GENETIC DIVERSITY IN POPULATIONS OF KINCAID'S LUPINE, HOST PLANT OF FENDER'S BLUE BUTTERFLY

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ABSTRACT

Kincaid's lupine (*Lupinus sulphureus* ssp. *kincaidii*) is the primary host plant of the endangered Fender's blue butterfly (*Icaricia icarioides fenderi*). Both species are found in remnant upland prairies in the Willamette Valley of western Oregon, a habitat that is threatened by agriculture and urbanization. Enzyme electrophoresis was used to characterize the levels and distribution of genetic diversity in Kincaid's lupine. Eight populations of Kincaid's lupine exhibited high genetic identities (mean interpopulational $I = 0.984$) and low levels of genetic differentiation among populations ($G_{ST} = 0.119$). Although gene flow between the disjunct populations is limited at present, the species may have had a more continuous distribution prior to the agriculturalization of the past 150 years. At the Baskett Butte site, all plants considered to represent Kincaid's lupine had alleles that were otherwise restricted to spur lupine (*L. arbustus*). In addition, two clones composed of genetically identical individuals with fixed heterozygosity at four loci were identified at Baskett Butte. We suggest that hybridization between the two species is responsible for these findings.

Grasslands dominated by native bunch grasses extended over at least one million acres in the Willamette Valley prior to the introduction of agriculture, livestock grazing and urban development in the nineteenth century (Franklin and Dyrness 1973). Today, fewer than 1000 acres of high quality native grasslands are known to remain in the Willamette Valley. These upland prairies support a variety of native grasses and perennial forbs. An animal species of particular conservation interest, the endemic Fender's blue butterfly (*Icaricia icarioides fenderi*), is known from only twelve upland prairie sites within Oregon's Willamette Valley (Hammond and Wilson 1993).

The primary larval host plant of this lycaenid butterfly is Kincaid's lupine (*Lupinus sulphureus* Hooker ssp. *kincaidii* (Smith) Phillips). This member of the legume family is known from approximately 40 sites in the Willamette Valley of Oregon, south to Douglas County, and from a single location in southern Washington's Puget Trough (Kuykendall and Kaye 1993). Kincaid's lupine is present at all twelve known Fender's blue populations. However, at three of the sites, spur lupine (*L. arbustus* Douglas ex Lindley) or sickle-keeled lupine (*L. albicaulis* Hooker) also support Fender's blue (Hammond and

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Wilson 1993). The nomenclature of *L. arbustus* follows Barneby (1989); the name *L. laxiflorus* Douglas ex Lindley has been misapplied to this species in the past.

Kincaid's lupine is a potentially long-lived perennial herb (at least 25 years old in one excavated individual, M. Wilson unpublished data) that can spread vegetatively. Populations are disjunct and generally small (< 10 individuals), although a few populations may have more than 1000 individuals. The fragmented distribution of the species in the Willamette Valley is apparently due to the conversion of suitable habitat to agricultural and urban uses. Kincaid's lupine is self-incompatible, and is pollinated by native bees and flies (T. Kaye unpublished data; P. Hammond personal communication). Measured seed production rates are low: 1.8 seeds/fruit in 1990 (T. Kaye unpublished data) and 0.3–1.2 seeds/fruit in 1992 (Kuykendall and Kaye 1993). A chromosome count of $n=24$ has been reported for a Willamette Valley population of Kincaid's lupine (Phillips 1957). This chromosome number is the most common in North America *Lupinus* (Goldblatt 1981). Although this number is considered "tetraploid" (Phillips 1957), isozyme studies in *Lupinus* suggest that most loci do not show duplicate expression (Wolko and Weeden 1989).

Fender's blue butterfly and Kincaid's lupine are both considered "threatened throughout [their] range" (Oregon Natural Heritage Program 1993). The survival of Fender's blue butterfly will require the careful management and perhaps restoration of its unique habitat. Efforts to preserve Fender's blue will benefit from a full understanding of the biology of the primary host plant. In this study we use the technique of enzyme electrophoresis to estimate the levels and distribution of genetic variation in Kincaid's lupine. This technique is now widely used to infer the genetic structure of plant species of conservation concern (Karron 1987; Waller et al. 1987; Lesica et al. 1988; Pleasants and Wendel 1989; Prober et al. 1990; Les et al. 1991; Soltis et al. 1992; Godt and Hamrick 1993). The present study is unique in that the genetic characterization of a rare plant species is also of relevance to the conservation of the endangered Fender's blue butterfly.

Enzyme electrophoresis is used in this study to determine 1) if genetic differentiation exists among populations; 2) which populations contain the most genetic variation; 3) the patterns of genetic partitioning within populations; and 4) whether hybridization takes place when Kincaid's lupine is sympatric with other lupines.

Methods

Populations of Kincaid's lupine were sampled at eight sites in the Willamette Valley of Oregon and the single known location in south-

TABLE 1. ALLOZYME VARIATION AND MEASURES OF GENETIC DIVERSITY AT THE POPULATION LEVEL IN KINCAID'S LUPINE. N = average number of individuals scored per locus. P = percentage polymorphic loci at the 0.99 level. A_p = average number of alleles at polymorphic loci. H_o = observed heterozygosity. H_u = expected heterozygosity, unbiased for sample size, under Hardy-Weinberg equilibrium. F = fixation index, calculated from observed and expected heterozygosity for polymorphic loci. No significant deviations from Hardy-Weinberg expectations were observed.

Abbreviation	Population Subpopulation	Location	Fender's Blue	N	P (%)	A_p	H_o	H_u	F
BC	Boistfort Cemetery	Lewis Co., WA	absent	22.3	13.3	2.50	0.081	0.070	−0.156
OR	Oak Ridge	Yamhill Co., OR	present	18.7	33.3	2.40	0.150	0.135	−0.048
MC	Mill Creek	Polk Co., OR	absent	16.9	26.7	2.50	0.130	0.107	−0.301
MV	McTimmonds Valley	Polk Co., OR	present	14.0	26.7	2.25	0.100	0.096	−0.153
MF	McDonald Forest	Benton Co., OR	present	38.3	33.3	2.20	0.090	0.096	−0.037
MF-1	Upper			19.6	20.0	2.33	0.078	0.082	−0.107
MF-2	Lower			18.7	33.3	2.20	0.103	0.105	−0.065
WH	West Hills Road	Benton Co., OR	present	18.7	26.7	2.25	0.111	0.111	0.063
HR	Hillaire Road	Lane Co., OR	absent	21.2	40.0	2.33	0.135	0.126	−0.136
WC	Willow Creek	Lane Co., OR	present	22.2	33.3	2.20	0.153	0.146	−0.152
BB	Baskett Butte	Polk Co., OR	present				see Table 2		
Mean				21.5	29.2	2.33	0.134	0.111	−0.115

ern Washington (Table 1). Fender's blue butterfly is present at six of these sites, representing half of its known occurrences. The other three sites do not support Fender's blue. The McDonald Forest site of Kincaid's lupine was represented by an upper and lower subpopulation separated by 100 meters. The site at Baskett Butte (within the Baskett Slough National Wildlife Refuge) was sampled as five subpopulations (Table 2). These represented *L. arbustus* (BB-arb-1, BB-arb-2), *L. s. kincaidii* (BB-kin-1, BB-kin-2), and a small group of morphologically intermediate plants considered putative hybrids (BB-int). The north meadow subpopulation (BB-arb-2) is located ca. 500 meters from the remaining four subpopulations, which are located on the summit and southwest slope of Baskett Butte proper.

Population samples were made in the spring of 1991 and 1992. A total of 18–24 individuals were sampled per population (Table 1). A single leaf was collected from plants separated by at least one meter. Leaves were transported to the laboratory on ice, and frozen at −80°C until analysis. The following ten enzymes were analyzed for electrophoretically detectable genetic variation: aconitase (ACN; E.C. 4.2.1.3), aldolase (ALD; E.C. 4.1.2.13), aspartate aminotransferase (AAT; E.C. 2.6.1.1), endopeptidase (ENP; E.C. 3.4.-.-), glucose-6-phosphate dehydrogenase (GPD; E.C. 1.1.1.49), malic enzyme (ME; E.C. 1.1.1.40), phosphoglucose isomerase (PGI; E.C. 5.3.1.9), phosphoglucomutase (PGM; E.C. 5.4.2.2); superoxide dismutase (SOD; E.C. 1.15.1.1) and triosephosphate isomerase (TPI; E.C. 5.3.1.1). Electrophoretic procedures followed the general methodology of Wendel and Weeden (1989). Enzymes were extracted in cold tris-HCl buffer (Soltis et al. 1983) and resolved in 12.0% horizontal starch gels at 4°C. Three gel/electrode buffer combinations were used: AAT, ALD, ENP, ME, PGI, SOD, and TPI were examined in a pH 8.3 lithium-borate/tris-citrate system; GPD and PGM were examined in a pH 5.7 histidine-citrate system; and ACN was examined in a pH 6.1 morpholine citrate system. Enzyme/buffer combinations which provided high activity, clear bands, and consistent results were selected. Enzyme activity was assayed following the protocols of Wendel and Weeden (1989) and photographed with color print film.

Multiple loci for an enzyme were numbered sequentially with the most anodally migrating isozyme designated "1". Likewise, enzyme variants at a locus were given letters with the fastest allozyme designated "a". Standard measures of genetic polymorphism (Tables 1, 2, and 3) were calculated by hand or with Genestat-PC 3.3 (Lewis 1993). The calculation of expected heterozygosity (H_u) was unbiased for sample size (Nei 1987). This program was also used to calculate Nei's genetic identity (I) unbiased for sample size (Nei 1978) for all pair-wise sample comparisons and Nei's (1973) gene diversity statistics (H_T, H_S, and G_{ST}), unbiased for sample size and population

TABLE 2. ALLOZYME VARIATION AND MEASURES OF GENETIC DIVERSITY AT THE SUBPOPULATION LEVEL IN KINCAID'S AND SPUR LUPINE (*L. ARBUSTUS*) AT BASKETT BUTTE. Legend as in Table 1. Significant deviations ($P < 0.05$) from Hardy-Weinberg expectations are marked by an asterisk.

Abbreviation	Taxon	Location	Fender's Blue	N	P (%)	A_p	H_o	H_u	F
BB-kin-1	*kincaidii*	butte top	present	15.1	26.7	2.00	0.251	0.139	−1.000*
BB-kin-2	*kincaidii*	southwest base	present	9.5	26.7	2.00	0.289	0.140	−1.000*
BB-int	*kincaidii* × *arbustus*	southwest slope	present	6.8	33.3	2.20	0.186	0.151	−0.324
BB-arb-1	*arbustus*	butte top	present	14.5	40.0	2.50	0.138	0.180	0.204
BB-arb-2	*arbustus*	north meadow	present	13.9	46.7	2.43	0.177	0.179	0.078

TABLE 3. GENETIC POLYMORPHISM AND GENE DIVERSITY STATISTICS (NEI 1973) AT THE SPECIES LEVEL FOR *LUPINUS SULPHUREUS* SSP. *KINCAIDII* AND OTHER SPECIES WITH SIMILAR CHARACTERISTICS (HAMRICK AND GODT 1990). P_s = Percentage of polymorphic loci; A_s = average number of alleles per locus; A_{es} = effective number of alleles per locus; H_{es} = total gene diversity, averaged over all loci; H_{ET} = total gene diversity; H_{ES} = gene diversity within populations; G_{ST} = the proportion of total gene diversity found among populations. Estimates of H_{ET} and H_{ES} are biased for sample size and population number, and include polymorphic loci only. For unbiased estimates (Nei 1973), see text. The number of taxa included in each category is given separately for P_s, A_s, A_{es}, H_{es} (N_{pol}) and H_{ET}, H_{ES}, G_{ST} (N_{div}). Standard errors are in parentheses.

Categories	N_{pol}	P_s	A_s	A_{es}	H_{es}	N_{div}	H_{ET}	H_{ES}	G_{ST}
L. s. kincaidii		46.7	1.67	1.22	0.123		0.264	0.231	0.119
long-lived perennial herb	4	39.6	1.42	1.28	0.205	2	0.346	0.282	0.213
		(16.5)	(0.13)	(0.12)	(0.084)		(0.018)	(0.024)	(0.144)
short-lived perennial herb	152	41.3	1.70	1.15	0.116	119	0.300	0.222	0.233
		(2.2)	(0.06)	(0.01)	(0.009)		(0.013)	(0.013)	(0.019)
narrow distribution	101	45.1	1.83	1.17	0.137	82	0.300	0.215	0.242
		(2.8)	(1.17)	(0.02)	(0.011)		(0.015)	(0.013)	(0.024)
outcrossing, animal	172	50.1	1.99	1.24	0.167	124	0.310	0.243	0.197
		(2.0)	(0.07)	(0.02)	(0.010)		(0.010)	(0.010)	(0.017)

number. In addition, biased gene diversity statistics (H_{ET} and H_{ES}) were calculated for polymorphic loci only in order to facilitate comparison with the summary values of Hamrick and Godt (1989). All loci were included in the calculation of G_{ST}, as this results in the same value as excluding them (Lewis 1993).

The fixation index ($F = 1 - H_o/H_e$, where H_o is observed heterozygosity and H_e is expected heterozygosity) measures deviations from Hardy-Weinberg equilibrium and was calculated for polymorphic loci within each population (Wright 1965). Negative values indicate an excess of heterozygotes, and positive values a deficiency. A chi-square test was used to measure significant deviations from the expected value ($F = 0$). Estimates of interpopulational gene flow (number of migrants per generation, Nm) were calculated for eight populations of Kincaid's lupine (excluding Baskett Butte). The value of Nm was calculated using two methods as described in Godt and Hamrick (1993). The method of Wright (1978) as modified by Crow and Aoki (1984) is based on the coefficient of gene differentiation (G_{ST}). The method of Slatkin (1985) is based on the frequency of "private" alleles restricted to a single population.

Results

Fifteen putative loci with 28 alleles (numbers in parentheses) were resolved: *Aat-1* (4), *Acn-1* (3), *Ald-1* (1), *Enp-1* (2), *Gpd-1* (2), *Me-1* (1), *Pgi-1* (1), *Pgi-2* (4), *Pgm-1* (1), *Pgm-2* (1), *Pgm-3* (2), *Sod-1* (1), *Tpi-1* (1), *Tpi-2* (2), and *Tpi-3* (2). The complete data set is available from the authors upon request. Measures of genetic polymorphism at the population and subpopulation level are summarized in Tables 1 and 2. Since population samples were generally small, the measures of genetic polymorphism presented below have relatively high standard errors associated with them. For this reason, the results are interpreted conservatively.

The Baskett Butte subpopulations assigned to *L. s. kincaidii* were treated separately due to the complicating effects of fixed heterozygosity (see below). The eight remaining populations of *L. s. kincaidii* had a percentage of polymorphic loci (P) ranging from 13.3% to 40.0% (Table 1). The average number of alleles at a polymorphic locus (A_p) ranged from 2.2 to 2.5. Observed heterozygosity (H_o) averaged 0.134 and ranged from 0.078 to 0.153. Values of unbiased expected heterozygosity (H_u) averaged 0.111 and ranged from 0.070 to 0.146. The mean fixation index over these eight populations of Kincaid's lupine was $F = -0.115$ and ranged from 0.063 to -0.301. None of these values were significantly different from the expectations of Hardy-Weinberg equilibrium.

Gene diversity statistics (Nei 1973; Hamrick and Godt 1989) and measures of genetic polymorphism at the species level are sum-

marized in Table 3. Estimates of H_{ET} and H_{ES} (excluding polymorphic loci and variation in sample number and population size) were 0.264 and 0.231, respectively. The unbiased estimate of total gene diversity over all loci (H_T) equalled 0.126, and the majority of gene diversity was distributed within populations (H_S = 0.111). On average, only 11.9% of the genetic variation resided among populations (G_{ST} = 0.119). Calculating interpopulational gene flow *Nm* with the unbiased estimate of G_{ST} resulted in *Nm* = 0.843. Two private alleles (both in the Hillaire Road population) at a mean frequency of 0.033 resulted in an estimate of *Nm* = 3.92.

The distribution of genetic diversity within a population was measured at the McDonald Forest site (Table 1). Nei's genetic identity between the two subpopulations was 0.995. Two alleles (*Pgi-2c* and *Pgm-3b*) with a mean frequency of 0.063 were present in the lower and absent in the upper subpopulation. A total of 4.9% of the genetic variation was distributed between the subpopulations (G_{ST} = 0.049). The number of migrants per generation (*Nm*) as estimated with G_{ST} was 1.03 and with private alleles, *Nm* = 1.14.

The mean genetic identity value between the two subpopulations of *L. arbustus* (BB-arb-1 and BB-arb-2) and the eight allopatric populations of *L. s. kincaidii* was *I* = 0.947 (Table 4). These two subpopulations of *L. arbustus* possess four alleles (*Aat-1a* [0.211], *Enp-1b* [0.378], *Pgi-2a* [0.276], *Pgm-3b* [0.104]) absent in allopatric *L. s. kincaidii.* In addition, two alleles were present at a relatively higher mean frequency in *L. arbustus* than in allopatric *L. s. kincaidii* (*Acn-1a,* 0.388 vs. 0.100; *Gpd-1b,* 0.530 vs. 0.111). Five of these six alleles (excluding *Pgm-3b*) were also found in the morphologically intermediate subpopulation (BB-int), and one locus exhibited the fixed heterozygote pattern *Acn-1a/b.* One of these six alleles (*Pgm-3b*) was found in one of the Baskett Butte subpopulations considered to represent Kincaid's lupine (BB-kin-1); and one allele was found in the other subpopulation (*Gpd-1b* in BB-kin-2). Thus all three Baskett Butte subpopulations considered to represent Kincaid's lupine or intermediates had some of the characteristic alleles of spur lupine.

A fixation index of −1.00 ($P < 0.05$) was observed in BB-kin-1 and BB-kin-2 (Table 2). This result reflects the fixed heterozygosity at four loci in each population. Three of these fixed heterozygote patterns were shared by BB-kin-1 and BB-kin-2 (*Aat-1b/c, Acn-1b/c, Pgi-2b/c*). The heterozygote *Pgm-3a/b* was found in all individuals of BB-kin-1; *Gpd-1a/b* was found in all BB-kin-2 individuals. Fixed heterozygosity was not observed in populations of *L. s. kincaidii* outside of Baskett Butte.

The two subpopulations of spur lupine had a higher percentage of polymorphic loci and more alleles per polymorphic locus than nearly all of the Kincaid's lupine populations (Tables 1 and 2).

TABLE 4. NEI'S GENETIC IDENTITY, UNBIASED FOR SAMPLE SIZE (1978). Mean values for between and within group comparisons are given. N = number of samples. Eight populations of Kincaid's lupine (excluding Baskett Butte) are included under "Kincaid's" and the range of genetic identity values is given for their 36 pairwise comparisons.

Taxon	N	BB-kin	BB-int	BB-arb	Kincaid's
BB-kin	2	0.968	0.928	0.935	0.986
BB-int	1		1.000	0.972	0.930
BB-arb	2			0.968	0.947
Kincaid's	8				0.984 (0.954–1.000)

Positive values of the fixation index were observed in the two subpopulations of spur lupine (Table 2); however these were not significantly different from the expectations of Hardy-Weinberg equilibrium.

DISCUSSION

Levels and distribution of genetic variability in Kincaid's lupine. Hamrick and Godt (1989) have summarized the patterns of allozyme variation found in 653 studies representing 449 plant species. The levels of genetic polymorphism in Kincaid's lupine as measured by percentage of polymorphic loci (P_S), average number of alleles per locus (A_S), effective number of alleles per locus (A_{es}), and total gene diversity over all loci (H_{es}), are generally similar to those found in short-lived perennial herbs, species with a narrow geographic distribution, and animal-pollinated species (Table 3). Kincaid's lupine is a long-lived perennial, but comparisons to this category are inconclusive, since the summary is based on only four studies vs. 152 for short-lived perennial herbs (Table 3, Hamrick and Godt 1989).

Although the levels of genetic variation observed in Kincaid's lupine are near the mean for species with similar growth form, breeding system, and geographic distributions, it does have a lower value of G_{ST} than comparable species (Table 3, Hamrick and Godt 1989). The fact that only 11.9% of the observed genetic variation resides among populations is reflective of a relatively low level of population differentiation. Similarly the mean genetic identity of Kincaid's lupine populations ($I = 0.984$) is relatively high for conspecific plant populations (Crawford 1989).

Low values of G_{ST} and high interpopulational I are characteristic of species with significant gene flow among populations. However, the estimated level of gene flow based on G_{ST} is only $Nm = 0.843$. For neutral genes, Nm needs to be greater than one to prevent divergence due to genetic drift (Wright 1978). Although a higher value of Nm is estimated using private alleles ($Nm = 3.92$), the fact that this method can be inaccurate when population sizes and the number

of rare alleles are low (Slatkin and Barton 1989) leads us to prefer the first estimate. Due to its fragmented distribution, actual gene flow between the disjunct populations of Kincaid's lupine is presently unlikely. However, populations may have been distributed continuously in the past (as recently as 150 years ago), providing opportunities for gene flow and explaining the low levels of genetic differentiation and high genetic identities among populations.

The levels of genetic variation observed between populations with and without Fender's Blue did not substantially differ (Table 1). For example, the two populations with the highest (Hillaire Road) and lowest (Boistfort Cemetery) number of polymorphic loci did not host Fender's blue. Among populations which do host the butterfly, McDonald Forest, Willow Creek, and Oak Ridge had the highest number of polymorphic loci. Within-population differentiation at McDonald Forest was observed (Table 1). The lower McDonald Forest subpopulation has two alleles not observed in the upper subpopulation. Although suggestive of differentiation, these results could also be attributed to sampling error resulting from the relatively small subpopulation sizes.

Seven of the eight allopatric populations of Kincaid's lupine had positive values of *F*, the fixation index (Table 1). However no population had a value of *F* significantly different from zero, suggesting that the populations can be considered to be in Hardy-Weinberg equilibrium. This is indicative of an outcrossing breeding system, and is consistent with the observed self-incompatibility in Kincaid's lupine (Kuykendall and Kaye 1993). The two subpopulations of spur lupine had negative (but not significantly different from zero) values of *F*. The consistent (but non-significant) differences in the direction of the deviations from Hardy-Weinberg equilibrium may be indicative of differing breeding systems in the two species.

Evidence for hybridization on Baskett Butte. Spur lupine (*L. arbustus*) and Kincaid's lupine are generally allopatric. The two species have a mean genetic identity of $I = 0.947$, and no fixed allelic differences (alternate alleles at a frequency of $P = 1.00$) separate the two. However four spur lupine alleles at average frequencies of 0.146–0.378 are absent in Kincaid's lupine, while two additional loci are present at high frequency in spur lupine (0.388 and 0.530) and low frequency (0.100 and 0.111) in Kincaid's. These six alleles can be used to document introgression between the two species. On Baskett Butte, where the two species are sympatric, all surveyed individuals considered to represent Kincaid's lupine or intermediates had some of the alleles characteristic to spur lupine.

Hybridization and introgression are very widespread phenomena in the genus *Lupinus* (Phillips 1957), and it is possible that the high genetic identity observed between Kincaid's and spur lupine may be a consequence of past genetic interchange. Likewise, the relatively

high levels of genetic diversity in spur lupine on Baskett Butte may have resulted from past introgression with Kincaid's lupine or other species. Additional allopatric populations of spur lupine would need to be examined in order to confirm this hypothesis.

Considering the high frequency of hybridization in the genus, it is not surprising that individuals suggestive of a hybrid origin were identified prior to the isozyme study. The subpopulation (BB-int) comprised of seven morphologically intermediate individuals has all four alleles that are restricted to spur lupine. Interestingly, all seven individuals exhibit the heterozygous pattern *Acn-1a/b*. Unfortunately, the ploidy level of these individuals is unknown. Thus it cannot be determined if this "fixed heterozygosity" is the result of two loci in a hybrid tetraploid or allelic heterozygosity.

The genetic situation found in the two subpopulations considered to represent Kincaid's lupine (BB-kin-1 and BB-kin-2) is rather different. Within each subpopulation, individuals are identical at the sampled loci and have fixed heterozygote patterns at four loci. This "heterozygote excess" results in values of $F = -1.00$ ($P < 0.05$) in these two subpopulations. The only difference between the two subpopulations is that one of the fixed heterozygote genotypes within BB-kin-1 is *Pgm-3a/b* while BB-kin-2 has *Gpd-1a/b*. It is at these latter two loci that each population has one of the characteristic alleles of spur lupine (*Pgm-3b* and *Gpd-1b*). The simplest explanation for the uniform genotypes in each subpopulation is that the "individuals" sampled in BB-kin-1 and BB-kin-2 are actually ramets of large vegetative clones. Furthermore, each clone must have had an independent origin due to the different fixed heterozygote genotypes at PGM and GPD. It is likely that each clone resulted from a separate hybridization event between spur and Kincaid's lupine. Observations of BB-kin-1 over four years (A. Liston unpublished data) suggest that this "individual" is sterile. The majority of the ramets do not produce inflorescences, and there is no evidence that flowering ramets produce seed. Pollen viability in these individuals is low as measured by lactophenol staining (K. St. Hilaire unpublished data). Other populations of Kincaid's lupine show high viability (nearly 100% pollen staining, K. St. Hilaire unpublished data).

Wolko and Weeden (1989) tested ten enzyme systems in five *Lupinus* species and found duplicate isozyme expression at 2–7 loci within individuals from each species. These data were interpreted as consistent with an ancient tetraploid origin of the *Lupinus* genome (Wolko and Weeden 1989). In Kincaid's and spur lupine three loci were observed for PGM and TPI (two are expected in diploid plants, Weeden and Wendel 1989). Thus duplicate gene expression is found in only two of the ten surveyed enzyme systems, suggesting that although these species are cytologically tetraploid, their genome is effectively diploidized (Wolko and Weeden 1989).

Duplicate gene expression (fixed heterozygosity) at four loci in

BB-kin-1 and BB-kin-2 suggests that these plants have a higher ploidy level than spur lupine and allopatric Kincaid's lupine. Both $n=24$ "tetraploids" and $n=48$ "octoploids" have been reported from spur lupine (as *L. laxiflorus*) (Phillips 1957). Thus it is predicted that BB-kin-1 and BB-kin-2 are $n=48$. Cytological confirmation of the inferred ploidy level of these clones would provide further evidence for their putative hybrid origin. In addition, the total extent of each clone could be determined by the genotyping of all putative ramets in the subpopulations.

Management implications. The relatively low level of genetic differentiation among populations of Kincaid's lupine suggests that only normal precautions need to be taken in choosing seed sources used in revegetation of native prairie. Thus while seeds from a nearby source are preferable, geographic differentiation does not appear to be significant in Kinaid's lupine. Since populations with and without Fender's blue are undifferentiated, it might be desirable to collect seeds in non-host populations in order not to risk impacting the butterfly. It would also be desirable to collect seeds from populations with the highest levels of observed diversity.

Despite evidence of hybridization between Kincaid's lupine and spur lupine on Baskett Butte, this does not appear to pose a threat to the survival of the endangered Fender's blue butterfly. In fact, the sterile clones of presumed hybrid origin are used by the butterfly for oviposition (A. Liston unpublished observation). Hybridization between a rare plant and a widespread congener can be considered a threat to the "genetic integrity" of the rare plant (Rieseberg 1991). In some cases, it has even been suggested that the widespread species be physically removed from the site of a rare species (Rieseberg et al. 1989). However, the fact that no "pure" Kincaid's lupine individuals were found on Baskett Butte suggests that such manipulation would be impossible in this situation.

Although spur lupine is widespread in central Oregon, it is only known from two additional sites in the Willamette Valley. Preliminary isozyme results also suggest that spur lupine from Baskett Butte is genetically isolated from central Oregon populations (K. St. Hilaire and A. Liston unpublished data). Spur lupine serves as an alternative host of Fender's blue, and on Baskett Butte supports the largest known population of the butterfly (Hammond and Wilson 1993). Thus although conservation efforts have focused on Kincaid's lupine, spur lupine in the Willamette Valley is also deserving of protection.

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