicots. Proceedings of the National Academy of Sciences 103:9101-9106.

Hughes, C. and R. Eastwood. 2006. Island radiation on a continental scale: exceptional rates of plant diversification after uplift of the Andes. Proceedings of the National Academy of Sciences 103:10334-10339.

Husband, B. C. and D. W. Schemske. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. Evolution 50:54-70.

Juncosa, A. M. and B. D. Webster. 1989. Pollination in *Lupinus nanus* subsp. *latifolius* (Leguminosae). American Journal of Botany 76:59-66.

Karoly, K. 1994. Inbreeding effects on mating system traits for two species of *Lupinus* (Leguminosae). American Journal of Botany 81:1538-1544.

Kaye, T. N. 1999. Obligate insect pollination of a rare plant: *Lupinus sulphureus* ssp. *kincaidii*. Northwest Science 73:50-52.

Kaye T. N. and K. Kuykendall. 1993. *Lupinus sulphureus* ssp. *kincaidii*: survey and reproductive studies. Report submitted to the USDA Bureau of Land Management, Roseburg District, Roseburg, OR.

Kaye T. N. and K. Kuykendall. 2001. Effects of scarification, cold stratification, and source population on germination of *Lupinus sulphureus* ssp. *kincaidii*. Seed Science and Technology 29:663-668.

Keller, L. F. and D. M. Waller. 2002. Inbreeding effects in wild populations. Trends in Ecology and Evolution 17:230-241.

Kéry, M., Matthies, D., and M. Fischer. 2001. The effect of plant population size on the interactions between the rare plant *Gentiana cruciata* and its specialized herbivore *Maculinea rebeli*. Journal of Ecology 89:418-427.

Kéry, M., Matthies, D., and H. H. Spillmann. 2000. Reduced fecundity and offspring performance in small populations of the declining grassland plants *Primula veris* and *Gentiana lutea*. Journal of Ecology 88:17–30.

Kunin, W. E. 1997. Population size and density effects in pollination: pollinator foraging and plant reproductive success in experimental arrays of *Brassica kaber*. Journal of Ecology 85:225-234.

Lamont, B. B., Witkowski, E. T. F., and N. J. Enright. 1993. Post-fire litter microsites: safe for seeds, unsafe for seedlings. Ecology 74:501-512.

Lande, R. 1994. Risk of population extinction from fixation of new deleterious mutations. Evolution 48:1460-1469.

Lande, R. 1995. Mutation and conservation. Conservation Biology 9:782-291.

Leimu, R., Mutikainen, P., Koricheva, J., and M. Fischer. 2006. How general are positive relationships between plant population size, fitness and genetic variation? Journal of Ecology 94:942-952.

Lewis, G. M., Schrire, B., Mackinder, B., and M. Lock. 2005. Legumes of the world. Royal Botanic Gardends, Kew, UK.

Liston, A., St. Hilaire, K., and M. V. Wilson. 1995. Genetic diversity in populations of Kincaid's lupine, host plant of fender's blue butterfly. Madroño 42:309-322.

Maron, J. L. and S. N. Gardner. 2000. Consumer pressure, seed versus safe-site limitation, and plant population dynamics. Oecologia 124:260-269.

Montalvo, A. M. and N. C. Ellstrand. 2001. Nonlocal transplantation and outbreeding depression in the subshrub *Lotus scoparius* (Fabaceae). American Journal of Botany 88:258–269.

Morgan, M. T., Schoen, D. J., and T. M. Bataillon. 1997. The evolution of self-fertilization in perennials. The American Naturalist 150:618-638.

Mustajärvi, K., Siikamäki, P., Rytkönen, S., and A. Lammi. 2001. Consequences of plant population size and density for plant-pollinator interactions and plant performance. Journal of Ecology 89:80-87.

Newman, D. and D. A. Tallmon. 2001. Experimental evidence for the beneficial fitness effects of gene flow in recently isolated populations. Conservation Biology 15:1054-1063.

Peakall, R., Gilmore, S., Keys, W., Morgante, M., and A. Rafalski. 1998. Cross-species amplification of soybean (*Glycine max*) simple sequence repeats (SSRs) within the genus and other legume genera: implications for the transferability of SSRs in plants. Molecular Biology and Evolution 15:1275-1287.

Pendergrass, K. L., P. M. Miller, J. B. Kauffman, and T. N. Kaye. 1999. The role of prescribed burning in maintenance of an endangered plant species *Lomatium bradshawii*. Ecological Applications 9:1420–1429.

Pfab, M. F. and E. T. F. Witkowski. 2000. A simple population viability analysis of the critically endangered *Euphorbia clivicola* R.A. Dyer under four management scenarios. Biological Conservation 96:263-270.

Pickup, M. and A. G. Young. 2008. Population size, self-incompatibility and genetic rescue in diploid and tetraploid races of *Rutidosis leptorrhynchoides* (Asteraceae). Heredity 100:268-274.

Ree, R. H., Citerne, H. L., Lavin, M., and Q. B. C. Cronk. 2004. Heterogenous selection on *LEGCYC* paralogs in relation to flower morphology and phylogeny of *Lupinus* (Leguminosae). Molecular Biology and Evolution 21:321-331.

Reed, D. H. and R. Frankham. 2003. Correlation between fitness and genetic diversity. Conservation Biology 17:230-237.

Richards, C. M. 2000. Inbreeding depression and genetic rescue in a plant metapopulation. The American Naturalist 155:383-394.

Schaal, B. A. 1980a. Reproductive capacity and seed size in *Lupinus texensis*. American Journal of Botany 67:703-709.

Schaal, B. A. 1980b. Measurement of gene flow in *Lupinus texensis*. Nature 284:450-451.

Schemske, D. W., Husband, B. C., Ruckelshaus, M. H., Goodwillie, C., Parker, I. M., and J. G. Bishop. 1994. Evaluating approaches to the conservation of rare and endangered plants. Ecology 75:584-606.

Schulke, B. and N. M. Waser. 2001. Long-distance pollinator flights and pollen dispersal between populations of *Delphinium nuttallianum*. Oecologia 127:239-245.

Severns, P. 2003a. Inbreeding and small population size reduce seed set in a threatened and fragmented plant species, *Lupinus sulphureus* ssp. *kincaidii* (Fabaceae). Biological Conservation 110:221-229.

Severns, P. M. 2003b. Propagation of a long-lived and threatened prairie plant, *Lupinus sulphureus* ssp. *kincaidii*. Restoration Ecology 11:334-342.

Severns, P. M. 2006. Seasonality, habitat preference and life history of some Willamette Valley wet prairie terrestrial molluscs in western Oregon, USA. The Veliger 48:220-227.

Severns, P. M. 2008a. Exotic grass invasion impacts fitness of an endangered prairie butterfly, *Icaricia icarioides fenderi*. Journal of Insect Conservation 12:651-661

Severns, P. M. 2008b. Seedling population size and microhabitat association in *Lupinus oreganus* A. Heller var. *kincaidii* C.P. Sm. (Fabaceae), a threatened plant of western Oregon grasslands. Native Plants Journal 9:358-365.

Severns, P. M. and B. Lewis. 2007. Is it appropriate to rely on seed set to assess candidate plant populations for genetic rescue? A case study with a threatened species. Natural Areas Journal 27:313-319.

Sheridan, P. M. and D. N. Karrowe. 2000. Inbreeding, outbreeding, and heterosis in the yellow pitcher plant, *Sarracenia flava* (Sarraceniaceae) in Virginia. American Journal of Botany 87:1628-1633.

Silvertown, J. 2008. The evolutionary maintenance of sexual reproduction: evidence from the ecological distribution of asexual reproduction in clonal plants. International Journal of Botany 169:157-168.

Stehlik, I. and R. Holderegger. 2000. Spatial genetic structure and clonal diversity of *Anemone nemorosa* in late successional deciduous woodlands of central Europe. Journal of Ecology 88:425-435.

Stockwell, C. A., Hendry, A. P., and M. T. Kinnison. 2003. Contemporary evolution meets conservation biology. Trends in Ecology and Evolution 18:94-101.

Tallmon, D. A., Luikart, G. and R. S. Waples. 2004. The alluring simplicity and complex reality of genetic rescue. Trends in Ecology and Evolution 19:489-496.

Tomlinson, P. B. 1973. Branching in monocotyledons. The Quarterly Review of Biology 48:458-466.

Valtonen, A., Jantunen, J., and K. Saarinen. 2006. Flora and Lepidoptera fauna adversely affected by invasive *Lupinus polyphyllus* along road verges. Biological Conservation 133:389-396.

Waser, N. M. 1988. Comparative pollen and dye transfer by natural pollinators of *Delphinium nelsonii*. Functional Ecology 2:41-48.

Waser, N. M. and M. V. Price. 1994. Crossing distance effects in *Delphinium nelsonii*: outbreeding and inbreeding depression in progeny fitness. Evolution 48:842-852.

Willi, Y., Van Buskirk, J. and M. Fischer. 2005. A threefold genetic allee effect: population size affects cross-compatibility, inbreeding depression and drift load in the self-incompatible *Ranunculus reptans*. Genetics 169:2255-2266.

Williams, C. F. 2007. Effects of floral display size and biparental breeding on outcrossing rates in *Delphinium barbeyi* (Ranunculaceae). American Journal of Botany 94:1696-1705.

Wilson, M. V., Erhart, T., Hammond, P. C., Kaye, T. N., Kuykendall, K., Liston, A., Robinson, A. F. Jr., Schultz, C. B., and P. M. Severns. 2003. Biology of Kincaid's lupine (*Lupinus sulphureus* ssp. *kincaidii* [Smith] Phillips), a threatened species of western Oregon native prairies. Natural Areas Journal 23:72-83.

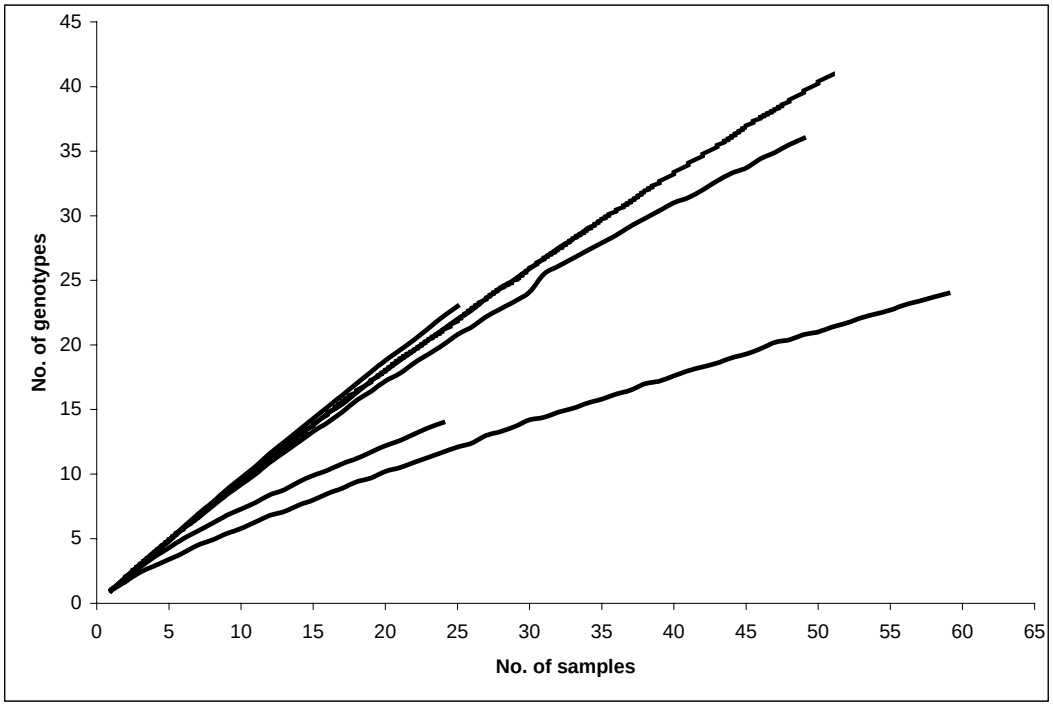

Figure 2.1. Rarefaction curves for the accumulation of multiple locus genotypes with increasing number of plantlets genotyped in all six lupine study patches.

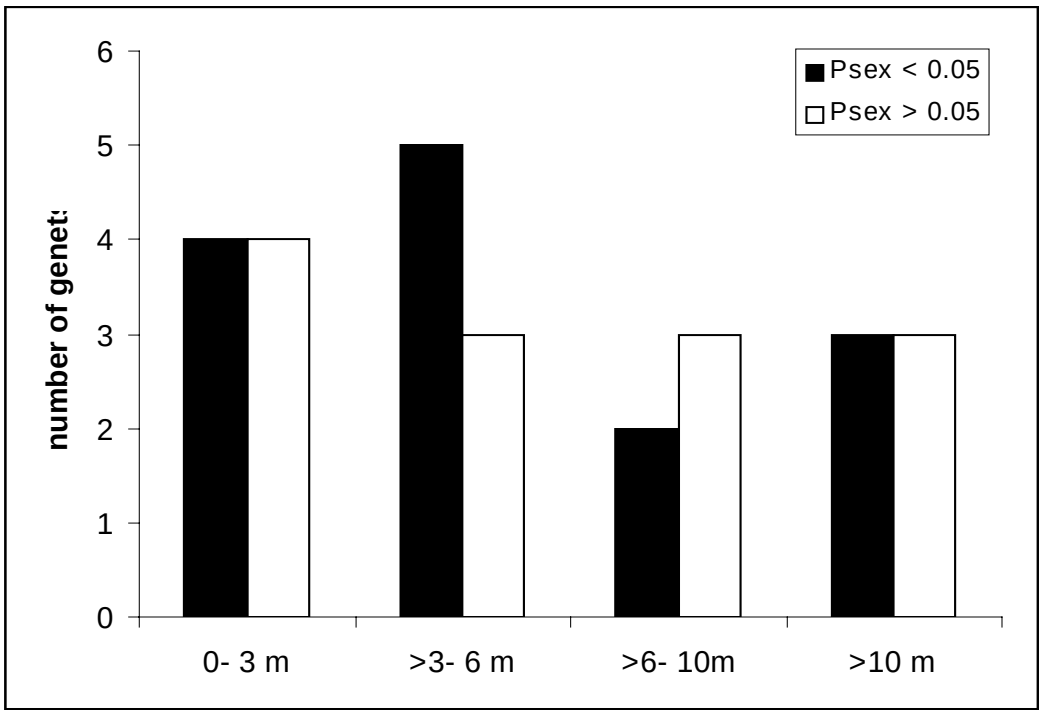

Figure 2.2. Distribution of maximum geographic distance classes between plantlets that appeared to be part of a shared genet ($P_{sex} < 0.05$) and when plantlets with shared multilocus genotypes had a probability of occurring independently from sexual reproduction ($P_{sex} > 0.05$).

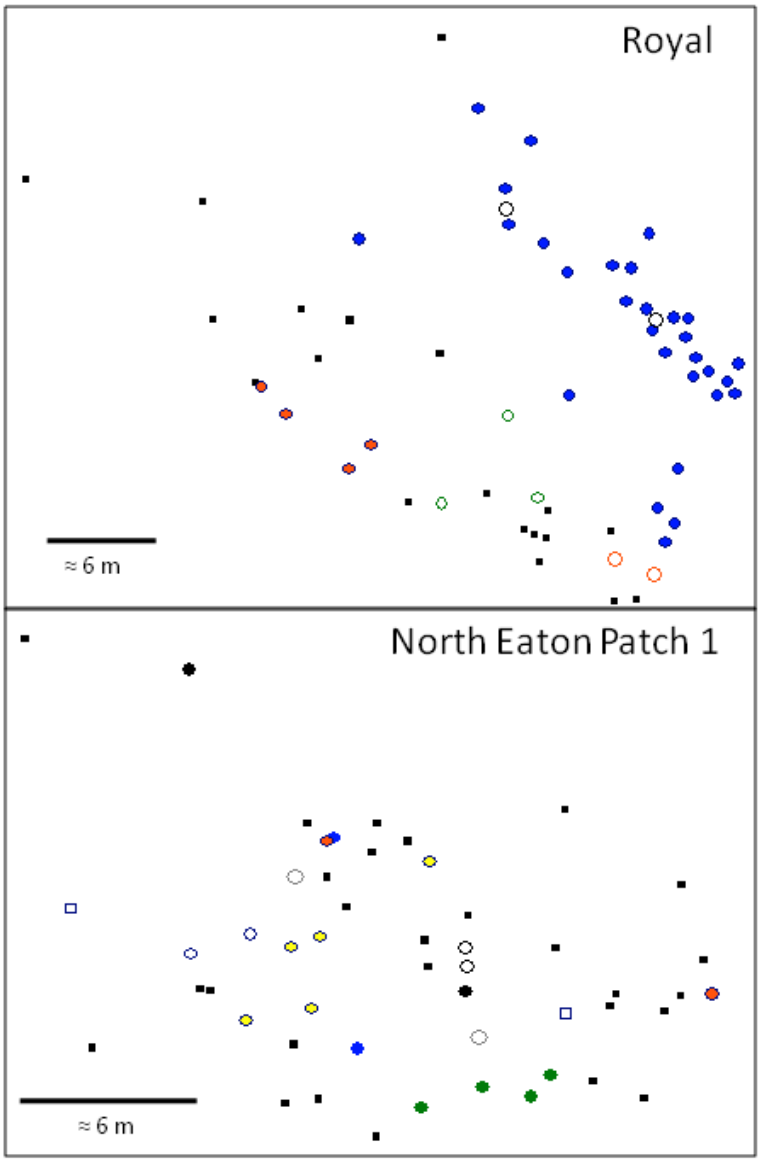

Figure 2.3. Distribution of plantlets sharing multiple locus genotypes at Royal and the North Eaton oaks patch. Smaller, solid black squares ■ represent plantlets with unmatched multiple locus genotypes (genets) within the study patch; solid, filled, circles ● of matching colors represent groups of 2 or more plantlets that belong to a genet (P_{sex} < 0.05); hollow circles ○ or squares □ of different colors represent groups of plantlets with identical multiple locus genotypes that possibly occurred independently through sexual reproduction (P_{sex} > 0.05).

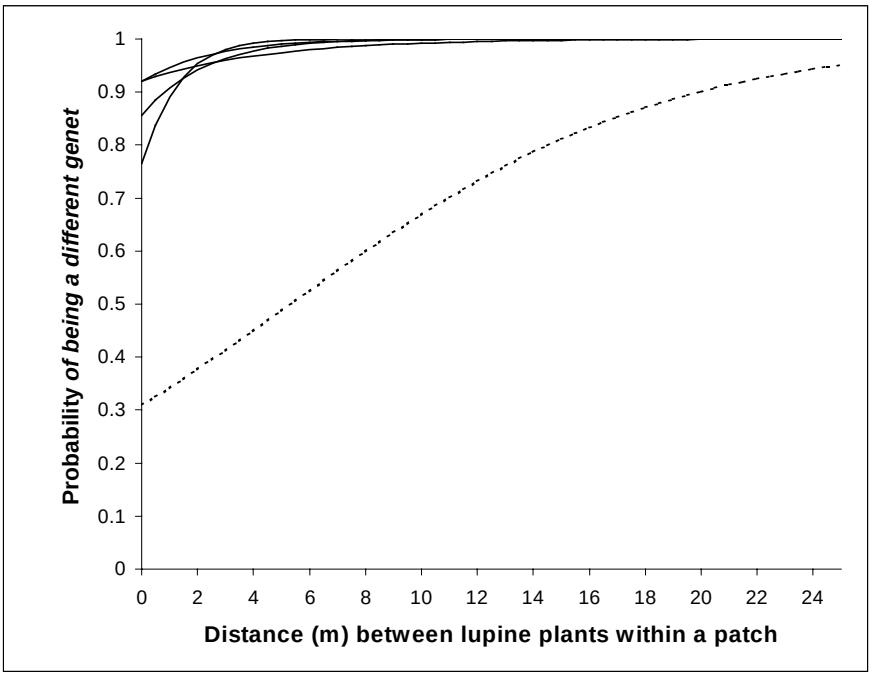

Figure 2.4. Probability of plantlets belonging to a shared genet with increasing interplantlet distance at Royal (dotted line) and North Eaton middle, North Eaton forest edge, North Eaton oaks, and South Eaton main patches (solid lines) calculated from logistic regression models for each study patch.

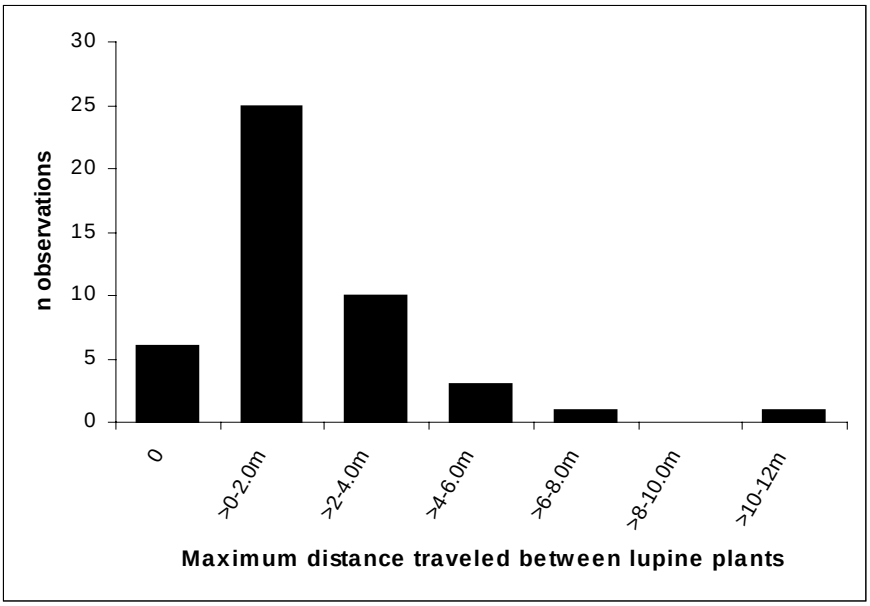

Figure 2.5. Summary of *Bombus* spp. (bumblebee) movements from Severns and Lewis (2007) by maximum interplantlet foraging flight distance classes.

Table 2.1. SSR allelic richness for each nSSR locus in three study populations.

	AG55-26-16	*AG81*	*CAC60-13-16*	*LEGCYC 1B-PolyR*	*LEGCYC 1B-TCP1*
North Eaton	3	12	4	4	6
South Eaton	2	8	4	4	4
Royal	2	7	4	2	3
Total # alleles amplified	**4**	**16**	**4**	**5**	**6**

Table 2.2. Genet number inferred from P_{sex} calculations for all genotyped plantlets in the six study patches.

Plantlet Category	No. of MLGs	No. of genets	No. of plantlets
Plantlets with unmatched MLGs	140	140	140
Plantlets with identical MLGs			
$P_{sex} < 0.05$	16	16	77
$P_{sex} > 0.05$	12	26	26
Total	**168**	**182**	**243**

Table 2.3. Maximum median geographic distance between Kincaid's lupine plantlets with identical shared multilocus genotypes (MLGs) that potentially arose from sexual reproduction ($P_{sex} > 0.05$) and plantlets that appeared to be part of a genet ($P_{sex} < 0.05$).

	No. identical MLGs shared by ≥ 2 plantlets	Lower 75th quartile	Median	Upper 25th quartile
$P_{sex} < 0.05$	16	3.1 m	5.6 m	10.6 m
$P_{sex} > 0.05$	12	3.6 m	5.1 m	8.8 m
Total	28	3.1 m	5.2 m	9.4 m

Table 2.4. Sample intensity, multilocus genotype (MLG) number, slope at the top of MLG accumulation curve, aggregation index (A_c), and associated probability of the estimate from 1,000 Monte Carlo permutations from six Kincaid's lupine study patches.

Study group	No. of plants genotyped	No. of MLGs	Slope at the top of the accumulation curve	A_c	Prob. of A_c estimate
Royal	59	24	0.45	0.28	<0.00001
N. Eaton Patch 1	50	36	0.45	0.12	<0.0001
N. Eaton Patch 2	34	29	0.54	0.11	0.004
N. Eaton Patch 3	25	23	0.48	0.08	0.05
S. Eaton Patch 1	24	14	0.42	0.36	<0.0001
S. Eaton Patch 2	51	41	0.44	0.11	0.001

Table 2.5. Mean probability (± 95% CI) of bumblebee flights (from Severns and Lewis 2007) occurring between different genets (n = 49) calculated from the logistic regression model of the probability of a plantlet belonging to a different genet given interplantlet distance (Fig. 4).

Study Patch	Lower 95% CI	Mean	Upper 95% CI
N.E. forest	94.4%	95.6%	96.1%
N.E. middle	94.0%	94.5%	95.0%
N.E. oaks	88.8%	90.9%	93.0%
S.E. main	91.2%	92.4%	93.6%
Royal	35.6%	37.9%	40.1%

Chapter 3: Conflicts between larval host abundance measurements for an endangered butterfly and population size estimates for its threatened larval host plant

Paul M. Severns and Mark V. Wilson

Abstract. Larvae of the endangered Fender's blue butterfly feed on the leaves of Kincaid's lupine, a threatened plant of western Oregon grasslands, that spreads through extensive, non-adventitious, rhizome growth. Decisions for status down-listing and species recovery for both the butterfly and larval host plant rests on estimates of population size, but such estimates are difficult for Kincaid's lupine because rhizomes spread > 10 m and genets intermingle. Kincaid's lupine population size estimates are commonly determined by measurements of leaf abundance (cover), which is more pertinent for estimating larval resource abundance than plant population size. We used simple sequence repeat DNA markers to identify lupine plantlets belonging to genets that vegetatively spread, to generate a genet to plantlet ratio that could be used to estimate population size by measuring mean lupine plantlet density and lupine patch area. We also compared lupine plantlet density to leaf cover and raceme number to investigate the reliability of transforming previously collected abundance data to genet population size estimates. Relationships between leaf cover, raceme number, and plantlet density were highly reliable within patches of Kincaid's lupine, but differed among plant patches within and between populations. Genet population size estimates with ACE (abundance-based coverage estimator) were close to size estimates from plantlet density and genet to plantlet ratios in some patches but not others. Genet population sizes in Kincaid's lupine can be estimated from cover, provided that plantlet density is first calibrated to cover within each study patch. Variation in genet spatial dominance, plantlet density, and plantlet size was too great between lupine patches and populations to create a reliable general model for converting leaf cover to genet number.

Introduction

Rare, threatened, and endangered butterflies depend upon the abundance of host plants under appropriate conditions for population persistence and colonization (Thomas et al. 1986, Dennis et al. 2003, Severns 2008, Severns and Warren 2008). For most rare butterflies, primary larval host plants are not rare enough to be considered at risk for extinction throughout their range (New et al. 1995, Warren et al. 1997, Maes and Van Dyke 2001), although there are a few instances in which rare butterflies also rely upon globally rare host plants (Kéry et al. 2001, Wilson et al. 2003, Krauss et al. 2005). As habitat fragmentation, degradation, loss, and ecosystem perturbance from global climate change is likely to increase, it is conceivable that butterfly host plant species may become rare enough to garner special conservation status in the foreseeable future. Plant demographic studies may become an integral part of rare butterfly conservation because long-term stability of butterfly populations depends on the continuous availability of abundant, useable larval resources (Thomas et al. 1996).

Population size changes are important and commonly measured properties of rare plant populations that influence conservation strategies (Schemske et al. 1996, Menges 2000, Kaye et al. 2001, Matthies et al. 2004). Recovery plans for United States Fish and Wildlife Service threatened and endangered plants often require estimates of population size and growth rates to update species listing status and guide management decisions. In non-clonal plants, simply counting or estimating the number of plants yields the genet population size, which is essential for estimating population growth rate, extinction risk, and effective population size. These population parameters and projections are important tools for enhancing rare plant population conservation and understanding the mechanisms regulating population persistence (Menges 2000). However, for plants that either spread vegetatively or clonally proliferate, simply counting plant number is unlikely to account for the number of genets in the population because the spatial patterns of aboveground plant tissues may not directly relate to genet distribution.

For example, ramets may be dispersed over a relatively large, discontinuous area (Kudoh et al. 1999, Persson and Gustavsson 2001), ramet turnover rates may differ from those of genets (Damman and Cain 1998, Tamm et al. 2002), and ramets from different genets may be so thoroughly intermingled that genet identification is nearly impossible without intensive genotyping (Kudoh et al. 1999) or excavation. Destructive sampling of above- and under-ground tissue to determine the patterns of vegetative spread may not be justifiable for rare plants, nor is repeated genotyping throughout all populations likely to be a sustainable and efficient method for plant population monitoring. However, if aboveground vegetative measurements correlate well with patterns of vegetative spread, then aboveground vegetation measurements may provide an acceptable method for estimating genet number (*e.g.* Fischer et al. 2000).

Plebejus icarioides fenderi Macy (= *Icaricia icarioides fenderi*), Fender's blue butterfly, is an endangered lycaenid of western Oregon, USA, whose larvae feed on the leaves of a threatened plant species, *Lupinus oreganus* Heller = *Lupinus sulphureus* ssp. *kincaidii* (Smith) Phillips (hereafter Kincaid's lupine) (Wilson et al. 2003). A draft recovery plan for Fender's blue and Kincaid's lupine requires population size estimates to calculate population growth rates for the butterfly and relative changes in aboveground abundance for the plant, which determine downlisting and recovery (U.S. Fish and Wildlife Service 2008). While butterfly population sizes can be estimated through annual counts (*e.g.* Schultz and Hammond 2003), Kincaid's lupine growth habit presents a problem to genet number estimates because it has non-adventitious rhizome spread > 25 m and genets appear to be intermixed (Chapter 2). Kincaid's lupine produces distinct clusters of leaves that arise either from the original tap-rooted plant or from rhizome nodes, both appearing aboveground as a "plant" (hereafter termed a plantlet). The growth form of Kincaid's lupine appears to be similar to the crown of a flowering tree, except that all tissues other than the leaves and racemes are below ground. Because Kincaid's lupine is not known to ramify, the relative plant population size is commonly estimated from either leaf number, lupine cover, or

raceme number (Schultz and Dlugosch 1999, Wilson et al. 2003). Both leaf and raceme number correlate positively with lupine cover, and leaf cover is also a convenient measurement for Fender's blue larval resource abundance (Schultz and Dlugosch 1999, Wilson et al. 2003). Cover, leaf, and raceme counts are also commonly employed methods for plant population monitoring that are relatively easy and efficient to use (Elzinga et al. 1998). Despite frequent use, there are inconsistencies and drawbacks to ocular cover estimates; they can be influenced by observer bias and cover classes (Sykes et al. 1983, Floyd and Anderson 1987, Wilson in prep.). Moreover, correlates with cover, such as flower production, can vary between years and among populations due to differences in abiotic (Cox and McEvoy 1983, Young 1984, Pendergrass et al. 1999, Severns 2003a) and biotic conditions (Brown and Gange 1989, Blossey and Hunt-Joshi 2003), potentially rendering the interpretation of population size from vegetative abundance measurements inconsistent.

We investigated the between and within population transferability of common aboveground lupine abundance measurements by comparing cover and inflorescence number estimates within different lupine patches in the same population and between populations. If there is a consistent relationship between and within populations in vegetative measures of aboveground lupine abundance, then historical plant abundance data could be converted to a single abundance variable for comparisons across populations and within populations over time. In counting the number of lupine plantlets, we attempted to both capture the modular unit of vegetative plant growth (*sensu* Harper and White 1974) and have a unit useful for identifying a genet arising from seed. We compared lupine plantlet number to cover and inflorescence number to determine whether a useful relationship between vegetative abundance measurements and modular lupine growth exists. Last of all, we used nuclear DNA simple sequence repeat markers (SSRs or microsatellites) to identify lupine plantlets belonging to genets that vegetatively spread. Using genet to plantlet ratios, plantlet density, and lupine

patch area we estimated lupine genet population size and evaluated the utility of our projections.

Methods

Study sites. We selected three study sites, Royal, North Eaton, and South Eaton, which have been the subjects of several past studies involving Kincaid's lupine (Severns 2003a, b, Severns 2008). Three patches of lupine plants in North Eaton, two patches in South Eaton, and one large patch at Royal were selected for investigation. To be consistent with prior studies, we considered a lupine patch as a group of plants in a shared field that is separated from another group of plants by at least 10 m (Severns 2003a). Under this definition of a lupine patch, genets do not extend from one patch to another. Both the North and South Eaton sites are adjacent to each other and share similar degraded upland prairie habitats but are separated by a dense deciduous forest of native and non-native trees. The Royal population occupies a different grassland habitat, a seasonally flooded wetland prairie (see Severns 2006 for a more detailed habitat description), but the lupines grow on slightly elevated mounded areas just above the high water table in the wet season.

Definitions for vegetative growth and measurement methods. Kincaid's lupine non-adventitious spread and apparent lack of asexual reproduction means that modular units of vegetative spread from a genet can not be referred to as a ramet, as they are in clonal plants (Harper and White 1974). We use the term plantlet to refer to a situation where a cluster of leaves appears to arise from a point distinct and separate from adjacent lupine leaf clusters. A plantlet may either represent a portion of a rhizomatously spreading genet or an entire genet. To be classified as a plantlet, a cluster of leaves must arise from a single point on the ground but be separated from another cluster of leaves by at least 10 cm from the margin of where the petioles emerged from the ground. We selected plantlets as a

measurement because physical delimitation was straightforward and it also appeared to reflect units of modular plant growth in several genets with vegetative spread that were followed from seeds (Severns unpublished data, Severns 2003b). We use the term genet to refer to a genetic individual; a genet may be represented in genotyping by multiple plantlets.

Cover was the proportion of a 1-m^2 quadrat occupied by lupine leaf crowns viewed from above. Lupine leaf cover was estimated to the nearest 1% in all quadrats. Racemes, a single inflorescence of flowers arranged vertically without side branching, were counted directly if they originated within the boundary of the quadrat. The number of lupine plantlets within a quadrat were also directly counted if more than half of the plantlet fell within the boundary of the quadrat.

Aboveground lupine abundance and analysis. We recorded plantlet number, raceme abundance, and lupine cover within 1-m ×1-m quadrats, selected at random from a 1-m grid superimposed on each lupine patch. Quadrat numbers ranged between 23 and 31 quadrats depending upon the size of the six study lupine patches. Quadrat reading was performed by the first author in order to reduce multiple observer bias in cover estimates.

We were interested in representing how vegetative measures relate to each other and we also desired a model where axis invertability was straightforward. We used eigenanalysis regression (instead of least-squares regression) to quantify the relationships among abundance measurements. Calculating the eigenvalue for each coordinate gives equal weight to each vegetation measurement and thus renders the relationship of a dependent y-variable on an independent x-variable invertible. In our eigenvalue regression model where $y = a + bx$, a is the y-intercept and b is the slope, inverting the relationship yields $x = -a/b + y/b$.

We calculated two different eigenvalue regression models to compare the consistency of vegetative relationships between and within lupine populations. We created a patch-specific model, where only within patch data was used, and a full model, where all patch data was combined. We quantified the difference in

performance using the predictions from the combined full model with estimates from the patch specific model by calculating root mean square errors (RMSE) for each study patch. The root mean square error approximates the order of magnitude difference in the two eigenvalue regression model predictions given the same set of lupine patch abundance data.

Tissue sampling for genetic analysis. Only leaves from distinct lupine plantlets were collected for genotyping. To select plantlets for tissue sampling, the boundaries of the lupine patches were identified and a coordinate position was randomly selected as a starting point. From this starting point a random compass bearing and distance between 0.5 m and 10 m was selected for each plantlet. Depending upon the size of the lupine patch and sampling effort, there were from one to several random starting points selected.

Molecular markers. Young leaf tissue was collected from each plantlet, placed on ice, and then transported to a -20°C freezer where the tissue was stored until DNA was extracted. We used five variable nuclear simple sequence repeats (SSRs) to indentify plantlets belonging to different genets. Two of the five loci are considered neutral (*AG55-26-16*, *CAC60-13-16*), with no known relationship to a known coding region (Drummond and Hamilton 2005). The locus *AG-81* is immediately downstream of a seryl-tRNA synthetase gene in *Glycine*, and has been treated as neutral (Peakall et al. 1998). The last two SSR loci are a pair of linked, exonic, trinucleotide repeats from a *CYCLOIDEA* paralog, *LEGCYC1B* (Citerne et al. 2003, Citerne 2005). *LEGCYC1B* contains a glycine (GGT) repeat near the 5′ of the exon and an asparagine repeat (AAC) near the 3′ end (Ree et al. 2004). The amino acid codon repeats are separated by approximately 800 basepairs (Ree et al. 2004).

Genomic DNA from 50-100 mg of frozen leaf tissue per individual was extracted with the FastDNA® kit and FastPrep® instrument according to manufacturer recommended protocols (QBiogene, Inc., CA). Marker loci were

amplified from 0.5-1.2 μL (10 – 30 ng) of genomic DNA, 1.2 μL of 10X Thermopol Buffer (New England Biolabs), 0.6 μL of 100X BSA, 1.0 μL (0.2mM of each dNTP), 0.5 μL of each forward and reverse primer (5 pM), 0.5 to 1.0 units of *Taq* polymerase (New England Biolabs) and 7.0 μL of ddH_20 for an approximate 11μL reaction volume. Marker loci were amplified with a BIO-RAD MyCycler™ thermal cycler at 94 °C for 4 min., 36 cycles of 94 °C for 1 min., 54 °C for 1 min., 72 °C for 1 min and 30 sec.

Marker primers were fluorescently labeled and the amplicon lengths were determined on an ABI3100 sequencer with GS500Rox as an internal standard. The ABI3100 output data was visualized and SSR marker lengths were scored using the program Genographer 1.6 (Benham 2001 http://hordeum.oscs.montana.edu/genographer).

Genet determination. Within a lupine patch, plantlets with identical shared multilocus genotypes (hereafter MLG) could belong to the same genet due to rhizome spread. A shared multilocus genotype among a group of plantlets could also arise independently from different sexual reproduction events. We used the estimator P_{sex} (Arnaud-Haond et al. 2007) as a filter to separate groups of two or more plantlets with identical multiple locus genotypes into two categories: plantlets with identical genotypes due to rhizome spread and plantlets with identical genotypes due to different sexual reproduction events. *Psex* gives an estimate of the probability of obtaining *n* samples with a shared multilocus genotype from the pool of genotyped samples through sexual reproduction under the assumption of random mating. (We assumed the absence of somatic mutations at SSR loci.) The *Psex* estimator also accounts for the influence of inbreeding on the occurrence of identical multiple locus genotypes through independent sexual reproduction events (Arnaud-Haond et al. 2007). We performed the calculations with the program Genclone 2.0 (Arnaud-Haond and Belkhir 2007) and selected a 5% cutoff probability for lupine plantlets. Two or more lupine plantlets within a patch sharing identical genotypes with $P_{sex} > 0.05$ we considered as having arose

from independent sexual reproduction events, and groups of two or more lupine plantlets within a patch sharing identical genotypes with $P_{sex} \leq 0.05$ we identified as belonging to a single vegetatively spreading genet.

Genet population size estimates. Estimation of genet population size within each patch was calculated by multiplying the mean number of lupine plantlets/m^2 by the study patch size (m^2) and either the within-patch ratio of genets to plantlets (patch specific) or the combined genet-to-plantlet ratio across patches (full model). We then compared the number of plants to within-patch estimates of cover and inflorescence number to arrive at a population estimate given specific patch information and overall, combined ratios.

We selected an algorithm commonly used for estimating species richness, the abundance-based coverage estimator (ACE) in the program EstimateS 8.0 (Colwell 2006 http://viceroy.eeb.uconn.edu/estimates) as an alternative method for estimating genet population size. The ACE species richness estimator uses a modified Jaccard Index to account for undetected species in the community sample based in part on the relative occurrence of taxa that occur in only once or twice (Chao et al. 2005). By analogy, we treated the number of genets detected through genotyping and their relative frequency among the genotyped plantlets within each patch, as an analog to species in a community, and estimated the number of genets.

Results

Relationship of aboveground abundance vegetative measurements. Within study patch relationships between different measures of abundance -- lupine cover/ m^2 vs. racemes/ m^2, cover/ m^2 vs. plantlet number/ m^2, and racemes/ m^2 vs. plantlet number/ m^2 -- were all positively correlated with each other, both within patches as well as when all patch data were combined (Table 3.1). The relationship of leaf cover/ m^2 vs. racemes/ m^2 appeared to be the most consistent within and between populations, while the relationship of plantlet number/ m^2 vs. cover/ m^2 and

plantlet number/ m^2 vs. racemes/ m^2 varied widely in both slope and intercept (Table 3.1). Within study patches, the patch-specific regression model explained more than 90% of the variation between measures of abundance, and in some instances explained nearly all of the variation (Table 3.1). However, there were differences in the slopes of all modeled vegetative measurements between patches within the same study population and between populations (Table 3.1) that appeared large enough to yield quantitatively different estimates as well as biologically different interpretations. Root mean square errors between the full model and patch specific models for individual lupine patches ranged from approximately 0.4 to 13.5 orders of magnitude differences in vegetative relationships depending upon the study patch (Table 3.1). There also appeared to be no patches or vegetative measurements with consistently low root mean square errors in our study patches (Table 3.1).

Genet to plantlet ratio. The ratio of genets to plantlets varied from 0.46 to 0.95 depending upon the study patch (Table 3.2). The combined full model genet to plantlet ratio was approximately three-quarters (0.75 genets/ plantlet) of the genotyped plantlets and the mean genet to plantlet ratio when patches were averaged was 0.79 genets/ plantlet (± 0.07 SEM).

Genet population size estimates. Estimates of population size ranged from about 50 to nearly 1,900 genets depending upon method of estimation and study patch (Table 3.3). ACE genet number estimates were generally lower than those of the vegetation based approach, although two study patches (North Eaton middle and North Eaton forest) were similar among all methods used (Table 3.3). Both the patch specific and combined genet-to-plantlet ratio population size estimates were positively strongly correlated with patch area (combined genet-to-plantlet ratio: $r = 0.887$, $p = 0.019$; patch specific genet-to-plantlet ratio: $r = 0.948$, $p = 0.004$) while the relationship between patch area and ACE values were positively related but not statistically significant ($r = 0.726$, $p = 0.102$). Since the number of plantlets/m^2

was directly involved in the calculation of vegetation based genet population size, lupine plantlet density might be expected to be strongly positively correlated with genet number estimates. Genet population size estimates with combined full model ($r = 0.753$, $p = 0.084$) and patch specific ($r = 0.50$, $p = 0.31$) were both positively correlated with genet to plantlet ratios but neither relationship was statistically significant.

Discussion

One of our goals in this study was to assess the efficacy of relating different methods of lupine abundance to each other so that data collected prior to this experiment could be transformed into a common abundance measurement for between site and year comparisons. Our eigenvalue regression models explained more than 90% of the data variation (Table 3.1) suggesting that land managers could convert between number of plantlets, number of racemes, and leaf cover estimates within lupine patches and some populations. While within-patch vegetative relationships appeared reliable, there was a marked difference in slope and intercept between different patches within a population as well as between populations (Table 3.1). Furthermore, the combined, full model (all patch data merged) glossed over important patch-specific vegetative relationships, despite R^2 values that exceeded 0.90 (Table 3.1), indicating that statistical significance does not necessarily equate to biological relevance.

We also found noteworthy differences in vegetation abundance between lupine study patches within the same population. For example, the three study patches in North Eaton, despite occurring within a relatively small site (< 1.5 ha), had different mean genet density, number of racemes per unit of cover, racemes per plantlet, and cover per plantlet (Table 3.1). Despite their close spatial proximity, the three patches in North Eaton grow in different habitat conditions, possibly explaining the between-patch inconsistency in vegetative abundance measurement relationships. The forest edge patch is partially shaded throughout

the day while the other two study patches receive full sunlight. The oaks patch is dominated by a tall exotic grass, *Arrhenatherum elatius*, that overtops plants and likely results in above ground competition hampering leaf production (Wilson and Clark 2001, Severns 2008a) and also likely inhibits seed germination (Severns 2008b). The middle patch is dominated by another exotic grass, *Anthoxanthum odoratum*, that does not overtop lupine plants nor does it appear to inhibit seedling germination relative to *A. elatius* (Severns 2008b).

Habitat conditions in this study encompassed some, but not all of the environmental conditions in which Kincaid's lupine occurs and spanned only one growing season. Given the variation in vegetative abundance measurements between different lupine patches and the large root mean square errors between the combined and patch specific eigenvalue regression models, cover data will need to be calibrated to plantlet number in each population, and potentially each patch, to yield comparable estimates of genet population size. Repeated multiyear measurements of lupine abundance and plantlet number may reveal that the relationship between plantlet number and cover or raceme number is consistent enough that a one-time calibration of vegetative abundance to plantlet number is sufficient for genet population size projections. If this relationship between cover or number of racemes and plantlet number proves reliable within populations, then historically collected lupine abundance data could be used to estimate changes in genet population size over time.

We have presented an additional vegetative measurement in this manuscript that is not yet incorporated into routine abundance monitoring of Kincaid's lupine, plantlet number. We chose to measure lupine plantlet density because it is relatively easy to delimit and is likely to represent a unit of modular vegetative growth as well as sexual reproduction. Within study patches, plantlet number correlated well with cover and raceme number (Table 3.1), but the number of lupine plantlets is likely to be a better indicator of genet population size than either cover or inflorescence number alone. Because Kincaid's lupine vegetative spread appears to be slow (Wilson et al. 2003), between-year differences in

plantlet number is more likely to reflect differences in recruitment or vegetative spread than either cover or raceme number, while cover and raceme number are more likely to reflect differences in plant vigor. Since substantial changes in cover or raceme number could occur from changes in the environment, such as weather (Severns 2003a), multiple observer bias in cover estimation (Sykes et al. 1983), use of cover classes (Sykes et al. 1983), nutrient loading (Helenurum and Schaal 1996, Theodose and Bowman 1997), fire (Abrahamson 1984, Hartnett 1991, Pendergrass et al. 1999), and competition (Goldberg 1987, Graham and Turkington 2000), we advise that Kincaid's lupine plantlet number be integrated into future vegetation monitoring protocols. Simultaneous estimates of plantlet number and plantlet vigor would yield more refined information concerning trends in population status over time and under different vegetation management treatments.

We genotyped plantlets to estimate genet number through ACE and to extrapolate genet number from genet to plantlet ratios within lupine patches. Genet number estimates from ACE were generally lower than that of vegetation based estimates unless the lupine patch was relatively small in area (Table 3.3). For biodiversity studies, ACE appears to yield relatively accurate species richness predictions compared with other species richness projection methods when as little as 65% of the community is sampled (Chao et al. 2005). Our genet population size estimates from ACE and the genet to plantlet ratio projection are similar in some study patches but not in others (Table 3.3), suggesting that genotyping effort was suitable for ACE in some small patches but fell short in larger ones. ACE may provide a worthwhile method to estimate genet population size in clonal and vegetatively spreading plants, but only if the study populations are genotyped at an appropriate level for ACE reliability.

The accuracy and precision of the methods used in our study to estimate genet population size can be further tested only through intensive genotyping, or excavation of physically connected individuals. Differences in genet population size estimates between the patch specific and combined, full model genet to plantlet ratios highlight the variation inherent in our method, even within the same

study patch. Two of the study patches with the largest difference between genet population size estimates were Royal and South Eaton main. Both study patches had a relatively dense number of genets/m^2 and occupied a relatively large area when compared with other study patches (Table 3.3). The influence of one large expansive genet at Royal spanning 27 meters (Chapter 2) appeared to greatly influence the patch specific genet to plantlet ratio, which resulted in a large different in calculated genet number when compared with a full model estimate (Table 3.3). While vegetative measurements may be insufficiently calibrated for precise and accurate genet population size estimates, land managers can still use the vegetative relationships presented in this manuscript to calculate a "ball-park" population size and fulfill their Endangered Species Act Recovery Plan requirements for population size monitoring.

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Literature Cited

Abrahamson, W. G. 1984. Species responses to fire on the Florida Lake Wales Ridge. American Journal of Botany 71:35-43.

Arnaud-Haond, S. and K. Belkhir. 2007. GENCLONE 1.0: a new program to analyse genetics data on clonal organisms. Molecular Ecology Notes 7:15-17.

Arnaud-Haond, S., Duarte, C. M., Alberto, F., and E. A. Serrão. 2007. Standardizing methods to address clonality in population studies. Molecular Ecology 16:5115-5139.

Blossey, B. and T. R. Hunt-Joshi. 2003. Belowground herbivory by insects: influence on plants and aboveground herbivores. Annual Review of Entomology 48:521-547.

Brown, V. K. and A. C. Gange. 1989. Differential effects of above- and below-ground insect herbivory during early plant succession. Oikos 54:67-76.

Chao, A., Chazdon, R. L., Colwell, R. K., and T. Shen. 2005. A new statistical approach for assessing similarity of species composition with incidence and abundance data. Ecology Letters 8:148-159.

Citerne, H. L., Möller, M., and Q. C. B. Cronk. 2000. Diversity of *CYCLOIDEA*-like genes in Gesnerianaceae in relation to floral symmetry. Annals of Botany 86:167-176.

Citerne, H. L., Luo, D., Pennington, R. T., Coen, E. and Q. C. B. Cronk. 2003. A phylogenomic investigation of *CYCLOIDEA* like TCP genes in the Leguminosae. Plant Physiology 131:1042–1053.

Citerne, H. L. 2005. A primer set for specific amplification of two *CYCLOIDEA*-like genes in the genistoid clade of *Leguminosae* subfam. *Papilionideae*. Edingburgh Journal of Botany 62:119-126.

Colwell, R. K. 2006. ESTIMATES: Statistical estimation of species richness and shared species from samples, Version 8.0.

Cox, S. C. and P. B. McEvoy. 1983. Effect of summer moisture stress on the capacity of tansy ragwort (*Senecio jacobaea*) to compensate for defoliation by cinnabar moth *(Tyria jacobaeae*). Journal of Applied Ecology 20:225-234.

Damman, H. and M. L. Cain. 1998. Population growth and viability analysis of the clonal woodland herb, *Asarum canadense*. Journal of Ecology 86:13-26

Dennis, R. L. H., Shreeve, T. G., and H. Van Dyck. 2003. Towards a functional resource-based concept for habitat: a butterfly biology viewpoint. Oikos 102:417-426.

Drummond, C. S. and M. B. Hamilton. 2005. Isolation and characterization of nuclear microsatellite loci in *Lupinus* group *Microcarpi* (Leguminosae). Molecular Ecology Notes 5:510-513.

Elzinga, C. L., Salzer, D. W., and J. W. Willoughby. 1998. Measuring and monitoring plant populations. U.S. Department of the Interior, Bureau of Land Management, Technical Reference 1730–1, Denver, Colorado, USA.

Fischer, M., Husi, R., Prati, D., Peintinger, M., van Kleunen, M., and B. Schmid. 2000. RAPD variation among and within small and large populations of the rare clonal plant *Ranunculus reptans* (Ranunculaceae). American Journal of Botany 87:1128-1137.

Floyd, D. A. and J. E. Anderson. 1987. A comparison of the three methods for estimating plant cover. Journal of Ecology 75:221-228.

Goldberg, D. E. 1987. Neighborhood competition in an old-field plant community. Ecology 68:1211-1223.

Graham, S. A. and R. Turkington. 2000. Population dynamics response of *Lupinus arcticus* to fertilization, clipping, and neighbour removal in the understory of the boreal forest. Canadian Journal of Botany 78:753-758.

Haper, J. L. and J. White. 1974. The demography of plants. Annual Review of Ecology and Systematics 5:419-463.

Hartnett, D. C. 1991. Effects of fire in tallgrass prairie growth and reproduction on prairie coneflower (*Ratibida columnifera*: Asteraceae). American Journal of Botany 78:429-435.

Helenurum, K. and B. A. Schaal. 1996. Genetic load, nutrient limitation, and seed production in *Lupinus texensis* (Fabaceae). American Journal of Botany 83:1585-1595.

Kaye, T. N., Pendergrass, K. L., Finley, K., and J. B. Kauffman. 2001. The effect of fire on the population viability of an endangered prairie plant. Ecological Applications 11:1366-1380.

Kéry, M., Matthies, D., and M. Fischer. 2001. The effect of plant population size on the interactions between the rare plant *Gentiana cruciata* and its specialized herbivore *Maculinea rebeli*. Journal of Ecology 89:418-427.

Krauss, J., Steffan-Dewenter, I., Müller, C. B., and T. Tscharntke. 2005. Relative importance of resource quality, isolation and habitat quality for landscape distribution of a monophagous butterfly. Ecography 28:465-474.

Kudoh, H., Shibaike, H., Takasu, H., Whigham, D. F., and S. Kawano. 1999. Genet structure and determinants of clonal structure in a temperate deciduous woodland herb, *Uvularia perfoliata*. Journal of Ecology 87:244-257.

Maes, D. and H. Van Dyck. 2001. Butterfly diversity loss in Flanders (north Belgium): Europe's worst case scenario? Biological Conservation 99:263-276.

Matthies, D., Bräuer, I., Maibom, W., and T. Tscharntke. 2004. Population size and the risk of local extinction: empirical evidence from rare plants. Oikos 105:481-488.

Menges, E. S. 2000. Population viability analysis in plants: challenges and opportunities. Trends in Ecology and Evolution 15:51-56.

New, T. R., Pyle, R. M., Thomas, J. A., Thomas, C. D. and P. M. Hammond. 1995. Butterfly conservation management. Annual Review of Entomology 40:57-83.

Peakall, R., Gilmore, S., Keys, W., Morgante, M. and A. Rafalski. 1998. Cross-species amplification of soybean (*Glycine max*) simple sequence repeats (SSRs) within the genus and other legume genera: implications for the transferability of SSRs in plants. Molecular Biology and Evolution 15:1275-1287.

Pendergrass, K. L., Miller, P. M., Kauffman, J. B., and T. N. Kaye. 1999. The role of prescribed burning in maintenance of an endangered plant species, *Lomatium bradshawii*. Ecological Applications 9:1420-1429.

Persson, H. A. and B. A. Gustavsson. 2001. The extent of clonality and genetic diversity in lingonberry (*Vaccinium vitis-idaea* L.) revealed by RAPDs and leaf shape analysis. Molecular Ecology 10:1385-1397.

Ree, R. H., Citerne, H. L., Lavin, M., and Q. B. C. Cronk. 2004. Heterogenous selection on *LEGCYC* paralogs in relation to flower morphology and phylogeny of *Lupinus* (Leguminosae). Molecular Biology and Evolution 21:321-331.

Schultz, C. B. and K. M. Dlugosch. 1999. Nectar and host plant scarcity limit populations of an endangered Oregon butterfly. Oecologia 119:231-238.

Schultz, C. B. and P. C. Hammond. 2003. Using population viability analysis to develop recovery criteria for endangered insects: case study of the Fender's blue butterfly. Conservation Biology 17:1372-1385.

Severns, P. 2003a. Inbreeding and small population size reduce seed set in a threatened and fragmented plant species, *Lupinus sulphureus* ssp. *kincaidii* (Fabaceae). Biological Conservation 110:221-229.

Severns, P. M. 2003b. Propagation of a long-lived and threatened prairie plant, *Lupinus sulphureus* ssp. *kincaidii*. Restoration Ecology 11:334-342.

Severns, P. M. 2006. Seasonality, habitat preference and life history of some Willamette Valley wet prairie terrestrial molluscs in western Oregon, USA. Veliger 48:220-227.

Severns, P. M. 2008a. Exotic grass invasion impacts fitness of an endangered prairie butterfly, *Icaricia icarioides fenderi*. Journal of Insect Conservation 12:651-661

Severns, P. M. 2008b. Seedling population size and microhabitat association in *Lupinus oreganus* A. Heller var. *kincaidii* C.P. Sm. (Fabaceae), a threatened plant of western Oregon grasslands. Native Plants Journal 9:358-365.

Severns, P. M. and A. D. Warren. 2008. Selectively eliminating and conserving exotic plants to save an endangered species from local extinction. Animal Conservation 11:476-483.

Sykes, J. M., Horrill, A. D., and M. D. Mountford. 1983. Use of visual cover assessments as quantitative estimators of some British woodland taxa. Journal of Ecology 71:437-450.

Tamm, A., Kull, K., and M. Sammul. 2002. Classifying clonal growth forms based on vegetative mobility and ramet longevity: a whole community analysis. Evolutionary Ecology 15:383-401.

Theodose, T. A. and W. D. Bowman. 1997. Nutrient availability, plant abundance, and species diversity in two alpine tundra communities. Ecology 78:1861-1872.

Thomas, J. A., Thomas, C. D., Simcox, D. J., and R. T. Clarke. 1986. Ecology and declining status of the silver-spotted skipper butterfly (*Hesperia comma*) in Britain. Journal of Applied Ecology 23:365-380.

Thomas, C. D., Singer, M. C., and D. A. Boughton. 1996. Catastrophic extinction of population sources in a butterfly metapopulation. American Naturalist 148:957-975.

United States Fish and Wildlife Service. 2008. Draft recovery plan for the prairie species of western Oregon and southwestern Washington. U.S. Fish and Wildlife Service, Portland, Oregon.

Warren, M. S., Barnett, L. K., Gibbons, D. W., and M. I. Avery. 1997. Assessing national conservation priorities: an improved red list of British butterflies. Biological Conservation 82:317-328.

Wilson, M. V., and D. L. Clark. 2001. Controlling invasive *Arrhenatherum elatius* and promoting native prairie grasses through mowing. Applied Vegetation Science 4:129-138.

Wilson, M. V., Erhart, T., Hammond, P. C., Kaye, T. N., Kuykendall, K., Liston, A., Robinson, A. F. Jr, Schultz, C. B. and P. M. Severns. 2003. Biology of Kincaid's lupine (*Lupinus sulphureus* ssp. *kincaidii* [Smith] Phillips), a threatened species of western Oregon native prairies. Natural Areas Journal 23:72-83.

Young, T. P. 1984. The comparative demography of semelparous *Lobelia telekii* and iteroparous *Lobelia keniensis* on Mount Kenya. Journal of Ecology 72:637-650.

Table 3.1. Eigenvalue regression predicted aboveground vegetation abundance measurement relationship and variance explained by study patch and a full model (all patch data combined) and root mean square error (RMSE) between the full model and patch specific model.

Vegetative Relationship	Royal (n=30 quadrats)	N. Eaton forest edge (n=24 quadrats)	N. Eaton middle (n=31 quadrats)	N. Eaton oaks (n=28 quadrats)	S. Eaton main (n=30 quadrats)	S. Eaton bike path (n=23 quadrats)	Full model (n=166 quadrats)
cover (y) vs. raceme # (x)	Y=4.02+1.92x R^2 = 0.94 RMSE = 0.69	Y=7.65+1.99x R^2 = 0.96 RMSE = 3.65	Y=5.99+1.08x R^2 = 0.93 RMSE = 13.5	Y=4.61+2.04x R^2 = 0.95 RMSE = 2.41	Y=4.27+2.32x R^2 = 0.96 RMSE = 4.56	Y=7.26+2.05x R^2 = 0.96 RMSE = 3.57	Y=5.0+1.89x R^2 = 0.93
plantlet # (y) vs. raceme # (x)	Y=4.38+0.35x R^2 = 0.91 RMSE = 4.27	Y=1.75+0.10x R^2 = 0.98 RMSE = 1.62	Y=1.37+0.07x R^2 = 0.99 RMSE = 2.69	Y=3.58+0.04x R^2 = 0.97 RMSE = 1.41	Y=2.24+0.33x R^2 = 0.96 RMSE = 1.80	Y=2.86+0.11x R^2 = 0.93 RMSE = 0.41	Y=3.04+0.14x R^2 = 0.91
plantlet # (y) vs. cover (x)	Y=3.19+0.20x R^2 = 0.97 RMSE = 1.98	Y=1.28+0.05x R^2 = 0.99 RMSE = 1.98	Y=0.56+0.08x R^2 = 0.99 RMSE = 1.74	Y=2.84+0.04x R^2 = 0.99 RMSE = 1.78	Y=1.64+0.14x R^2 = 0.99 RMSE = 1.08	Y=1.84+0.08x R^2 = 0.98 RMSE = 0.53	Y=1.96+0.10x R^2 = 0.97

Table 3.2. Sample size, number of genets among the genotyped plantlets, and genet to plantlet ratio in six Kincaid's lupine study patches.

Study Patch	*n* plantlets genotyped	# genets	Genet to plantlet ratio
Royal	59	24	0.46
N. Eaton forest edge	25	23	0.95
N. Eaton middle	34	30	0.88
N. Eaton oaks	50	40	0.80
S. Eaton main	51	46	0.90
S.Eaton bike path	24	18	0.75
Combined, full model	243	181	0.76

Table 3.3. Genet population size estimates, genet density, relative plantlet size, and cover per genet in m^2 vegetation sampling quadrats in six Kincaid's lupine study patches. Shown are the mean estimate, with 95% confidence intervals in parentheses.

	Royal (300 m^2)	N.E. Middle (130 m^2)	N.E. Oaks (312 m^2)	N.E. Forest (71 m^2)	S.E. Main (295 m^2)	S.E. Bike Path (42 m^2)
ACE	228	224	300	185	531	49
Genet pop. size (full model)	1878 (1518, 2244)	217 (135, 380)	967 (771, 1167)	141 (112, 173)	1221 (997, 1449)	115 (84, 147)
Genet pop. size (patch specific)	1158 (932, 1377)	256 (160, 449)	1035 (827, 1248)	180 (141, 220)	1475 (1204, 1749)	114 (83, 145)
# genets/m^2 (full model)	6.26 (5.06, 7.48)	1.67 (1.04, 2.92)	3.10 (2.47, 3.74)	1.99 (1.57, 2.43)	4.14 (3.38, 4.91)	2.70 (1.96, 3.45)
# genets/m^2 (patch specific)	3.86 (3.12, 4.61)	1.97 (1.23, 3.45)	3.32 (2.65, 4.0)	2.54 (1.99, 3.1)	5.0 (4.08, 5.93)	2.70 (1.96, 3.45)
cover/plantlet	3.4 (2.7, 4.2)	10.5 (8.1, 12.9)	9.2 (6.7, 11.6)	10.4 (8.2, 12.6)	5.1 (4.4, 5.7)	7.1 (5.0, 9.2)
cover/genet/m^2 (full model)	4.6 (3.6, 5.6)	14.0 (10.8, 17.2)	12.3 (9.0, 15.5)	13.9 (10.9, 16.8)	6.8 (5.9, 7.6)	9.5 (6.7, 12.3)
cover/genet/m^2 (patch specific)	7.4 (5.8, 9.0)	11.8 (9.1, 14.5)	11.5 (8.4, 14.5)	10.9 (8.6, 13.2)	5.6 (4.9, 6.3)	9.5 (6.7, 12.3)

Chapter 4: Is interpopulation seed mixing necessary for effective genetic conservation of a threatened, perennial grassland lupine?

Paul M. Severns, Aaron Liston, and Mark V. Wilson

Abstract. Interpopulation seed mixing for the rescue of populations harboring genetic load is a potentially effective mitigation strategy that can enhance the likelihood of long-term rare plant population persistence. Kincaid's lupine, a threatened plant of western Oregon grasslands, has small, isolated populations that appear to suffer from inbreeding depression. But, a recent study suggested that low seed set may instead be the byproduct of frequent selfing calling into question suppositions of widespread inbreeding depression. We used simple sequence repeat (SSR) DNA loci, both nuclear and chloroplast, to screen populations of Kincaid's lupine for evidence of severe inbreeding that would suggest a genetic rescue strategy could be effective. Most study populations did not present evidence of a recent bottleneck, fixation coefficients from nSSRs that approached Hardy-Weinberg Equilibrium expectations in nearly half of the screened populations, and genetic diversity was relatively high. Chloroplast DNA haplotype number averaged over all populations, from very small to large, averaged about 4.2 haplotypes/ population, a relatively high number of maternal lineages for an animal pollinated plant. Chloroplast haplotypes were also distributed fairly evenly within lupine populations, suggesting that maternal lineages and genetic diversity is not rapidly being lost to recent habitat fragmentation. Even Kincaid's lupine patches shared similar patterns of genetic diversity to populations: evidence for recent genetic bottlenecks was lacking, genetic diversity was high, and there were typically more than three cpDNA haplotypes per study patch (n = 5). Genotyped progeny from a common garden experiment revealed that even small founding populations of Kincaid's lupine can maintain both nuclear and chloroplast genetic diversity through balanced contributions to the F1 cohort. Moreover, a heterosis-like effect may accompany mixing seeds from different patches within populations as seeds produced by seven

surviving progeny collected from two source patches outperformed the source population by 30-fold in a common garden. We recommend within-population seed transfer for population enhancement. This approach may be conservative, but it decreases the chances of outbreeding depression through accidental mixing of seeds populations with non-matching ploidy and populations with different hybridization histories, both of which are known to occur in Kincaid's lupine. Given the centuries-long life of Kincaid's lupine genets, overlap of multiple generations, high penalty for inbreeding in lowered seed set (possibly germination rates), and large amounts of within-population genetic diversity, land managers could likely maintain genetic diversity by improving habitat conditions that favor natural recruitment.

Introduction

A major challenge in rare plant conservation is to develop and implement conservation plans that enhance the chance of long-term population (multi-generational) persistence. In some populations, pollinator service and habitat conditions appear to limit natural recruitment (Jennerston 1988, Kearns et al. 1998), while in others small effective population size and limited genetic diversity may be the culprit (Ellstrand and Elam 1993, Kéry et al. 2000, Keller and Waller 2002, Willi et al. 2005, Pickup and Young 2008). Several mechanisms may simultaneously limit plant population growth and persistence over time and under changing habitat conditions, but proximate causes that repeatedly limit rare plant population growth should be targeted in conservation plans (Schemske et al. 1994). Inbreeding depression is often an explanation for low seed production, progeny vigor, and recruitment in plant populations, especially those that are relatively small and isolated from immigrants (Ellstrand and Elam 1993, Richards 2000). In outcrossing species, multiple generations of inbreeding reduce population genetic diversity and heterozygosity, leading to declines in population fitness from the accumulation and fixation of deleterious alleles, genetic load (Barrett and Charlesworth 1991, Hedrick 1994, Charlesworth and Charlesworth 1999). In the

absence of migration or beneficial mutations, inbreeding may ultimately lead to mutational meltdown, where nearly all recombinants from within population matings harbor sufficiently large levels of genetic load that natural recruitment ceases (Lynch et al. 1995).

Spielman et al. (2004) argue that most populations of rare species are affected by genetic factors well before stochastic events lead to population extinction. If this assertion is true, then genetic rescue of inbred populations through the introduction of unrelated genotypes that result in a reduction of genetic load is a valuable mitigating measure for conservation biologists seeking the persistence of small, rare plant populations (Brown and Kodric-Brown 1977, Richards 2000, Newman and Tallmon 2001, Tallmon et al. 2004). However, for genetic rescue to be effective, the targeted populations must harbor enough genetic load that when new genotypes are introduced outcrossing results in heterosis. Interpopulation seed transfer for genetic rescue carries a risk of outbreeding depression, a process where crossing between incompatible genotypes of the same species results in lower fitness and progeny health (Waser and Price 1979). While instances of outbreeding depression are known to occur within natural plant populations (Waser and Price 1979, 1989), it appears to be more frequently associated with the intentional movement of individuals between populations for the purpose of conservation or habitat restoration (*e.g.* Fischer and Matthies 1997, Keller et al. 2000, Montalvo and Ellstrand 2001, Holmes et al. 2008).

Relatively small, recently fragmented plant populations that appear to be isolated from migrants may be candidates for genetic rescue (reviewed by Honnay and Jacquemyn 2007, Aguilar et al. 2008). Outward symptoms of population level inbreeding depression such as relatively low observed seed set (Ågren 1996, Husband and Schemske 1996, Kéry et al. 2000), and diminished progeny vigor and fitness (Karoly 1994, Husband and Schemske 1996, Buza et al. 2000, Sheridan and Karrowe 2000) often correspond with small population size. Due to the frequent observations of suppressed reproduction with small populations, small population size may be considered as sufficient evidence to recommend genetic rescue in the

absence of direct inbreeding depression evaluation (*e.g.* Kéry et al. 2000). While the link between small population size and the potential for inbreeding depression is well-documented (reviewed by Leimu et al. 2006), the presumption that relatively small populations are genetically impoverished and that genetic load is high without direct evidence can yield an inaccurate assignment of population genetic status (*e.g.* Field et al. 2008). Even an index of population fitness, such as low seed set, may be explained by mechanisms other than inbreeding depression (Severns and Lewis 2007, Chapter 2).

Controlled crossing experiments to determine whether a single rare plant population harbors enough genetic load that it requires genetic rescue is unlikely to require a large investment of resources (Severns 2009 in revision). However, assessing a number of populations for genetic rescue through hand crosses is a far more daunting task and may not occur over an acceptable time frame for land managers. Molecular markers provide a means by which populations can be more efficiently screened for the telltale signs of severe inbreeding. Allelic patterns in DNA marker diversity, such as simple sequence repeats (SSRs), can identify processes that may be affecting genetic diversity across the genome. In the case of genetic rescue, the expectation of a plant population that is a candidate for seed transfer compared to one that does not appear to be highly inbred is fairly straight forward. When compared with a population that does not require genetic rescue, a severely inbred population would have relatively low genetic diversity and there would be a notable shift from Hardy-Weinberg Equilibrium (HWE) expectations towards an excess of homozygosity. Most molecular markers, such as SSRs, are considered to be selectively neutral and they may therefore not reflect genetic load because the marker loci themselves are not fitness or vigor related genes (Jaquiéry et al. 2009). However, selectively neutral DNA markers can identify processes that will eventually lead to an accumulation of genetic load, such as genetic bottlenecks (Luikart et al. 1998) or founder events (Rasner et al. 2004).

Lupinus oreganus Heller (= *Lupinus sulphureus* ssp. *kincaidii* [Smith] Phillips), hereafter Kincaid's lupine, is an endemic grassland legume to restricted

to western Oregon and southwest Washington, U.S.A. (Wilson et al. 2003). In the Willamette Valley of western Oregon, Kincaid's lupine is the primary larval host plant of *Plebejus icarioides fender* Macy, Fender's blue butterfly, a lycaenid that was once thought to be extinct. Fender's blue butterfly and Kincaid's lupine are protected by the U.S. Endangered Species Act, and listed as endangered and threatened, respectively. Both species occupy highly fragmented native grasslands that have been reduced to around 1% of their former expanse over the last century from increasing urbanization, agricultural development, habitat degradation, and loss (Alverson 2005).

Kincaid's lupine populations manifest the typical symptoms associated with inbreeding depression. All of the populations surveyed for seed production have low natural seed set (Kaye and Kuykendall 1993, Severns 2003a). In a group of locally fragmented populations, seed set was correlated with the number of lupine patches within a population (Severns 2003a), suggesting that small population size influences fitness. A hand pollination experiment indicated that a relatively small, single-patched population was inbred (Severns 2003a). Seeds collected from a small, single-patched population germinated at about half the rate of seeds collected from a large, multiple-patched population under optimal germination laboratory conditions (Kaye and Kuykendall 2001). Last of all progeny from five populations within 0.7 km of each other grown in a common garden varied widely in survival and vigor, suggesting genetic differentiation between populations, perhaps due to habitat fragmentation (Severns 2003b). While the conclusions from multiple Kincaid's lupine studies suggest that relatively small, fragmented populations harbor genetic load, one pair of populations grown in a common garden suggest otherwise. Seeds collected from seven individuals in an artificially created population outperformed its source population by nearly 30 fold when survivorship, leaf area, and fitness were considered together (Severns 2003b). This large difference in progeny performance between the parent seed source population and the progeny produced from a small number of source population individuals should not occur if the

source population accumulated the abundance of sublethal mutations typical for populations with inbreeding depression (*e.g.* Newman and Tallmon 2001).

We used nuclear and chloroplast DNA simple sequence repeats (SSRs) to screen Kincaid's lupine populations of different relative sizes across the species range for evidence of severe inbreeding and recent genetic bottlenecks due to habitat fragmentation. If Kincaid's lupine populations require genetic rescue because they are severely inbred, the SSR marker genetic diversity should be relatively diminished in small populations and there should be evidence of recent genetic bottlenecks. To address the potential genetic impacts of transferring seeds within different patches from the same lupine population we compared patterns of genetic diversity within augmented and non-augmented patches. We also employed SSRs to unravel the genetic situation surrounding fitness differences in the common garden performance of source population and a small number of selected progeny. Insight into the disparity in population level performance of seed sources derived from the same population in the common garden experiment may lend important insight into the effectiveness of local seed transfer.

Methods

Study species. Approximately 60 populations of Kincaid's lupine are found primarily in remnant grasslands throughout the 200 km long Willamette Valley, of western Oregon, USA, but groups of outlying populations are known from southwest Washington State and from Douglas, Co. Oregon (Wilson et al. 2003). Populations south of the Willamette Valley, in Douglas County, grow under and on the edge of mixed deciduous forests canopy or in recent forest clear cuts, habitats unoccupied by populations to the north.

Kincaid's lupine is a long-lived perennial, that is potentially hundreds of years old (Wilson et al. 2003) and has relatively heavy seeds (0.27-0.30 g) that are dispersed through explosive fruit dehiscence. On average, 40 to 100 flowers are borne on unbranched racemes that are elevated above a cluster of long-petioled

leaves that arise directly from the ground. Kincaid's lupine is genetically self-compatible and has a mixed mating system (Severns and Lewis 2007), but pollen viability does not overlap in time with stigma receptivity within each flower, so pollinators are required for both self and outcross seed set (Kaye 1999). Using the calculation for stage-specific inbreeding depression and individual seed set from prior controlled crossing experiments (Severns 2003a, Severns and Lewis 2007); $\delta = 1 - (\omega_{self} / \omega_{outcross})$, where ω = seed set from self pollination and outcrossing (Husband and Schemske 1996), the inbreeding coefficient for Kincaid's lupine seed set ranges from 0.53-0.64, categorizing it as a primarily outcrossing species (Husband and Schemske 1996). The δ inbreeding coefficient values for Kincaid's lupine seed set may be an underestimate because in one case there was no geitonogamous selfing treatment (Severns 2003a) and in the other there was no pure outcross treatment (Severns and Lewis 2007). We do not have data that would allow us to calculate an inbreeding coefficient for germination and early life stage survival. But, seeds from a large, multi-patched population, germinated (under controlled laboratory conditions) at a much higher rate (95%) than seeds from a small, single patch population (55%) (Kaye and Kuykendall 2001), suggesting that inbreeding may also impact germination.

Kincaid's lupine appears to be unique among all *Lupinus* worldwide in that it produces non-adventitious rhizomes that can spread to at least 27 m (Chapter 2). Clusters of leaves and racemes appear to be distinct plants but they may be a solitary, non-spreading genet or the reproductive tissues of spreading genets. We refer to clusters of aboveground leaves, separated from the nearest cluster of leaves at the petiole bases by more than 10 cm a "plantlet" because Kincaid's lupine is not known to ramify and refer to a genetic individual (with all of its plantlets) as a genet (Chapter 2).

Study populations. Study sites for genotyping were spread throughout the species range from Douglas Co. (n = 8 populations), throughout the Willamette Valley (n = 16 populations), to the northernmost extant populations known in southwest

Washington State (n = 3 populations). Two multiple-patched study sites in the southern Willamette Valley, ≈ 10 km west of Eugene, Oregon, (North Eaton and South Eaton) were genotyped to determine the potential impact of within population transfer of genets on patch diversity and heterozygosity. One of these two study sites, North Eaton, was also the seed source for the Office population that outperformed the parental source population in a common garden (Severns 2003b). North Eaton has three patches of lupine that are separated by more than 10 m from each other (Severns 2003a) in this manuscript referred to as the middle, oaks, and forest edge patches. Of these three patches, the middle patch and oaks patch were replanted with seedlings that were grown from seeds originally collected from the two patches. Seeds from North Eaton were grown in a 10 m x 4 m area adjacent to the U.S. Army Corps of Engineers Fern Ridge Reservoir Project Office (hereafter the Office population). After the seeds were germinated and seedlings had grown for a year all but a handful of the Office plants were dug and transplanted back into the two source patches in the spring of 1992. The plants left at the Office grow-out site appeared to be less vigorous than the others and the surviving individuals founded the Office population. Seeds from the Office population were collected and grown in a common garden with seeds from the two North Eaton seed source patches (middle and oaks patches). The Office progeny outperformed the North Eaton progeny by nearly 30-fold in a common garden (Severns 2003b). South Eaton, a prairie parcel adjoining North Eaton, is separated by a physical barrier of conifers and deciduous trees, but otherwise has comparable habitat conditions to North Eaton. There are three lupine patches in South Eaton, but only two patches were genotyped, the main patch and one that falls within a proposed bike path right-of-way. Neither of the study patches in South Eaton have experienced supplemental planting.

Categorizing Kincaid's lupine population size. Estimates of population size in Kincaid's lupine are confounded by extensive spread of non-adventitious rhizomes. For the purpose of this study we categorized population size by ranking

each population into a size class. Four size classes, very small, small, medium, and large were relative rankings based on subjective estimates of plantlet number in each population. Very small populations, are among the smallest known and have < 100 plantlets. Small populations have between 100 to 500 plantlets and very rarely support Fender's blue butterfly populations unless there is a larger population of lupines nearby. Medium size populations contain > 500 plantlets but < 5,000 plantlets, and large populations appear to easily number > 5,000 plantlets and represent some of the largest Kincaid's lupine populations known.

Molecular markers. We used five variable nuclear DNA (nDNA) simple sequence repeats (SSRs) to investigate patterns of biparentally inherited allelic diversity. Two of the five nSSR loci are considered neutral (*AG55-26-16*, *CAC60-13-16*), with no known relationship to a coding region (Drummond and Hamilton 2005). The locus *AG-81* is immediately downstream of a seryl-tRNA synthetase gene in *Glycine*, and has been treated as neutral (Peakall et al. 1998). The last two nSSR loci are linked, exonic, trinucleotide repeats from a *CYCLOIDEA* paralog, *LEGCYC1B* (Citerne et al. 2003, Citerne 2005). *LEGCYC1B* contains a glycine (GGT) repeat near the 5' end of the exon and an asparagine repeat (AAC) near the 3' end (Ree et al. 2004). The amino acid repeat codons are separated by approximately 800 basepairs (Ree et al. 2004).

Genomic DNA from 50-100 mg of frozen leaf tissue per individual was extracted with the FastDNA® kit and FastPrep® instrument according to manufacturer recommended protocols (QBiogene, Inc., CA). Nuclear SSR loci were amplified from 0.5-1.2 µL (10 – 30 ng) of genomic DNA, 1.2 µL of 10X Thermopol Buffer (New England Biolabs), 0.6 µL of 100X BSA, 1.0 µL (0.2mM of each dNTP), 0.5 µL of each forward and reverse primer (5 pM), 0.5 to 1.0 units of *Taq* polymerase (New England Biolabs) and 7.0 µL of ddH_20 for an approximate 11µL reaction volume. Marker loci were amplified with a BIO-RAD MyCycler™ thermal cycler at 94 °C for 4 min., 36 cycles of 94 °C for 1 min., 54 °C for 1 min., 72 °C for 1 min and 30 sec.

A total of four variable chloroplast DNA (cpDNA) SSR loci were used to quantify maternal lineage diversity. Two of the loci *ccmp5*, *ccmp10* (Weising and Gardener 1999) are universal cpDNA SSR loci and two other loci within the *trnT-L* intergenic region were found through sequencing. All four variable cpDNA loci were mononucleotide A or T repeats. Chloroplast SSR loci were amplified from 0.5 µL (10-20 ng) of genomic DNA, 2.0 µL of 10X Thremopol Buffer (New England Biolabs), 1.0 µL of 100X BSA, 1.0 µL (0.25mM of each dNTP), 0.5 µL (5pM) of each forward and reverse primer, and 0.5 units of Taq polymerase (New England Biolabs) and 6.5 µL of dd H_20 for an 11 µL reaction volume. Chloroplast loci were amplified with the following thermocycler protocol: 94 °C for 4 min, 36 cycles of 94 °C for 1 min, 50 °C for 1 min, 72 °C for 1 min, followed by 72°C for 10 minutes. The amplicon of the entire *trnT-L* region was cut with *Cla*I (New England Biolabs) following manufacturer's protocol, which yielded two different sized chloroplast fragments, each with a mononucleotide repeat.

Both nuclear and chloroplast SSR primers were fluorescently labeled and the amplicon lengths were determined on an ABI3100 sequencer with GS500Rox as an internal standard. The ABI3100 output data was visualized and SSR amplicon lengths were scored with the program Genographer 1.6 (Benham 2001 http://hordeum.oscs.montana.edu/genographer).

Landscape genetic analysis. Since Kincaid's lupine is capable of extensive rhizomatous spread, leaf samples from different plantlets may arise from the same genet and, if genotyped, could skew genetic diversity estimates because the same individual is represented multiple times. To decrease the chances of selecting leaf tissue from the same genet, we sampled lupine plantlets that were at least 2 m apart from each other, a distance from which the probability of sampling another plantlet from the same genet was < 10% in 5 of 6 Kincaid's lupine study patches (Chapter 2). Plantlets with identical shared multilocus genotypes could belong to the same genet due to rhizome spread or arise independently from different sexual reproduction events. We used the estimator P_{sex} (Arnaud-Haond et al. 2007) as a

filter to separate groups of two or more plantlets with identical multiple locus genotypes into two categories: plantlets with identical genotypes due to rhizome spread and plantlets with identical genotypes due to different sexual reproduction events. *Psex* gives an estimate of the probability of obtaining *n* samples with a shared multilocus genotype from the pool of genotyped samples through sexual reproduction under the assumption of random mating. (We assumed the absence of somatic mutations at SSR loci.) The *Psex* estimator also accounts for the influence of inbreeding on the occurrence of identical multiple locus genotypes through independent sexual reproduction events (Arnaud-Haond et al. 2007). We performed the calculations with the program Genclone 2.0 (Arnaud-Haond and Belkhir 2007) and selected a 5% cutoff probability for lupine plantlets. Two or more lupine plantlets within a patch sharing identical genotypes with $P_{sex} > 0.05$ we considered as having arose from independent sexual reproduction events, and groups of two or more lupine plantlets within a patch sharing identical genotypes with $P_{sex} \leq 0.05$ we identified as belonging to a single vegetatively spreading genet. Only one individual from groups of plantlets with $P_{sex} \leq 0.05$ was included in the genetic analyses because plantlets appeared to belong to a shared genet whereas plantlets with $P_{sex} > 0.05$ were treated as individual genets.

For all study patches and populations throughout the range of Kincaid's lupine, we calculated the fixation index ($F = (H_E\text{-}H_O)/H_E$), where H_E is the expected heterozygosity ($H_E = 1\text{-}\Sigma p_i^2$) and H_O (no. of heterozygotes/ N samples in the population) is the observed heterozygosity. Fixation values range from –1 to 1; *F*-values > 0 indicate homozygote excess, F< 0 indicate heterozygote excess, and a value of 0 indicates that allelic diversity is in Hardy-Weinberg Equilibrium (HWE) expectations. The expected heterozygosity (H_E) was calculated for each locus and averaged over all loci to generate a mean estimate of genetic diversity within each population. The total allele number (Na_{tot}) in each population was directly counted. Some populations appeared to be comprised of genets with mixed ploidy levels (Severns 2009ab). In these populations the individuals amplifying more than three alleles per locus per individual were excluded from the statistical

analysis. We realize that even discarding apparent polyploid individuals from the analysis may not remove all polyploid individuals and this may affect calculations. However, we still present the summary population statistics to represent the overall patterns in genetic diversity across the range of the species.

We also used nSSRs to test for evidence of recent population bottlenecks with the program BOTTLENECK (Piry et al. 1999). In populations that have experienced a recent bottleneck, the expected heterozygosity (H_e) is greater than the heterozygosity expected within the population while in mutation-drift equilibrium (H_{eq}) because rare allele number diminishes more rapidly than population heterozygosity (Cornuet and Luikart 1996). We performed a one-tailed sign-rank test at each locus in all populations and the Eaton study patches for $H_e > H_{eq}$ under the infinite alleles model (IAM) and the stepwise mutation model (SMM). The SMM assumes that all length mutations occur sequentially and cannot skip a length step, which is a false assumption for simple sequence repeats (Li et al. 2002). Tests for recent genetic bottlenecks under SMM assumptions are more likely to give a false positive test result if there is any deviation from the stepwise mutation model in our SSR marker system. If tests revealed that $H_e > H_{eq}$ in either the IAM or SMM ($\alpha < 0.05$), then we considered there to be potential evidence suggesting a recent population bottleneck.

Chloroplast SSR repeat number was determined for each of the four loci and combined into one haplotype with complete linkage because the chloroplast is not known to recombine. We summed the number of haplotypes in each population as an estimate for the number of maternal lineages and calculated genetic diversity, $H_E = 1-\Sigma p_i^2$, from the haplotype proportions in each population. We investigated the relationship between population size class ranking and cpDNA haplotype richness with a Kruskal-Wallis ANOVA and the relationship between population size and the Fixation index (F) with a one-way ANOVA.

Office population. We used F-statistics to assess whether the Office population was a distinct genetic segregate of the maternal source population (North Eaton)

by comparing the seed donor patches in North Eaton to the Office population. We estimated F_{ST} from AMOVA (Peakall et al. 1995) under the infinite alleles model and each between population pairwise value was assessed for statistical significance with 1,000 permutations in GenalEx 6.1 (Peakall and Smouse 2006). To address the possibility of a single mother producing the vigorous progeny in the common garden experiment we calculated cpDNA haplotype frequencies in the Office population and office progeny cohort surviving in the common garden experiment. We also calculated F_{ST} values between the Office population and office progeny cohort to investigate whether the cohort was a subset of the founding Office population individuals.

Results

Nuclear and chloroplast SSR marker diversity. We amplified no fewer than six alleles from any nSSR or cpSSR locus among the populations sampled (Table 4.1). For the 26 populations (excluding North Eaton and South Eaton), the mean observed population heterozygosity (H_E) was relatively high, 0.403 (±0.025 SE), the fixation index (F) was near HWE expections, 0.007 (±0.021 SE), and the mean number of migrants per generation (Nm) was estimated at 1.013 (±0.21 SE). The number of patches within a study population was not statistically correlated with the fixation index (r = 0.29, p = 0.12), observed heterozygosity (r = 0.02, p = 0.90), or the number of nSSR alleles (r = 0.24, p = 0.20). However, there was a positive correlation between the number of lupine patches and the number of cpDNA haplotypes (r = 0.40, p = 0.03). Although, H_O is involved in the calculation of the fixation index (F), we found that there was no correlation between the two population values (Fig. 4.1). Most (85% ±3% SE) of the genotyped plantlets within each population had non-matching multilocus genotypes or had identical shared genotypes that likely arose through different sexual reproduction events (P_{sex}>0.05). This large number of genets per genotyped plantlet indicates that we did not frequently resample the same genet (Table 4.2).

Statistical tests in 22 of 24 Kincaid's lupine populations, and all lupine patches within North Eaton and South Eaton, did not yield strong evidence for recent genetic bottlenecks, although some populations and study patches had *P*-values < 0.10 in the stepwise mutation model (Table 4.3). Three of the 22 populations tested had test results suggesting a recent population bottleneck under the stepwise mutation model. Only one population, China Ditch, had statistical support for a recent genetic bottleneck in both the infinite alleles and stepwise mutation models (Table 4.3). A little of half of the study populations had fixation coefficients > 0.1 and < -0.1, but the remaining populations and study patches had nSSR allelic variation near HWE expectations (Table 4.2). Mean nSSR genetic diversity was relatively high in study populations and patches, 0.42 ± 0.03 95%CI (Table 4.2) as was mean chloroplast haplotype number, 4.2 ± 1.0 95%CI and mean haplotype diversity, 0.52 ± 0.09 95%CI (Table 4.4).

We found no evidence that relative population size was related to the fixation coefficient values. All population size categories (*n* very small = 5, *n* small =6, *n* medium = 10, *n* large = 5) had mean *F*-values close to zero (ANOVA: F = 0.5, p = 0.69, means; very small = -0.022, small = -0.015, medium = 0.016, large = 0.056). Both the very small and small sized populations had about half the number of cpDNA haplotypes of the medium and large populations, which averaged about 5.2 haplotypes/ population (Fig. 4.2).

Augmented versus non-augmented Kincaid's lupine patches. Both augmented patches of Kincaid's lupine in North Eaton did not appear to have experienced a recent bottleneck (Table 4.3) and the fixation index values were also close to zero (Table 4.2), suggesting that marker loci were close to HWE expectations in the two augmented patches. There were at least eight chloroplast DNA haplotypes in the two augmented North Eaton patches and haplotype diversity was relatively evenly distributed in both patches (Table 4.3). The unmanipulated lupine patches in North Eaton (forest patch) and both study patches in the adjacent parcel of South Eaton, shared similar properties to the augmented patches in North Eaton. In these

unmanipulated patches, tests for a recent bottleneck were negative (Table 2) and the fixation coefficient was near HWE expectations, with the exception of the South Eaton bike path patch which had an excess of heterozygous loci (Table 4.2). The number of maternal lineages in the non-augmented patches were lower than in the augmented patches but there were still three or more cpDNA haplotypes in each unmanipulated study patch (Table 4.3). Similar to the augmented patches, cpDNA haplotype diversity was relatively evenly distributed in the unmanipulated patches (Table 4.3).

North Eaton seed donor patches, Office population, and Office population progeny. The Office plants appeared to be a different genetic subset than either of the two seed donor patches, North Eaton oaks and North Eaton middle. F_{ST}-values between the Office population and the North Eaton seed donor patches statistically differed from each other and the between patch F_{ST} value was > 0.15 (Table 4.5). Additionally, all three chloroplast haplotypes from the seven Office population plants were not found in the North Eaton seed donor patches. The Office progeny in the common garden did not appear to be a reduced genetic subset of the Office population because the F_{ST} value between the Office and Office progeny was zero (Table 4.5), and all three haplotypes from the Office population were represented in the Office progeny in the approximately same frequencies (Fig. 4.2).

Discussion

Our primary goal in this study was to use genetic markers in combination with a past common garden experiment and a prior population augmentation project to determine whether genetic rescue in Kincaid's lupine is a necessary genetic strategy. Molecular markers can be misleading if positive selection masks variation (Schlötterer 2004) and positive selection is plausible in exonic SSRs because the repeats may be linked to fitness or survival related traits (Verstrepen et al. 2005, Kashi and King 2006). In our study, the two amino acid repeats in

LEGCYCLOIDEA1B may be subject to positive selection but allelic diversity was relatively high at each locus despite linkage, suggesting that the locus is suitable for the types of questions we address in this study (Table 4.1). A comparison between *F*-statistics calculated from a group of eight populations where linkage between the two *LEGCYC1B* loci was accounted for against the same data set where linkage was ignored, revealed only small differences in the *F*-statistics (± 1.2 units), which did not change the biological interpretation of the data (Severns unpublished data). Linkage between all nSSRs was not statistically significant in most populations with the exception of the *AG-55* locus. AG-55 appears to have a relatively slow mutation rate and has different sets of alleles that are associated with other broadly sympatric *Lupinus* species (unpublished data).

To justify seed transfer for genetic rescue there should be evidence from the molecular markers, such as high population homozygosity, elevated fixation index values (*F*), and evidence suggesting founder effects or population bottlenecks. Averaged over all populations, the fixation index was near zero, suggesting that, in general, most Kincaid's lupine populations did not deviate from HWE expectations as they would if they were severely inbred. Moreover, there was no statistical relationship between population size class and the fixation coefficient which average close to HWE expectations (Fig. 4.2), suggesting that even the smallest of populations are unlikely to be severely inbred. Although the number of nSSR loci analyzed was low, tests for evidence of recent genetic bottlenecks did not suggest that they have yet occurred (Table 4.2), despite recent habitat fragmentation. Only China Ditch had positive tests for a recent genetic bottleneck in both the infinite alleles and stepwise mutation models. The other two populations that had a positive test result for a recent bottleneck, Rocking Easy and West Shore, were only supported by the stepwise mutation model, which likely overestimates the probability of a recent population bottleneck due to the violated assumptions of mutation in the stepwise mutation model. Rocking Easy and West Shore had fixation index values near zero (Table 4.2) and had four or more cpDNA haplotypes (maternal lineages) that were relatively well distributed within each

population (Table 4.3), suggesting that the status of these populations with respect to bottlenecking should be interpreted cautiously. The relatively large number and fairly even distribution of cpDNA haplotypes within genotyped Kincaid's lupine populations (Table 4.3) does not suggest either founder effects or bottlenecks. Surprisingly, the large mean number of maternal lineages in Kincaid's lupine populations and study patches (4.3 haplotypes per population or patch) compares more favorably to conifer cpDNA richness, which are wind pollinated and have paternally inherited chloroplasts (Wagner et al. 1987), than to perennial, animal pollinated, herbaceous plants (supplemental data from Petit et al. 2005). Haploid genetic diversity should be lost more quickly than diploid genetic diversity when populations experience generations of inbreeding (Charlesworth 2003), but Kincaid's lupine has relatively high and evenly distributed cpDNA haplotype diversity. Chloroplast DNA diversity and haplotype distribution indicates that severe inbreeding has not yet caused a loss in the number and diversity of maternal lineages. Although there are examples of plant populations that have molecular marker profiles implying that genetic rescue or seed mixing might be beneficial (Willi et al. 2005, Van Rossum 2008), our data suggest that Kincaid's lupine populations do not appear to be in need of genetic rescue.

Patterns in both nDNA and cpDNA SSRs suggest that most Kincaid's lupine populations should not be inbred nor manifest symptoms of inbreeding depression. One of the populations genotyped in this study, North Green Oaks, was demonstrated to be inbred by a controlled crossing experiment (Severns 2003a). Yet, unexpectedly, the fixation index for North Green Oaks suggested an excess of heterozygotes (F = -0.10), there was no evidence for a bottleneck (Table 4.3), and there were at least two cpDNA haplotypes detected (Table 4.4). Moreover, this population also appears to have the greatest number of natural germinants in any population measured so far (Severns 2008) and seeds planted in a common garden at the home site had a 17.6% establishment rate after three years (Severns 2003b). While natural seed set maybe low in North Green Oaks (Severns 2003a, 2008) and between population crosses more than doubled seed set (Severns

2003a), other population characteristics that should indicate inbreeding depression, such as natural recruitment, survival, and molecular marker allelic diversity, don't support the conclusion from the crossing study that the population is inbred.

A plausible explanation for contradictory conclusions from different Kincaid's lupine studies may be explained by the stage specific filtering effects of elevated inbreeding coefficients. The inbreeding coefficient (δ) for seed filling in Kincaid's lupine ranges from 0.53-0.64 (Severns 2003a, Severns and Lewis 2007) and indicates that there is a substantial penalty in lost seed production that accompanies inbreeding, which is common for outcrossing plant species (Husband and Schemske 1996). A byproduct of lowered seed set from inbreeding is to shift the seed cohort towards one that is more outcrossed. This inbreeding based filter may play an important role in shaping annual seed cohort genetic composition because one of the most common and active Kincaid's lupine pollinators, *Bombus* spp., spent about 80% of their foraging time performing geitonogamous flights (Severns and Lewis 2007), but interplantlet movements mae by foraging bees also had > 90% probability of occurring between at least two different genets (Chapter 2). Considering that protandry in lupines and controlled release of pollen appears to increase outcrossing rates (Haynes and Mesler 1984, Juncosa and Webster 1989, Harder 1990), a high penalty against selfing combined with mechanisms to promote pollinator outcrossing may filter genetic load harboring genotypes at the seed stage in Kincaid's lupine.

Although prior controlled crossing experiments with Kincaid's lupine have met with unfortunate, seemingly demonic intrusion events (*sensu* Hurlbert 1984) that prevented further investigation into germination and seedling survival, anecdotal evidence suggests that there may also be a reduction in germination associated with inbreeding (Kaye and Kuykendall 2001). If Kaye and Kuykendall's (2001) maximum germination rate of 95% from a large population represents largely outcrossed seed and the germination rate of 55% represents a small, highly inbred population, then the inbreeding coefficient (δ) for germination is approximately 0.40. Loss of germinability is another potentially significant

filter for eliminating progeny produced from inbreeding that would shift surviving cohorts towards outcrossed progeny. Often, outcrossing plant species do have higher inbreeding coefficients for the early plant lifestages, and the proposed penalty for reductions in germination is not unreasonable when compared with other outcrossing species (Husband and Schemske 1996, Washitani et al. 2005). However, two species of perennial lupines, *Lupinus arboreus* (Kittleson and Maron 2000) and *L. perennis* (Michaels et al. 2008) had very small inbreeding depression coefficients for lifestages after seed filling, indicating that the high inbreeding coefficients before and during seed filling may be great enough to yield largely outcrossed progeny. Elevated inbreeding penalties for Kincaid's lupine during seed filling and germination would likely result in the maintenance of genetic diversity and preserve allele distributions near HWE expectations (Table 4.2). Since we only gathered leaf tissue for genotyping from established plants, a study directed towards seed cohorts and the germinant population could lend support to this important hypothesis.

Maintenance of genetic diversity in a population is a combination of existing genetic diversity, the effective population size, generation time, and migration (Silvertown and Charlesworth 2001). In general, Kincaid's lupine population genetic diversity appears to be relatively high. In this study, the number and distribution of cpDNA haplotypes was high regardless of population size and there was a relatively large proportion of unmatched nSSR genotypes in screened populations, despite extensive vegetative spread (Chapter 4.2). Even within lupine patches, the number of unique multiple locus genotypes accrued at a rate of one additional genotype for every two plants sampled (Chapter 4.2), suggesting that genetic diversity in Kincaid's lupine populations are unlikely to be genetically impoverished. If we assume that migration between populations is unlikely to occur from this point forward, without human intervention (*e.g.* population augmentation), and that most populations harbor relatively large amounts of genetic variation that approach HWE expectations, then generation

time becomes a focal point for understanding how patterns of genetic diversity will pertain to lupine conservation.

Genet longevity in Kincaid's lupine may be on the order of centuries (Wilson et al. 2003) and there are likely to be many overlapping generations in lupine populations. Assuming that the genetic diversity we uncovered with cpDNA and nSSR markers are an approximate index of the genetic diversity within Kincaid's lupine populations and that progeny from outcrossing events dominate seedling cohorts, then it seems likely that the number of generations necessary to result in severely inbred populations is likely to outstrip the life span of land managers and property owners. Despite evidence suggesting that populations of most rare species are likely to experience reproductive consequences of genetic load before they become demographically extinct (Spielman et al. 2004), it seems more probable that habitat degradation or catastrophic stochastic events are more likely to result in population extinction in Kincaid's lupine than mutational meltdown. If the life history and reproductive strategy of Kincaid's lupine is viewed in its likely historical context of native grass dominated prairies and more continuous habitat, the long-lived trait with a high inbreeding coefficient for seed production, implies that an individual can afford to wait many years for a reproductive event to replace itself. Traditional rare plant conservation strategies frequently target populations with low reproductive output because these reductions will lower population growth rates which may lead to population extinction (Schemske et al. 1996, Menges 2000). However, evaluation of the information presented in our molecular marker study when combined with Kincaid's lupine life history, suggest that it may be perfectly natural for lupine populations not to mass-produce seeds on an annual basis. The generation time of Kincaid's lupine far surpasses our time frame of reference for population seed production, despite the fact that annual seed set estimates have been enumerated in some populations for nearly a decade (Severns unpublished data).

Is interpopulation seed transfer necessary for Kincaid's lupine? While most of the lupine populations genotyped in this study do not appear to require genetic rescue, it is likely that between population seed mixing will still occur because reintroduction and augmentation are common themes in threatened and endangered plant recovery plans (Severns and Liston 2008). With respect to a Kincaid's lupine seed transfer zone, there are three primary options to consider: 1) to not develop and use seed transfer zones, 2) establish geographically based seed transfer zones, or 3) primarily use within population seeds or the nearest neighboring population as a seed source until more detailed information about interpopulation genetic compatibility is gathered.

From recently gathered genetic data, the first two options may incur a larger risk of combining non-compatible genotypes than the third option. Some Kincaid's lupine populations appear to be comprised of individuals with mixed ploidy number, both diploid and tetraploid (Severns 2009ab, unpublished data). Mixing seed sources that are not matched within ploidy would likely lead to substantial population genetic instability if crosses between different cytotypes produced largely sterile or unfit progeny (Petit et al. 1999, Levin 2002, Severns and Liston 2008). Further, there is also evidence that even crosses between allopolyploids produced from different events produces a pool of genetically unstabe progeny (Gaeta et al. 2007, Lim et al. 2008). In the Kincaid's lupine populations genotyped so far, there appears to be no geographic pattern with respect to polyploid populations and mixed ploidy populations appear to be products of multiple independent hybridization events (manuscript in prep).

Similar to differences in ploidy, hyrbidization with different *Lupinus* taxa across the range of Kincaid's lupine also appears to have occurred with no obvious geographic patterns (manuscript in prep). Liston et al. (1995) presented evidence of hybridization between Kincaid's lupine and *L. arbustus* at one site, and until recently, it was thought that this was the only population with interspecific hybrids. It now appears that a number of populations have independent contemporary and past hybridization events between two different species of

broadly sympatric lupines, *L. albicaulis* and *L. arbustus* (manuscript in prep). Some evidence of acquiring genetic diversity from another species appeared when we compared the fixation index (*F*) values to observed population heterozygosity (*Ho*) and found no relationship between the two measurements (Fig. 4.1). One would expect that a plant species derived from a single or founding population to have a clear relationship between the fixation index and observed heterozygosity. But, this does not appear to be the case in Kincaid's lupine and suggests that some of the variation among populations in due to different evolutionary histories. While hybridization may impart novel genes and allelic diversity to populations, hybridization also creates maladapted gene complexes that result in pools of progeny with low fitness and vigor (Arnold and Hodges 1995, Ellstrand and Schierenbeck 2000, Rieseberg 2001, Welch and Rieseberg 2002), which is contrary to conservation goals. Because Kincaid's lupine appears to be very long lived, any surviving lower fitness genotypes produced through hybridization and introgression may persist within populations exceeding practical conservation timeframes, potentially taking several generations or hundreds to thousands of years to be lost. Thus, non-lethal, Dobzansky-Muller type gene-gene incompatibilities facilitated by seed mixing between evolutionarily divergent populations could create headaches for future land managers and jeopardize inappropriately "augmented" natural populations.

The third option for interpopulation seed transfer, to use seeds only from within population, is a restricted take on local seed transfer guidelines (McKay et al. 2005, Broadhurst et al. 2008, Ward et al. 2008). The common garden experiment with the Office progeny derived from a subset of North Eaton seed suggests that movement of seed within a population but between different patches may be an effective short-term (within a few generations) genetic strategy for Kincaid's lupine conservation. Even though the surviving population of the original seed cohort planted at the Office site was small (n = 7), the surviving Office progeny from one year's seed cohort represented all three founding Office maternal lineages and in the same relative proportions as the parental population

(Fig. 4.2). There also appeared to be little genetic differentiation between the Office population and the Office progeny (Table 4.4, Fig. 4.2), suggesting that the mating system of Kincaid's lupine will tend to aid in the maintenance of within population genetic diversity. Since the Office progeny vigor, health, and survival was greater than any other seed cohort tested, including the seed source population (Severns 2003b), moving seeds within existing plant populations among different patches may yield similar results.

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Literature Cited

Ågren, J. 1996. Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. Ecology 77:1779–1790.

Aguilar, R., Quesada, M., Ashworth, L., Herrerias-Diego, Y. and J. Lobo. 2008. Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. Molecular Ecology 17:5177-5188.

Alverson, E. 2005. Preserving prairies and savannas in a sea of forest: a conservation challenge in the Pacific Northwest. Plant Talk 40:23-27.

Arnaud-Haond, S. and K. Belkhir. 2007. GENCLONE 1.0: a new program to analyse genetics data on clonal organisms. Molecular Ecology Notes 7:15-17.

Arnaud-Haond, S., Duarte, C. M., Alberto, F., and E. A. Serrão. 2007. Standardizing methods to address clonality in population studies. Molecular Ecology 16:5115-5139.

Arnold, M. C. and S. A. Hodges. 1995. Are natural hybrids fit or unfit relative to their parents? Trends in Ecology and Evolution 10:67-71.

Barrett, S. C. H. and D. Charlesworth. 1991. Effects of change in the level of inbreeding on the genetic load. Nature 352:522-524.

Broadhurst, L.M., Lowe, A., Coates, D.J., Cunningham, S.A., McDonald, M., Vesk, P.A., and C. Yates. 2008. Seed supply for broadscale restoration: maximizing evolutionary potential. Evolutionary Applications 1:587-597.

Brown, J. H. and A. Kodric-Brown. 1977. Turnover rates in insular biogeography: effect of immigration and extinction. Ecology 58:445–449.

Buza, L., Young, A., and P. Thrall. 2000. Genetic erosion, inbreeding and reduced fitness in fragmented populations of the tetraploid pea *Swainsona recta*. Biological Conservation 93:177-186.

Charlesworth, B. and D. Charlesworth. 1999. The genetic basis of inbreeding depression. Genetical Research 74:329-340.

Charlesworth, D. 2003. Effects of inbreeding on the genetic diversity of populations. Philosophical Transactions: Biological Sciences 358:1051-1070.

Citerne, H. L., Luo, D., Pennington, R. T., Coen, E., and Q. C. B. Cronk. 2003. A phylogenomic investigation of *CYCLOIDEA* like TCP genes in the Leguminosae. Plant Physiology 131:1042–1053.

Citerne, H. L. 2005. A primer set for specific amplification of two *CYCLOIDEA*-like genes in the genistoid clade of *Leguminosae* subfam. *Papilionideae*. Edingburgh Journal of Botany 62:119-126.

Cornuet, J. M. and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001-2014.

Drummond, C. S. and M. B. Hamilton. 2005. Isolation and characterization of nuclear microsatellite loci in *Lupinus* group *Microcarpi* (Leguminosae). Molecular Ecology Notes 5:510-513.

Ellstrand, N. C. and D. R. Elam. 1993. Population genetic consequences of small population size: implications for plant conservation. Annual Review of Ecology and Systematics 24:217-242.

Ellstrand, N. C. and K. A. Schierenbeck. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? Proceedings of the National Academy of Sciences 97:7043-7050.

Field, D. L., Ayre, D. J., Whelan, R. J., and A. G. Young. 2008. Relative frequency of sympatric species influences rates of interspecific hybridization, seed production and seedling performance in the uncommon *Eucalyptus aggregata*. Journal of Ecology 96:1198-1210.

Fischer, M. and D. Matthies. 1997. Mating structure and inbreeding and outbreeding depression in the rare plant *Gentianella germanica* (Gentianaceae). American Journal of Botany 84:1685-1692.

Gaeta, R. T., Pires, J. C., Iniquez-Luy, F., Leon, E., and T. C. Osborn. 2007. Genomic changes in resynthesized *Brassica napus* and their effect on gene expression and phenotype. Plant Cell 19:3403-3417.

Harder, L. D. 1990. Behavioral responses by bumble bees to variation in pollen availability. Oecologia 85:41-47.

Haynes, J. and M. Mesler. 1984. Pollen foraging by bumblebees: foraging patterns and efficiency on *Lupinus polyphyllus*. Oecologia 61:249-253.

Hedrick, P. W. 1994. Purging inbreeding depression and the probability of extinction: full sib mating. Heredity 73:363-372.

Holmes, G. D., James, E. A., and A. A. Hoffmann. 2008. Limitations to reproductive output and genetic rescue in populations of the rare shrub *Grevillea repens* (Proteaceae). Annals of Botany 102:1031-1041.

Honnay, O. and H. Jacquemyn. 2007. Susceptibility of common and rare plant species to the genetic consequences of habitat fragmentation. Conservation Biology 21:823-831.

Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. Ecological Monographs 54:187–211.

Husband, B. C. and D. W. Schemske. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. Evolution 50:54-70.

Jaquiéry, J., Guillaume, F. and N. Perrin. 2009. Predicting the deleterious effects of mutation load in fragmented populations. Conservation Biology 23:207-218

Jennerston, O. 1988. Pollination in Dianthus deltoides (Caryophyllaceae): effects of habitat fragmentation on visitation and seed set. Conservation Biology 2:359–366.

Juncosa, A. M. and B. D. Webster. 1989. Pollination in *Lupinus nanus* subsp. *latifolius* (Leguminosea). American Journal of Botany 76:59-66.

Karoly, K. 1994. Inbreeding effects on mating system traits for two species of *Lupinus* (Leguminosae). American Journal of Botany 81:1538-1544.

Kashi, Y. and D. G. King. 2006. Simple sequence repeats as advantageous mutators in evolution. Trends in Genetics 22:253-259.

Kaye, T. N. 1999. Obligate insect pollination of a rare plant: Lupinus sulphureus ssp. kincaidii. Northwest Science 73:50-52.

Kaye, T. N. and K. Kuykendall. 2001. Effects of scarification, cold stratification, and source population on germination of *Lupinus sulphureus* ssp. *kincaidii*. Seed Science and Technology 29:663-668.

Kearns, C. A., Inouye, D. W., and N. M. Waser. 1998. Endangered mutualisms: the conservation of plant-pollinator interactions. Annual Review of Ecology and Systematics 29:83-112.

Keller, M., Kollmann, J., and P. J. Edwards. 2000. Genetic introgression from distant provenances reduces fitness in local weed populations. Journal of Applied Ecology 37:647-659.

Keller, L. F. and D. M. Waller. 2002. Inbreeding effects in wild populations. Trends in Ecology and Evolution 17:230-241.

Kéry, M., Matthies, D., and H. H. Spillmann. 2000. Reduced fecundity and offspring performance in small populations of the declining grassland plants *Primula veris* and *Gentiana lutea*. Journal of Ecology 88:17–30.

Kittleson, P. M. and J. L. Maron. 2000. Outcrossing rates and inbreeding depression in the perennial yellow bush lupine, *Lupinus arboreus* (Fabaceae). American Journal of Botany 87:652-660.

Leimu, R., Matikainen, P., Koricheva, J. and M. Fischer. 2006. How general are positive relationships between plant population size, fitness and genetic variation? Journal of Ecology 94:942-952.

Levin, D. A. 2002. The role of chromosomal change in plant evolution. Oxford University Press, New York.

Li, Y., Korol, A. B., Fahima, T., Beiles, A., and E. Nevo. 2002. Microsatellites: a genomic distribution, putative function and mutational mechanisms: a review. Molecular Ecology 11:2453-2465.

Lim, K. Y., Soltis, D. E., Soltis, P. S., Tate, J., Matyasek, R., Srubarova, H., Kovarik, A., Pires, J. C., Xiong, Z., and A. R. Leitch. 2008. Rapid chromosome evolution in recently formed polyploids in *Tragopogon* (Asteraceae). PloS ONE 3:e3353.

Liston, A., St. Hilaire, K., and M. V. Wilson. 1995. Genetic diversity in populations of Kincaid's lupine, host plant of fender's blue butterfly. Madroño 42:309-322.

Luikart, G., Allendorf, F. W., Cornuet, J. M., and W. B. Sherwin. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. Journal of Heredity 89:238-247.

Lynch, M., Conery, J. and R. Bürger. 1995. Mutation accumulation and the extinction of small populations. American Naturalist 146:489-518.

McKay, J. K., Christian, C. E., Harrison, S., and K. J. Rice. 2005. "How local is local?"—a review of practical and conceptual issues in the genetics of restoration. Restoration Ecology 13**:**432–440.

Menges, E. S. 2000. Population viability analyses in plants: challenges and opportunities. Trends in Ecology and Evolution 15:51-56.

Michaels, H. J., Shi, X. J., and R. J. Mitchell. 2008. Effects of population size on performance and inbreeding depression in *Lupinus perennis*. Oecologia 154:651-661.

Montalvo, A. M. and N. C. Ellstrand. 2001. Nonlocal transplantation and outbreeding depression in the subshrub *Lotus scoparius* (Fabaeceae). American Journal of Botany 88:258-269.

Newman, D. and D. A. Tallmon. 2001. Experimental evidence for the beneficial fitness effects of gene flow in recently isolated populations. Conservation Biology 15:1054–1063.

Peakall, R., Smouse, P. E., and D. R. Huff. 1995. Evolutionary implications of allozyme and RAPD variation in diploid populations of dioecious buffalo grass *Buchloe dactyloides*. Molecular Ecology 4:135-147.

Peakall, R., Gilmore, S., Keys, W., Morgante, M. and A. Rafalski. 1998. Cross-species amplification of soybean (*Glycine max*) simple sequence repeats (SSRs) within the genus and other legume genera: implications for the transferability of SSRs in plants. Molecular Biology and Evolution 15:1275-1287.

Peakall, R. and P. E. Smouse. 2006. GENALEX: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288-295.

Petit, C., Bretagnolle, F., and F. Felber. 1999. Evolutionary consequences of diploid-polyploid hybrid zones in wild species. Trends in Ecology and Evolution 14:306-311.

Petit, R. J., Duminil, J., Fineschi, S., Hampe, A., Salvini, D., and G. G. Vendramin. 2005. Comparative organization of chloroplast, mitochondrial and nuclear diversity in plant populations. Molecular Ecology 14:689-701.

Pickup, M. and A. G. Young. 2008. Population size, self-incompatibility and genetic rescue in diploid and tetraploid races of *Rutidosis leptorrhynchoides* (Asteraceae). Heredity 100:268-274.

Piry, S., Luikart, G., and J. M. Cornuet. 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. Genetics 90:502-503

Rasner, C. A., Yeh, P., Essert, L. S., Hunt, K. E., Woodruff, D. S., and T. D. Price. 2004. Genetic and morphological evolution following a founder event in the dar-eyed junco, *Junco hyemalis thurberi*. Molecular Ecology 13:671-681.

Ree, R. H., Citerne, H. L., Lavin, M. and Q. B. C. Cronk. 2004. Heterogenous selection on *LEGCYC* paralogs in relation to flower morphology and phylogeny of *Lupinus* (Leguminosae). Molecular Biology and Evolution 21:321-331.

Richards, C. M. 2000. Inbreeding depression and genetic rescue in a plant metapopulation. The American Naturalist 155:383–394.

Rieseberg, L. H. 2001. Chromosomal rearrangements and speciation. Trends in Ecology and Evolution 16:351-358.

Schemske, D. W., Husband, B. C., Ruckelshaus, M. H., Goodwillie, C., Parker, I. M., and J. G. Bishop. 1996. Evaluating approaches to the conservation of rare and endangered plants. Ecology 75:584-606.

Schlötterer. C. 2004. The evolution of nuclear markers – just a matter of fashion? Nature Reviews Genetics 5:63-69.

Severns, P. 2003a. Inbreeding and small population size reduce seed set in a threatened and fragmented plant species, *Lupinus sulphureus* ssp. *kincaidii* (Fabaceae). Biological Conservation 110:221-229.

Severns, P. M. 2003b. Propagation of a long-lived and threatened prairie plant, *Lupinus sulphureus* ssp. *kincaidii*. Restoration Ecology 11:334-342.

Severns, P. M. and B. Lewis. 2007. Is it appropriate to rely on seed set to assess candidate plant populations for genetic rescue? A case study with a threatened species. Natural Areas Journal 27:313-319.

Severns, P. M. 2008. Seedling population size and microhabitat association in *Lupinus oreganus* A. Heller var. *kincaidii* C.P. Smith (Fabaceae), a threatened plant of western Oregon grasslands. Native Plants Journal 9:358-365.

Severns, P. M. and A. Liston. 2008. Intraspecific chromosome number variation: a neglected threat to the conservation of rare plants. Conservation Biology 22:1641-1647

Severns, P. M. 2009a. Patterns of genetic diversity in Douglas County populations of Kincaid's lupine (*Lupinus oreganus* var *kincaidii* = *Lupinus sulphureus* ssp. *kincaidii*). Report to USDI Bureau of Land Management, Roseburg District, Oregon. 19pp.

Severns, P. M. 2009b. Patterns of genetic diversity in Washington State populations of Kincaid's lupine (*Lupinus oreganus* var *kincaidii* = *Lupinus sulphureus* ssp. *kincaidii*). Report submitted to USFWS, Lacey District, Washington. 15pp.

Sheridan, P. M. and D. N. Karrowe. 2000. Inbreeding, outbreeding, and heterosis in the yellow pitcher plant, *Sarracenia flava* (Sarraceniaceae) in Virginia. American Journal of Botany 87:1628-1633.

Silvertown, J. and D. Charlesworth. 2001. Introduction to plant population biology. 4th Ed. Blackwell Science, Oxford, UK. 347pp.

Spielman, D., Brook, B. W., and R. Frankham. 2004. Most species are not driven to extinction before genetic factors impact them. Proceedings of the National Academy of Sciences 101:15261-15264.

Tallmon, D. A., Luikart, G., and R. S. Waples. 2004. The alluring simplicity and complex reality of genetic rescue. Trends in Ecology and Evolution 19:489–496.

Van Rossum, F. 2008. Conservation of long-lived perennial forest herbs in an urban context: *Primula elatior* as study case. Conservation Genetics 9:119-128.

Verstrepen, K. J., Jansen, A., Lewitter, F. and G. R. Fink. 2005. Intragenic tandem repeats generate functional variability. Nature Genetics 37:986-990.

Wagner, D. B., Furnier, G. R., Saghai-Maroof, M. A., Williams, S. M., Dancik, B. P., and R. W. Allard. 1987. Chloroplast DNA polymorphisms in lodgepole and jack pine and their hybrids. Proceedings of the National Academy of Sciences 84:2097-2100.

Ward, K., Gisler, M., Fiegener, R., and A. Young. 2008. The Willamette Valley seed increase program: developing genetically diverse germplasm using an ecoregion approach. Native Plants Journal 9:334-339.

Waser, N. M. and M. V. Price. 1979. Pollen dispersal and optimal outcrossing in *Delphinium nelsoni*. Nature 277:294-297.

Waser, N. M. and M. V. Price. 1989. Optimal outcrossing in *Ipomopsis aggregata*: seed set and offspring fitness. Evolution 43:1097-1109.

Washitani, I, Ishihama, F., Matsumura, C., Nagai, M., Nishihito, J., and M. A. Nishihito. 2005. Conservation ecology of *Primula sieboldii*: synthesis of information toward the prediction of the genetic/ demographic fate of a population. Plant Species Biology 20:3-15.

Weising, K. and R. C. Gardener. 1999. A set of conserved PCR primers for the analysis of simple sequence repeat polymorphisms in chloroplast genes of dicotyledonous angiosperms. Genome 42:9-19.

Welch, M. E. and L. H. Rieseberg. 2002. Habitat divergence between a homoploid hybrid sunflower species *Helianthus paradoxus* (Asteraceae), and its progenitors. American Journal of Botany 89:472-478.

Willi, Y., Van Buskirk, J., and M. Fischer. 2005. A threefold genetic allee effect: population size affects cross-compatibility, inbreeding depression and drift load in the self-incompatible *Ranunculus reptans*. Genetics 169:2255-2266.

Wilson, M. V., Erhart, T., Hammond, P. C., Kaye, T. N., Kuykendall, K., Liston, A., Robinson, A. F., Schultz, C. B., and P. M. Severns. 2003. Biology of Kincaid's lupine (*Lupinus sulphureus* ssp. *kincaidii* [Smith] Phillips), a threatened species of western Oregon native prairies. Natural Areas Journal 23:72-83.

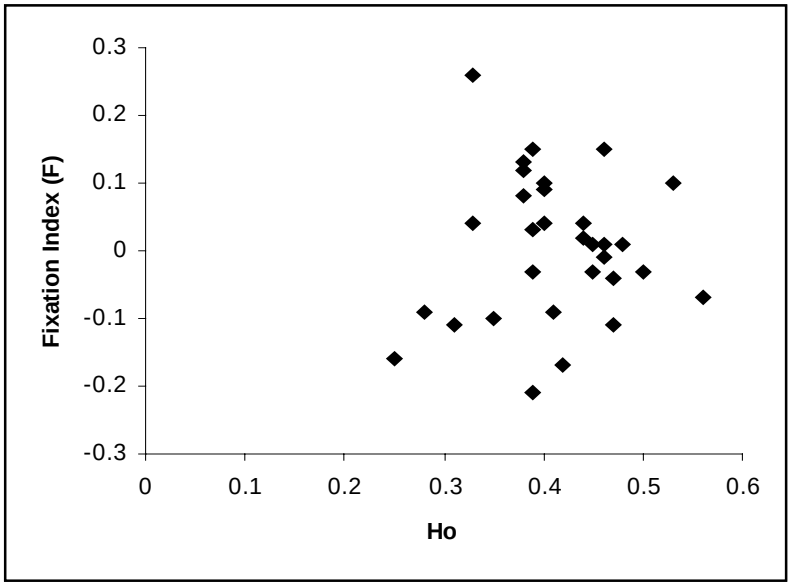

Figure 4.1. Scatterplot of observed population heterozygosity (*Ho*) versus the population fixation index *F*: Pearson correlation results: r = 0.30, p = 0.10.

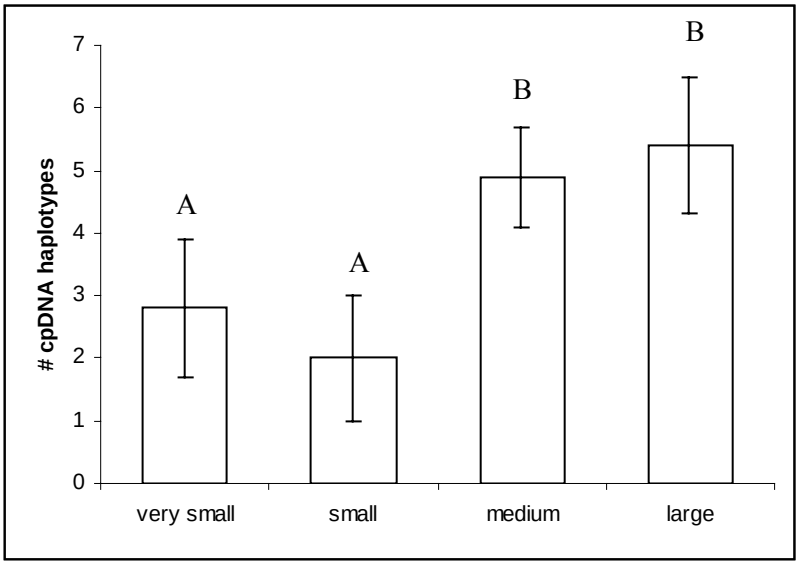

Figure 4.2. Mean (±SEM) for cpDNA haplotype number in four population size class categories. Kruskal-Wallis ANOVA results: $\chi^2 = 11.5$, p = 0.009.

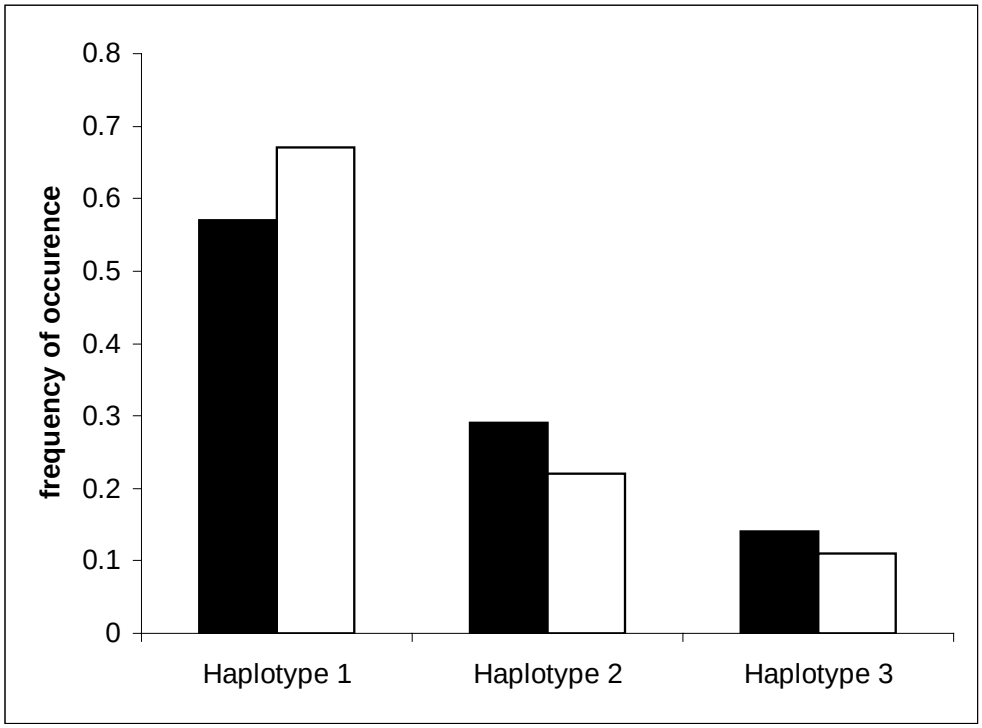

Figure 4.3. Frequency of cpDNA haplotypes in the Office population (n = 7 individuals, black bars) and the surviving Office progeny from the common garden experiment (n = 18 individuals, white bars).

Table 4.1. Number of alleles amplified at each locus for Kincaid's lupine populations and patches

nSSR loci					cpSSR loci			
LEGCYC 1B1	LEGCYC 1B2	CAC60	AG81	AG55	TrnT-L1	TrnT-L2	ccmp5	ccmp10
7	11	7	35	8	7	17	7	6

Table 4.2. Summary of nSSR allelic patterns for Kincaid's lupine populations and study patches within North and South Eaton (italics).

Population	Samples genotyped	Number of genets	Number of distinct MLGs	Na_{tot}	H_E	F
Stillman Creek*	35	35	32	16	0.45	0.15
Vader	15	15	14	11	0.48	-0.10
Boistfort	16	16	12	16	0.32	-0.08
Rocking Easy	47	47	33	23	0.49	0.01
Hecker Road	8	8	6	11	0.22	-0.16
Oak Ridge	7	7	4	17	0.31	-0.11
Butterfly Meadows*	74	71	59	27	0.42	0.12
West Hills Rd	43	43	33	18	0.34	0.04
Oak Basin (main)	25	25	23	23	0.48	0.01
Fir Butte	30	29	28	23	0.46	-0.04
East Shore	11	11	10	18	0.46	0.26
West Shore	25	25	23	23	0.42	-0.03
Spires	25	25	24	23	0.58	0.15
North Green Oaks	34	34	21	16	0.31	-0.10
South Green Oaks	25	25	22	24	0.44	0.13
Royal	59	31	24	17	0.47	0.08
Willow Creek W18th	25	25	23	16	0.44	0.04
Willow Creek Main	38	38	35	18	0.49	0.10
Khul	30	29	29	23	0.42	0.03
Dickerson Heights	20	20	14	12	0.35	-0.09
Lower Loose Laces	19	19	17	17	0.45	-0.01
Upper Loose Laces	34	34	27	17	0.44	-0.03
Dahl	20	20	16	13	0.32	-0.21
Callahan Ridge	25	25	15	14	0.27	-0.11
China Ditch	29	29	23	21	0.41	0.09
Stouts Creek*	36	36	34	23	0.46	0.04
North Eaton forest	25	24	23	24	0.47	-0.03
North Eaton middle	34	30	29	24	0.53	-0.07
North Eaton oaks	50	40	36	21	0.46	0.02
South Eaton bike path	24	18	14	16	0.39	-0.17
South Eaton main	51	46	41	19	0.43	0.01

* denotes a mixed ploidy population, MLG = multiple locus genotype, Na_{tot} = total number of alleles, H_E = expected genetic diversity, F = fixation coefficient

Table 4.3. Population characteristics and results of recent bottleneck statistical tests for Kincaid's lupine populations and North Eaton and South Eaton study patches (italics) with statistically significant tests ($\alpha < 0.05$) in bold.

Population	Approximate location	Relative population size	# lupine patches	*P*-values for (IAM; SMM) models for recent genetic bottlenecks
Stillman Creek*	Washington	Large	6	IAM 0.82; SMM 0.23
Vader	Washington	Small	3	IAM 0.63; SMM 0.81
Boistfort	Washington	Medium	2	IAM 0.13; SMM 0.09
Rocking Easy	North Valley	Medium	5	IAM 0.81; **SMM 0.03**
Hecker Road	North Valley	Very Small	1	Not tested
Oak Ridge (road patch)	North Valley	Very Small	1	Not tested
Butterfly Meadows*	Central Valley	Medium	4	IAM 0.56; SMM 0.11
West Hills Rd	Central Valley	Medium	5	IAM 0.63; SMM 0.06
Oak Basin (main)	South Valley, Cascades foothills	Medium	4	IAM 0.68; SMM 0.14
Fir Butte	South Valley	Large	4	IAM 0.56; SMM 0.09
East Shore	South Valley	Very small	1	IAM 0.44; SMM 0.16
West Shore	South Valley	Medium	2	IAM 0.10; **SMM 0.03**
Spires	South Valley	Medium	1	IAM 0.89; SMM 0.59
North Green Oaks	South Valley	Small	1	IAM 0.16; SMM 0.06
South Green Oaks	South Valley	Small	1	IAM 0.10; SMM 0.06
Royal	South Valley	Medium	1	IAM 0.95; SMM 0.50
Willow Creek W18th	South Valley	Small	2	IAM 0.13; SMM 0.88
Willow Creek Main	South Valley	Large	3	IAM 0.88; SMM 0.88
Khul	South Valley	Large	4	IAM 0.10; SMM 0.06
Dickerson Heights	Douglas Co.	Very small	1	IAM 0.91; SMM 0.44
Lower Loose Laces	Douglas Co.	Very small	2	IAM 0.16; SMM 0.16
Upper Loose Laces	Douglas Co.	Medium	3	IAM 0.89; SMM 0.81
Dahl	Douglas Co.	Small	1	IAM 0.88; SMM 0.19
Callahan Ridge	Douglas Co.	Medium	2	IAM 0.13; SMM 0.13
China Ditch	Douglas Co.	Small	1	**IAM 0.05**; **SMM 0.03**
Stouts Creek*	Douglas Co.	Large	5	IAM 0.56; SMM 0.18
North Eaton forest	South Valley	NA	1	IAM 0.13; SMM 0.08
North Eaton middle	South Valley	NA	1	IAM 0.89; SMM 0.08
North Eaton oaks	South Valley	NA	1	IAM 0.50; SMM 0.08
South Eaton bike path	South Valley	NA	1	IAM 0.31; SMM 0.11
South Eaton main	South Valley	NA	1	IAM 0.50; SMM 0.06

* denotes mixed ploidy populations

Table 4.4. Chloroplast haplotype richness and genetic diversity among Kincaid's lupine populations and North and South Eaton study patches (italics)

Population	N samples	# cpDNA haplotypes	H_E
Stillman Creek*	35	7	0.79
Vader	15	2	0.12
Boistfort	20	2	0.10
Rocking Easy	26	4	0.55
Hecker Road	8	3	0.63
Oak Ridge (road patch)	6	3	0.61
Butterfly Meadows*	48	14	0.88
West Hills Rd	24	6	0.71
Oak Basin (main)	25	5	0.72
Fir Butte	22	5	0.73
East Shore	6	3	0.61
West Shore	20	6	0.80
Spires	21	3	0.42
North Green Oaks	17	2	0.21
South Green Oaks	17	2	0.36
Royal	18	2	0.40
Willow Creek W18th	12	4	0.68
Willow Creek Main	22	4	0.57
Khul	21	7	0.64
Dickerson Heights	10	1	0
Lower Loose Laces	13	4	0.65
Upper Loose Laces	18	4	0.66
Dahl	11	1	0
Callahan Ridge	13	3	0.56
China Ditch	14	1	0
Stouts Creek*	21	4	0.59
North Eaton forest	24	5	0.57
North Eaton middle	30	8	0.67
North Eaton oaks	40	9	0.77
South Eaton bike path	18	3	0.36
South Eaton main	46	6	0.61

*denotes mixed ploidy populations, H_E = expected heterozygosity

Table 4.5. Above diagonal *P*-value of difference of pairwise F_{ST} values between North Eaton seed source patches, the Office population, and Office progeny (below diagnal) from 1,000 permutations. F_{ST} estimated through AMOVA with allelic distance.

	Office	**Office Progeny**	**NE oaks patch**	**NE middle patch**
Office	-	0.430	0.001	0.001
Office Progeny	0.000	-	0.001	0.001
NE oaks patch	0.160	0.137	-	0.001
NE middle patch	0.195	0.170	0.123	-

Chapter 5. Conclusions

The information presented in Chapters 2, 3, and 4 contribute important information that may impact the future conservation genetics strategy of Kincaid's lupine. Information at the individual level, like genet size and patterns of rhizome spread, suggest spatial genetic architecture within populations, but non-adventitious rhizome spread may increase the genetic neighborhood leading to outcrossing when numerous spreading genets overlap (Chapter 2). Genets dominating entire lupine patches or large areas of patches was not common, but did occur at one study site, Royal, where a single genet was approximately 27 meters long and dominated almost one third of the lupine patch area (Chapter 2). Clearly, in situations where a single genet dominates, most pollinator movements are unlikely to result in outcrossing events. However, a single individual within the center of a large genet would likely experience frequent outcrossing events. This suggests that genetic architecture within populations could be diminished and outcrossing encouraged by moving seeds between different areas within the same patch. Far more common than a single large dominant genet, many individuals that spread appeared interspersed, perhaps enabling more frequent outcrossing over the long life span of a Kincaid's lupine genet.

We found surprisingly high levels of genetic diversity within patches of lupines from the same population. Genotype diversity was well short of being accounted for by the genotyping effort and there were relatively large numbers of maternal lineages (inferred through the number of cpDNA haplotypes) within lupine patches (Chapter 2). Patch level characterization of genetic diversity indicates that many of the Kincaid's lupine populations thought to be relatively small, when assessed by area covered, harbor larger than expected amounts of genetic variation. Moreover, there was no evidence for genetic bottlenecking or severe inbreeding within genotyped study patches (Chapter 4), refuting the hypothesis that Kincaid's lupine populations are comprised of one or a few individuals.

Demographic estimates of Kincaid's lupine population size from cover or inflorescence number may be appropriate abundance measurements for Fender's blue butterfly larval resources, but these historically common measurements are not appropriate for plant demographics. Density of lupine plantlets, a cluster of leaves separated by another cluster of leaves by > 10 cm from the petiole base, correlates well ($R^2 > 0.90$) with lupine cover and raceme number within Kincaid's lupine patches (Chapter 3). But vegetative relationships vary greatly between populations and even among different patches within populations (Chapter 3). However, the relationship between lupine plant number and cover or inflorescence number is inconsistent between different lupine patches within the same population as well as between different populations (Chapter 3). The lack of a consistent relationship between vegetative measurements means that Kincaid's lupine monitoring should focus on a unit of modular plant growth if an estimation of population size is the goal. Land managers could use any of the genet to plantlet ratios provided in Chapter 3 to calculate genet number estimates, with the caveat that genet numbers should be interpreted cautiously.

Within population patterns of molecular marker allelic diversity all suggested that the majority of Kincaid's lupine populations genotyped are unlikely to require genetic rescue. Just three of 24 genotyped populations had statistical test results that suggested evidence of recent genetic bottlenecks, despite habitat loss, fragmentation, and degradation throughout the range of Kincaid's lupine. Furthermore, the number of cpDNA haplotypes recorded from study populations and patches was greater than most herbaceous animal pollinated plants with maternal chloroplast inheritance. Kincaid's lupine cpDNA haplotype diversity compares more favorably with cpDNA diversity found in conifer species, wind pollinated with paternal cpDNA inheritance, than herbaceous plants. Chloroplast DNA haplotypes were distributed fairly evenly within populations and even patches of lupine, suggesting that genetic diversity is not being lost very quickly. The high number of chloroplast haplotypes and relatively even distribution of cpDNA diversity within Kincaid's lupine populations also suggest that genetic

bottlenecking is unlikely to yet be a factor in Kincaid's lupine conservation genetics.

Loss of genetic diversity, justification for genetic rescue and interpopulation seed transfer, does not appear to be a concern within most of the Kincaid's lupine populations genotyped in this study. There is likely ample genetic variation within multipatch Kincaid's lupine populations to prevent mutational meltdown, barring sudden reductions in population size due to non-genetic factors. Whether or not land managers should mix different seed sources together for population augmentation or reintroduction depends upon the risks they are willing take with respect to combining incompatible genotypes. Some of these genetic incompatibilities, such as mismatch of ploidy number and combining plant populations with different hybridization histories, are possible outcomes without the appropriate level of genetic screening. There appears to be no immediate threat of mutational meltdown to Kincaid's lupine populations given the within population genetic diversity presented in this thesis.

Bibliography

Abrahamson, W. G. 1984. Species responses to fire on the Florida Lake Wales Ridge. American Journal of Botany 71:35-43.

Ågren, J. 1996. Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria*. Ecology 77:1779–1790.

Aguilar, R., Quesada, M., Ashworth, L., Herrerias-Diego, Y. and J. Lobo. 2008. Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. Molecular Ecology 17:5177-5188.

Alder, L. S. and R. E. Irwin. 2006. Comparison of pollen transfer dynamics by multiple floral visitors: experiments with pollen and fluorescent dye. Annals of Botany 97:141-150.

Alverson, E. 2005. Preserving prairies and savannas in a sea of forest: a conservation challenge in the Pacific Northwest. Plant Talk 40:23-27.

Antos, J. A. and C. B. Halpern. 1997. Root system differences among species: implications for early successional changes in forests of western Oregon. American Midland Naturalist 138:97-108.

Arnaud-Haond, S. and K. Belkhir. 2007. GENCLONE 1.0: a new program to analyse genetics data on clonal organisms. Molecular Ecology Notes 7:15-17.

Arnaud-Haond, S., Duarte, C. M., Alberto, F., and E. A. Serrão. 2007. Standardizing methods to address clonality in population studies. Molecular Ecology 16:5115-5139.

Arnold, M. C. and S. A. Hodges. 1995. Are natural hybrids fit or unfit relative to their parents? Trends in Ecology and Evolution 10:67-71.

Ashley, M. V., Willson, M. F., Pergams, O. R. W., O'Dowd, D. J., Gende, S. M., and J. S. Brown. 2003. Evolutionarily enlightened management. Biological Conservation 111:115-123.

Auld, T. D. and A. J. Denham. 2001. The impact of seed predation by mammals on post-fire seed accumulation in the endangered shrub *Grevillea caleyi* (Proteaceae). Biological Conservation 97:377-385.

Barrett, S. C. H. and D. Charlesworth. 1991. Effects of change in the level of inbreeding on the genetic load. Nature 352:522-524.

Barrett, S. C. H. and L. D. Harder. 1996. Ecology and evolution of plant mating. Trends in Ecology and Evolution 11:73-79.

Bishop, J. G. 2002. Early primary succession on Mount St. Helens: impact of insect herbivores on colonizing lupines. Ecology 83:191-202.

Blossey, B. and T. R. Hunt-Joshi. 2003. Belowground herbivory by insects: influence on plants and aboveground herbivores. Annual Review of Entomology 48:521-547.

Bosch, M. and N. M. Waser. 2001. Experimental manipulation of plant density and its effect on pollinators and reproduction of two confamilial montane herbs. Oecologia 126:76-83.

Broadhurst, L.M., Lowe, A., Coates, D.J., Cunningham, S.A., McDonald, M., Vesk, P.A., and C. Yates. 2008. Seed supply for broadscale restoration: maximizing evolutionary potential. Evolutionary Applications 1:587-597.

Brown, V. K. and A. C. Gange. 1989. Differential effects of above- and belowground insect herbivory during early plant succession. Oikos 54:67-76.

Brown, J. H. and A. Kodric-Brown. 1977. Turnover rates in insular biogeography: effect of immigration and extinction. Ecology 58:445–449.

Buza, L., Young, A., and P. Thrall. 2000. Genetic erosion, inbreeding and reduced fitness in fragmented populations of the tetraploid pea *Swainsona recta*. Biological Conservation 93:177-186.

Chao, A., Chazdon, R. L., Colwell, R. K., and T. Shen. 2005. A new statistical approach for assessing similarity of species composition with incidence and abundance data. Ecology Letters 8:148-159.

Charlesworth, B. and D. Charlesworth. 1999. The genetic basis of inbreeding depression. Genetical Research 74:329-340.

Charlesworth, D. 2003. Effects of inbreeding on the genetic diversity of populations. Philosophical Transactions: Biological Sciences 358:1051-1070.

Citerne, H. L., Luo, D., Pennington, R. T., Coen, E., and Q. C. B. Cronk. 2003. A phylogenomic investigation of *CYCLOIDEA* like TCP genes in the Leguminosae. Plant Physiology 131:1042–1053.

Citerne, H. L. 2005. A primer set for specific amplification of two CYCLOIDEA-like genes in the genistoid clade of *Leguminosae* subfam. *Papilionideae*. Edingburgh Journal of Botany 62:119-126.

Colwell, R. K. 2006. ESTIMATES: Statistical estimation of species richness and shared species from samples, Version 8.0.

Cook, R. E. 1983. Clonal plant populations. American Scientist 71:244-253.

Cox, S. C. and P. B. McEvoy. 1983. Effect of summer moisture stress on the capacity of tansy ragwort (*Senecio jacobaea*) to compensate for defoliation by cinnabar moth *(Tyria jacobaeae*). Journal of Applied Ecology 20:225-234.

Cornuet, J. M. and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001-2014.

Damman, H. and M. L. Cain. 1998. Population growth and viability analysis of the clonal woodland herb, *Asarum canadense*. Journal of Ecology 86:13-26.

Dennis, R. L. H., Shreeve, T. G., and H. Van Dyck. 2003. Towards a functional resource-based concept for habitat: a butterfly biology viewpoint. Oikos 102:417-426.

Dunn, D. B. and J. M. Gillett. 1966. The lupines of Canada and Alaska. Canada Department of Agriculture, Monograph No. 2, Ottawa, Canada.

Drummond, C. S. and M. B. Hamilton. 2005. Isolation and characterization of nuclear microsatellite loci in *Lupinus* group *Microcarpi* (Leguminosae). Molecular Ecology Notes 5:510-513.

Eckert, C. G. 2000. Contributions of autogamy and geitonogamy to selffertilization in a mass-flowering, clonal plant. Ecology 81:532-542.

Edmands, S. 2007. Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. Molecular Ecology 16**:**463-475.

Ehrlén, J. and K. Lehtilä. 2002. How perennial are perennial plants? Oikos 98:308-322.

Ellstrand, N. C. and D. R. Elam. 1993. Population genetic consequences of small population size: implications for plant conservation. Annual Review of Ecology and Systematics 24:217-242.

Ellstrand, N. C. and K. A. Schierenbeck. 2000. Hybridization as a stimulus for the evolution of invasiveness in plants? Proceedings of the National Academy of Sciences 97:7043-7050.

Elzinga, C. L., Salzer, D. W., and J. W. Willoughby. 1998. Measuring and monitoring plant populations. U.S. Department of the Interior, Bureau of Land Management, Technical Reference 1730–1, Denver, Colorado, USA.

Enright, N. J. and B. B. Lamont. 1989. Seed banks, fire season, safe sites and seedling recruitment in five co-occurring *Banksia* species. Journal of Ecology 77:1111-1122.

Feng, X., Zhao, Z., Tian Z., Xu, S., Luo, Y., Cai, Z., Wang, Y., *et al.* 2006. Control of petal shape and zygomorphy in *Lotus japonicus*. Proceedings of the National Academy of Sciences 103:4970-4975.

Field, D. L., Ayre, D. J., Whelan, R. J., and A. G. Young. 2008. Relative frequency of sympatric species influences rates of interspecific hybridization, seed production and seedling performance in the uncommon *Eucalyptus aggregata*. Journal of Ecology 96:1198-1210.

Fischer, M., Husi, R., Prati, D., Peintinger, M., van Kleunen, M., and B. Schmid. 2000. RAPD variation among and within small and large populations of the rare clonal plant *Ranunculus reptans* (Ranunculaceae). American Journal of Botany 87:1128-1137.

Fischer, M. and D. Matthies. 1997. Mating structure and inbreeding and outbreeding depression in the rare plant *Gentianella germanica* (Gentianaceae). American Journal of Botany 84:1685-1692.

Floyd, D. A. and J. E. Anderson. 1987. A comparison of the three methods for estimating plant cover. Journal of Ecology 75:221-228.

Gaeta, R. T., Pires, J. C., Iniquez-Luy, F., Leon, E., and T. C. Osborn. 2007. Genomic changes in resynthesized *Brassica napus* and their effect on gene expression and phenotype. Plant Cell 19:3403-3417.

Galloway, L. F., Cirigliano, T., and K. Gremski. 2002. The contribution of display size and dichogamy to potential geitonogamy in Campanula *americana*. International Journal of Plant Science 163:133-139.

García, M. B., Picó, F. X. and J. Ehrlén. 2008. Life span correlates with population dynamics in perennial herbaceous plants. American Journal of Botany 95:258-262.

Goldberg, D. E. 1987. Neighborhood competition in an old-field plant community. Ecology 68:1211-1223.

Gómez-Aparicio, Zamora, R., and J. M. Gómez. 2005. The regeneration status of the endangered *Acer opalus* subsp. *granatense* throughout its geographical distribution in the Iberian Peninsula. Biological Conservation 121:195-206.

Graham, S. A. and R. Turkington. 2000. Population dynamics response of *Lupinus arcticus* to fertilization, clipping, and neighbour removal in the understory of the boreal forest. Canadian Journal of Botany 78:753-758.

Hairston, N. G. Jr., Ellner, S. P., Geber, M. A., Takehito, Y., and J. A. Fox. 2005. Rapid evolution and the convergence of ecological and evolutionary time. Ecology Letters 8:1114-1127.

Harder, L. D. 1990. Behavioral responses by bumble bees to variation in pollen availability. Oecologia 85:41-47.

Haper, J. L. and J. White. 1974. The demography of plants. Annual Review of Ecology and Systematics 5:419-463.

Hartnett, D. C. 1991. Effects of fire in tallgrass prairie growth and reproduction on prairie coneflower (*Ratibida columnifera*: Asteraceae). American Journal of Botany 78:429-435.

Haynes, J. and M. Mesler. 1984. Pollen foraging by bumblebees: foraging patterns and efficiency on *Lupinus polyphyllus*. Oecologia 61:249-253.

Hedrick, P. W. 1994. Purging inbreeding depression and the probability of extinction: full sib mating. Heredity 73:363-372.

Helenurum, K. and B. A. Schaal. 1996. Genetic load, nutrient limitation, and seed production in *Lupinus texensis* (Fabaceae). American Journal of Botany 83:1585-1595.

Hickman, J. C. (ed) 1993. The Jepson manual: higher plants of California. University of California Press, Berkeley, California, USA.

Holmes, G. D., James, E. A., and A. A. Hoffmann. 2008. Limitations to reproductive output and genetic rescue in populations of the rare shrub *Grevillea repens* (Proteaceae). Annals of Botany 102:1031-1041.

Honnay, O. and H. Jacquemyn. 2007. Susceptibility of common and rare plant species to the genetic consequences of habitat fragmentation. Conservation Biology 21:823-831.

Howarth, D. G. and M. J. Donoghue. 2006. Phylogenetic analysis of the "ECE" (*CYC/TB1*) clade reveals duplications preceding the core eudicots. Proceedings of the National Academy of Sciences 103:9101-9106.

Hughes, C. and R. Eastwood. 2006. Island radiation on a continental scale: exceptional rates of plant diversification after uplift of the Andes. Proceedings of the National Academy of Sciences 103:10334-10339.

Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. Ecological Monographs 54:187–211.

Husband, B. C. and D. W. Schemske. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. Evolution 50:54-70.

Jaquiéry, J., Guillaume, F. and N. Perrin. 2009. Predicting the deleterious effects of mutation load in fragmented populations. Conservation Biology 23:207-218.

Jennerston, O. 1988. Pollination in Dianthus deltoides (Caryophyllaceae): effects of habitat fragmentation on visitation and seed set. Conservation Biology 2:359–366.

Juncosa, A. M. and B. D. Webster. 1989. Pollination in *Lupinus nanus* subsp. *latifolius* (Leguminosea). American Journal of Botany 76:59-66.

Karoly, K. 1994. Inbreeding effects on mating system traits for two species of *Lupinus* (Leguminosae). American Journal of Botany 81:1538-1544.

Kashi, Y. and D. G. King. 2006. Simple sequence repeats as advantageous mutators in evolution. Trends in Genetics 22:253-259.

Kaye, T. N. 1999. Obligate insect pollination of a rare plant: *Lupinus sulphureus* ssp. *kincaidii*. Northwest Science 73:50-52.

Kaye T. N. and K. Kuykendall. 1993. *Lupinus sulphureus* ssp. *kincaidii*: survey and reproductive studies. Report submitted to the USDA Bureau of Land Management, Roseburg District, Roseburg, OR.

Kaye, T. N. and K. Kuykendall. 2001. Effects of scarification, cold stratification, and source population on germination of *Lupinus sulphureus* ssp. *kincaidii*. Seed Science and Technology 29:663-668.

Kaye, T. N., Pendergrass, K. L., Finley, K., and J. B. Kauffman. 2001. The effect of fire on the population viability of an endangered prairie plant. Ecological Applications 11:1366-1380.

Kearns, C. A., Inouye, D. W., and N. M. Waser. 1998. Endangered mutualisms: the conservation of plant-pollinator interactions. Annual Review of Ecology and Systematics 29:83-112.

Keller, M., Kollmann, J., and P. J. Edwards. 2000. Genetic introgression from distant provenances reduces fitness in local weed populations. Journal of Applied Ecology 37:647-659.

Keller, L. F. and D. M. Waller. 2002. Inbreeding effects in wild populations. Trends in Ecology and Evolution 17:230-241.

Kéry, M., Matthies, D., and H. H. Spillmann. 2000. Reduced fecundity and offspring performance in small populations of the declining grassland plants *Primula veris* and *Gentiana lutea*. Journal of Ecology 88:17–30.

Kéry, M., Matthies, D., and M. Fischer. 2001. The effect of plant population size on the interactions between the rare plant *Gentiana cruciata* and its specialized herbivore *Maculinea rebeli*. Journal of Ecology 89:418-427.

Kittleson, P. M. and J. L. Maron. 2000. Outcrossing rates and inbreeding depression in the perennial yellow bush lupine, *Lupinus arboreus* (Fabaceae). American Journal of Botany 87:652-660.

Krauss, J., Steffan-Dewenter, I., Müller, C. B., and T. Tscharntke. 2005. Relative importance of resource quality, isolation and habitat quality for landscape distribution of a monophagous butterfly. Ecography 28:465-474.

Kudoh, H., Shibaike, H., Takasu, H., Whigham, D. F., and S. Kawano. 1999. Genet structure and determinants of clonal structure in a temperate deciduous woodland herb, *Uvularia perfoliata*. Journal of Ecology 87:244-257.

Kunin, W. E. 1997. Population size and density effects in pollination: pollinator foraging and plant reproductive success in experimental arrays of *Brassica kaber*. Journal of Ecology 85:225-234.

Lamont, B. B., Witkowski, E. T. F., and N. J. Enright. 1993. Post-fire litter microsites: safe for seeds, unsafe for seedlings. Ecology 74:501-512.

Lande, R. 1994. Risk of population extinction from fixation of new deleterious mutations. Evolution 48:1460-1469.

Lande, R. 1995. Mutation and conservation. Conservation Biology 9:782-291.

Leimu, R., Matikainen, P., Koricheva, J. and M. Fischer. 2006. How general are positive relationships between plant population size, fitness and genetic variation? Journal of Ecology 94:942-952.

Levin, D. A. 2002. The role of chromosomal change in plant evolution. Oxford University Press, New York.

Lewis, G. M., Schrire, B., Mackinder, B., and M. Lock. 2005. Legumes of the world. Royal Botanic Gardends, Kew, UK.

Li, Y., Korol, A. B., Fahima, T., Beiles, A., and E. Nevo. 2002. Microsatellites: a genomic distribution, putative function and mutational mechanisms: a review. Molecular Ecology 11:2453-2465.

Lim, K. Y., Soltis, D. E., Soltis, P. S., Tate, J., Matyasek, R., Srubarova, H., Kovarik, A., Pires, J. C., Xiong, Z., and A. R. Leitch. 2008. Rapid chromosome evolution in recently formed polyploids in *Tragopogon* (Asteraceae). PloS ONE 3:e3353.

Liston, A., St. Hilaire, K., and M. V. Wilson. 1995. Genetic diversity in populations of Kincaid's lupine, host plant of fender's blue butterfly. Madroño 42:309-322.

Luikart, G., Allendorf, F. W., Cornuet, J. M., and W. B. Sherwin. 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. Journal of Heredity 89:238-247.

Lynch, M., Conery, J. and R. Bürger. 1995. Mutation accumulation and the extinction of small populations. American Naturalist 146:489-518.

Maes, D. and H. Van Dyck. 2001. Butterfly diversity loss in Flanders (north Belgium): Europe's worst case scenario? Biological Conservation 99:263-276.

Maron, J. L. and S. N. Gardner. 2000. Consumer pressure, seed versus safe-site limitation, and plant population dynamics. Oecologia 124:260-269.

Matthies, D., Bräuer, I., Maibom, W., and T. Tscharntke. 2004. Population size and the risk of local extinction: empirical evidence from rare plants. Oikos 105:481-488.

McKay, J. K., Christian, C. E., Harrison, S., and K. J. Rice. 2005. "How local is local?"—a review of practical and conceptual issues in the genetics of restoration. Restoration Ecology 13**:**432–440.

Menges, E. S. 2000. Population viability analyses in plants: challenges and opportunities. Trends in Ecology and Evolution 15:51-56.

Michaels, H. J., Shi, X. J., and R. J. Mitchell. 2008. Effects of population size on performance and inbreeding depression in *Lupinus perennis*. Oecologia 154:651-661.

Montalvo, A. M. and N. C. Ellstrand. 2001. Nonlocal transplantation and outbreeding depression in the subshrub *Lotus scoparius* (Fabaeceae). American Journal of Botany 88:258-269.

Morgan, M. T., Schoen, D. J., and T. M. Bataillon. 1997. The evolution of selffertilization in perennials. The American Naturalist 150:618-638.

Mustajärvi, K., Siikamäki, P., Rytkönen, S., and A. Lammi. 2001. Consequences of plant population size and density for plant-pollinator interactions and plant performance. Journal of Ecology 89:80-87.

New, T. R., Pyle, R. M., Thomas, J. A., Thomas, C. D. and P. M. Hammond. 1995. Butterfly conservation management. Annual Review of Entomology 40:57-83.

Newman, D. and D. A. Tallmon. 2001. Experimental evidence for the beneficial fitness effects of gene flow in recently isolated populations. Conservation Biology 15:1054–1063.

Peakall, R., Smouse, P. E., and D. R. Huff. 1995. Evolutionary implications of allozyme and RAPD variation in diploid populations of dioecious buffalo grass *Buchloe dactyloides*. Molecular Ecology 4:135-147.

Peakall, R., Gilmore, S., Keys, W., Morgante, M. and A. Rafalski. 1998. Crossspecies amplification of soybean (*Glycine max*) simple sequence repeats (SSRs) within the genus and other legume genera: implications for the transferability of SSRs in plants. Molecular Biology and Evolution 15:1275-1287.

Peakall, R. and P. E. Smouse. 2006. GENALEX: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288-295.

Pendergrass, K. L., P. M. Miller, J. B. Kauffman, and T. N. Kaye. 1999. The role of prescribed burning in maintenance of an endangered plant species *Lomatium bradshawii*. Ecological Applications 9:1420–1429.

Persson, H. A. and B. A. Gustavsson. 2001. The extent of clonality and genetic diversity in lingonberry (*Vaccinium vitis-idaea* L.) revealed by RAPDs and leaf shape analysis. Molecular Ecology 10:1385-1397.

Petit, C., Bretagnolle, F., and F. Felber. 1999. Evolutionary consequences of diploid-polyploid hybrid zones in wild species. Trends in Ecology and Evolution 14:306-311.

Petit, R. J., Duminil, J., Fineschi, S., Hampe, A., Salvini, D., and G. G. Vendramin. 2005. Comparative organization of chloroplast, mitochondrial and nuclear diversity in plant populations. Molecular Ecology 14:689-701.

Pfab, M. F. and E. T. F. Witkowski. 2000. A simple population viability analysis of the critically endangered *Euphorbia clivicola* R.A. Dyer under four management scenarios. Biological Conservation 96:263-270.

Pickup, M. and A. G. Young. 2008. Population size, self-incompatibility and genetic rescue in diploid and tetraploid races of *Rutidosis leptorrhynchoides* (Asteraceae). Heredity 100:268-274.

Piry, S., Luikart, G., and J. M. Cornuet. 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. Genetics 90:502-503

Rasner, C. A., Yeh, P., Essert, L. S., Hunt, K. E., Woodruff, D. S., and T. D. Price. 2004. Genetic and morphological evolution following a founder event in the dark-eyed junco, *Junco hyemalis thurberi*. Molecular Ecology 13:671-681.

Ree, R. H., Citerne, H. L., Lavin, M. and Q. B. C. Cronk. 2004. Heterogenous selection on *LEGCYC* paralogs in relation to flower morphology and phylogeny of *Lupinus* (Leguminosae). Molecular Biology and Evolution 21:321-331.

Reed, D. H. and R. Frankham. 2003. Correlation between fitness and genetic diversity. Conservation Biology 17:230-237.

Richards, C. M. 2000. Inbreeding depression and genetic rescue in a plant metapopulation. The American Naturalist 155:383–394.

Rieseberg, L. H. 2001. Chromosomal rearrangements and speciation. Trends in Ecology and Evolution 16:351-358.

Schaal, B. A. 1980a. Reproductive capacity and seed size in *Lupinus texensis*. American Journal of Botany 67:703-709.

Schaal, B. A. 1980b. Measurement of gene flow in *Lupinus texensis*. Nature 284:450-451.

Schemske, D. W., Husband, B. C., Ruckelshaus, M. H., Goodwillie, C., Parker, I. M., and J. G. Bishop. 1996. Evaluating approaches to the conservation of rare and endangered plants. Ecology 75:584-606.

Schlötterer. C. 2004. The evolution of nuclear markers – just a matter of fashion? Nature Reviews Genetics 5:63-69.

Schulke, B. and N. M. Waser. 2001. Long-distance pollinator flights and pollen dispersal between populations of *Delphinium nuttallianum*. Oecologia 127:239-245.

Schultz, C. B. and K. M. Dlugosch. 1999. Nectar and host plant scarcity limit populations of an endangered Oregon butterfly. Oecologia 119:231-238.

Schultz, C. B. and P. C. Hammond. 2003. Using population viability analysis to develop recovery criteria for endangered insects: case study of the Fender's blue butterfly. Conservation Biology 17:1372-1385.

Severns, P. 2003a. Inbreeding and small population size reduce seed set in a threatened and fragmented plant species, *Lupinus sulphureus* ssp. *kincaidii* (Fabaceae). Biological Conservation 110:221-229.

Severns, P. M. 2003b. Propagation of a long-lived and threatened prairie plant, *Lupinus sulphureus* ssp. *kincaidii*. Restoration Ecology 11:334-342.

Severns, P. M. 2006. Seasonality, habitat preference and life history of some Willamette Valley wet prairie terrestrial molluscs in western Oregon, USA. Veliger 48:220-227.

Severns, P. M. 2008a. Seedling population size and microhabitat association in *Lupinus oreganus* A. Heller var. *kincaidii* C.P. Smith (Fabaceae), a threatened plant of western Oregon grasslands. Native Plants Journal 9:358-365.

Severns, P. M. 2008b. Exotic grass invasion impacts fitness of an endangered prairie butterfly, *Icaricia icarioides fenderi*. Journal of Insect Conservation 12:651-661.

Severns, P. M. 2009a. Patterns of genetic diversity in Douglas County populations of Kincaid's lupine (*Lupinus oreganus* var *kincaidii* = *Lupinus sulphureus* ssp. *kincaidii*). Report to USDI Bureau of Land Management, Roseburg District, Oregon. 19pp.

Severns, P. M. 2009b. Patterns of genetic diversity in Washington State populations of Kincaid's lupine (*Lupinus oreganus* var *kincaidii* = *Lupinus sulphureus* ssp. *kincaidii*). Report submitted to USFWS, Lacey District, Washington. 15pp.

Severns, P. M. and B. Lewis. 2007. Is it appropriate to rely on seed set to assess candidate plant populations for genetic rescue? A case study with a threatened species. Natural Areas Journal 27:313-319.

Severns, P. M. and A. Liston. 2008. Intraspecific chromosome number variation: a neglected threat to the conservation of rare plants. Conservation Biology 22:1641-1647.

Severns, P. M. and A. D. Warren. 2008. Selectively eliminating and conserving exotic plants to save an endangered species from local extinction. Animal Conservation 11:476-483.

Sheridan, P. M. and D. N. Karrowe. 2000. Inbreeding, outbreeding, and heterosis in the yellow pitcher plant, *Sarracenia flava* (Sarraceniaceae) in Virginia. American Journal of Botany 87:1628-1633.

Silvertown, J. 2008. The evolutionary maintenance of sexual reproduction: evidence from the ecological distribution of asexual reproduction in clonal plants. International Journal of Botany 169:157-168.

Silvertown, J. and D. Charlesworth. 2001. Introduction to plant population biology. 4th Ed. Blackwell Science, Oxford, UK. 347pp.

Spielman, D., Brook, B. W., and R. Frankham. 2004. Most species are not driven to extinction before genetic factors impact them. Proceedings of the National Academy of Sciences 101:15261-15264.

Stehlik, I. and R. Holderegger. 2000. Spatial genetic structure and clonal diversity of *Anemone nemorosa* in late successional deciduous woodlands of central Europe. Journal of Ecology 88:425-435.

Stockwell, C. A., Hendry, A. P., and M. T. Kinnison. 2003. Contemporary evolution meets conservation biology. Trends in Ecology and Evolution 18:94-101.

Sykes, J. M., Horrill, A. D., and M. D. Mountford. 1983. Use of visual cover assessments as quantitative estimators of some British woodland taxa. Journal of Ecology 71:437-450.

Tallmon, D. A., Luikart, G., and R. S. Waples. 2004. The alluring simplicity and complex reality of genetic rescue. Trends in Ecology and Evolution 19:489–496.

Tamm, A., Kull, K., and M. Sammul. 2002. Classifying clonal growth forms based on vegetative mobility and ramet longevity: a whole community analysis. Evolutionary Ecology 15:383-401.

Theodose, T. A. and W. D. Bowman. 1997. Nutrient availability, plant abundance, and species diversity in two alpine tundra communities. Ecology 78:1861-1872.

Thomas, J. A., Thomas, C. D., Simcox, D. J., and R. T. Clarke. 1986. Ecology and declining status of the silver-spotted skipper butterfly (*Hesperia comma*) in Britain. Journal of Applied Ecology 23:365-380.

Thomas, C. D., Singer, M. C., and D. A. Boughton. 1996. Catastrophic extinction of population sources in a butterfly metapopulation. American Naturalist 148:957-975.

Tomlinson, P. B. 1973. Branching in monocotyledons. The Quarterly Review of Biology 48:458-466.

United States Fish and Wildlife Service. 2008. Draft recovery plan for the prairie species of western Oregon and southwestern Washington. U.S. Fish and Wildlife Service, Portland, Oregon.

Valtonen, A., Jantunen, J., and K. Saarinen. 2006. Flora and Lepidoptera fauna adversely affected by invasive *Lupinus polyphyllus* along road verges. Biological Conservation 133:389-396.

Van Rossum, F. 2008. Conservation of long-lived perennial forest herbs in an urban context: *Primula elatior* as study case. Conservation Genetics 9:119-128.

Verstrepen, K. J., Jansen, A., Lewitter, F. and G. R. Fink. 2005. Intragenic tandem repeats generate functional variability. Nature Genetics 37:986-990.

Wagner, D. B., Furnier, G. R., Saghai-Maroof, M. A., Williams, S. M., Dancik, B. P., and R. W. Allard. 1987. Chloroplast DNA polymorphisms in lodgepole and jack pine and their hybrids. Proceedings of the National Academy of Sciences 84:2097-2100.

Ward, K., Gisler, M., Fiegener, R., and A. Young. 2008. The Willamette Valley seed increase program: developing genetically diverse germplasm using an ecoregion approach. Native Plants Journal 9:334-339.

Warren, M. S., Barnett, L. K., Gibbons, D. W., and M. I. Avery. 1997. Assessing national conservation priorities: an improved red list of British butterflies. Biological Conservation 82:317-328.

Waser, N. M. 1988. Comparative pollen and dye transfer by natural pollinators of *Delphinium nelsonii*. Functional Ecology 2:41-48.

Waser, N. M. and M. V. Price. 1979. Pollen dispersal and optimal outcrossing in *Delphinium nelsoni*. Nature 277:294-297.

Waser, N. M. and M. V. Price. 1989. Optimal outcrossing in *Ipomopsis aggregata*: seed set and offspring fitness. Evolution 43:1097-1109.

Washitani, I, Ishihama, F., Matsumura, C., Nagai, M., Nishihito, J., and M. A. Nishihito. 2005. Conservation ecology of *Primula sieboldii*: synthesis of information toward the prediction of the genetic/ demographic fate of a population. Plant Species Biology 20:3-15.

Weising, K. and R. C. Gardener. 1999. A set of conserved PCR primers for the analysis of simple sequence repeat polymorphisms in chloroplast genes of dicotyledonous angiosperms. Genome 42:9-19.

Welch, M. E. and L. H. Rieseberg. 2002. Habitat divergence between a homoploid hybrid sunflower species *Helianthus paradoxus* (Asteraceae), and its progenitors. American Journal of Botany 89:472-478.

Willi, Y., Van Buskirk, J., and M. Fischer. 2005. A threefold genetic allee effect: population size affects cross-compatibility, inbreeding depression and drift load in the self-incompatible *Ranunculus reptans*. Genetics 169:2255-2266.

Williams, C. F. 2007. Effects of floral display size and biparental breeding on outcrossing rates in *Delphinium barbeyi* (Ranunculaceae). American Journal of Botany 94:1696-1705.

Wilson, M. V., and D. L. Clark. 2001. Controlling invasive *Arrhenatherum elatius* and promoting native prairie grasses through mowing. Applied Vegetation Science 4:129-138.

Wilson, M. V., Erhart, T., Hammond, P. C., Kaye, T. N., Kuykendall, K., Liston, A., Robinson, A. F., Schultz, C. B., and P. M. Severns. 2003. Biology of Kincaid's lupine (*Lupinus sulphureus* ssp. *kincaidii* [Smith] Phillips), a threatened species of western Oregon native prairies. Natural Areas Journal 23:72-83.

Young, T. P. 1984. The comparative demography of semelparous *Lobelia telekii* and iteroparous *Lobelia keniensis* on Mount Kenya. Journal of Ecology 72:637-650.