# FINE-SCALE GENETICALLY BASED DIFFERENTIATION OF LIFE-HISTORY TRAITS IN THE PERENNIAL SHRUB *LUPINUS ARBOREUS*

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*Abstract.*—Across large spatial scales, plants often exhibit genetically based differentiation in traits that allow adaptation to local sites. At smaller spatial scales, sharp boundaries between edaphic conditions also can create strong gradients in selection that counteract gene flow and result in local adaptation. Few studies, however, have examined the degree to which continuous populations of perennial plants exhibit genetically based differentiation in life-history traits over small spatial scales. We quantified the degree of genetically based differentiation in adaptive traits among bush lupine (*Lupinus arboreus*) from nearby dune and grassland sites (sites separated by <0.75 km) that formed part of a larger continuous population of *L. arboreus*. We also investigated the spatial genetic structure of bush lupine by examining how genetic structure differed between seeds and juvenile plants that were less than two years old. We calculated *F*-statistics from gel electrophoresis of 10 polymorphic loci. We then used these values to infer levels of gene flow. To examine differentiation in adaptive traits, we created full-sibling/half-sibling families of lupine within each area and established reciprocal common gardens at each site. Across two years, we measured canopy volume, flowering time, seed set, and mortality of progeny planted in each garden. Spatial genetic structure among seeds was virtually nonexistent ($F_{ST} = 0.002$), suggesting that gene flow between the three areas could be quite high. However, genetic structure increased 20-fold among juvenile plants ($F_{ST} = 0.041$). We found strong evidence for fine-scale genetically based differentiation and local adaptation in adaptive traits such as plant size, flowering phenology, fecundity, and mortality. Thus, it is likely that strong but differing selection regimes within each area drive spatial differentiation in lupine life-history traits.

*Key words.*—*F*-statistics, gene flow, genetic differentiation, life-history traits, local adaptation, *Lupinus arboreus*, spatial genetic structure.

Intraspecific life-history variation in plants can arise for several reasons. Plants can be phenotypically plastic whereby underlying environmental variation results in a norm of reaction for traits with adaptive value. Subpopulations of plants also can become genetically differentiated as a result of spatial heterogeneity in selection regimes. Thus, plant genetic structure and phenotype can reflect variable edaphic conditions (Antonovics and Bradshaw 1970; Snaydon and Davies 1982; Linhart and Grant 1996) or spatially variable ecological interactions such as herbivory, competition, and pollen or seed dispersal (Levin and Kerster 1974; Linhart 1988; Fritz and Simms 1992; Waser and Price 1994). Early classic work demonstrated that large-scale variation in abiotic conditions can produce locally adapted ecotypes (Turesson 1922; Clausen et al. 1947). Selection coupled with restricted gene flow can maintain subpopulations or ecotypes that are adapted to local conditions (Levin and Kerster 1974; Slatkin 1987, 1994). Contemporary studies have shown that very fine-grained environmental variation can have similar diversifying effects on adaptive traits (Linhart 1974; Silander and Antonovics 1979; but see Tonsor 1990; Stanton and Galen 1997). Restricted gene flow may isolate subpopulations and create demes with small effective size; genetic drift then may fix particular allelic combinations and lead to spatial substructure (Wright 1969; Hartl 1988).

Although macroenvironmental features may appear relatively homogeneous, the microenvironment that sessile plants inhabit can change dramatically across relatively short geographic distances (Davies and Snaydon 1976). In plants, "the most convincing cases for selectively driven differentiation across short spatial distances are those caused by dramatic soil differences, either human induced (e.g. mine tailings) or natural (e.g. ultramafic outcrops)" (Linhart and Grant 1996, p. 265). In these cases, a sharp boundary between toxic and hospitable soil creates an extremely strong selection gradient (Jain and Bradshaw 1966; Antonovics and Bradshaw 1970; Bradshaw 1972; Jules and Shaw 1994). Gene flow between local populations can be substantial, but selection for tolerance to toxic soil is strong enough to create and maintain genetic structure.

In many other cases, however, high levels of gene flow between adjacent populations can have homogenizing effects on genetic structure, effectively retarding the evolution of fine-scale local adaptation (Levin and Kerster 1974; Tonsor 1990; Stanton et al. 1997). In situations such as these, genetic differentiation can only occur if selection is strong enough to overcome gene flow (Endler 1977; Loveless and Hamrick 1984).

Understanding the process of local adaptation involves determining how the forces of selection, gene flow, and drift operate in spatially structured populations. Few studies attempt to identify the influence of each process on spatial subdivision; as a result, controversy still exists over the relative importance of selection versus genetic drift and gene flow (Heywood 1991; Linhart and Grant 1996). At our study site along the California coast, bush lupine (*Lupinus arboreus*), a native perennial shrub, grows in two very different habitats (grasslands and dunes) that sharply abut each other along the San Andreas Fault. Grassland and dune environments are distinguished by distinct environmental conditions
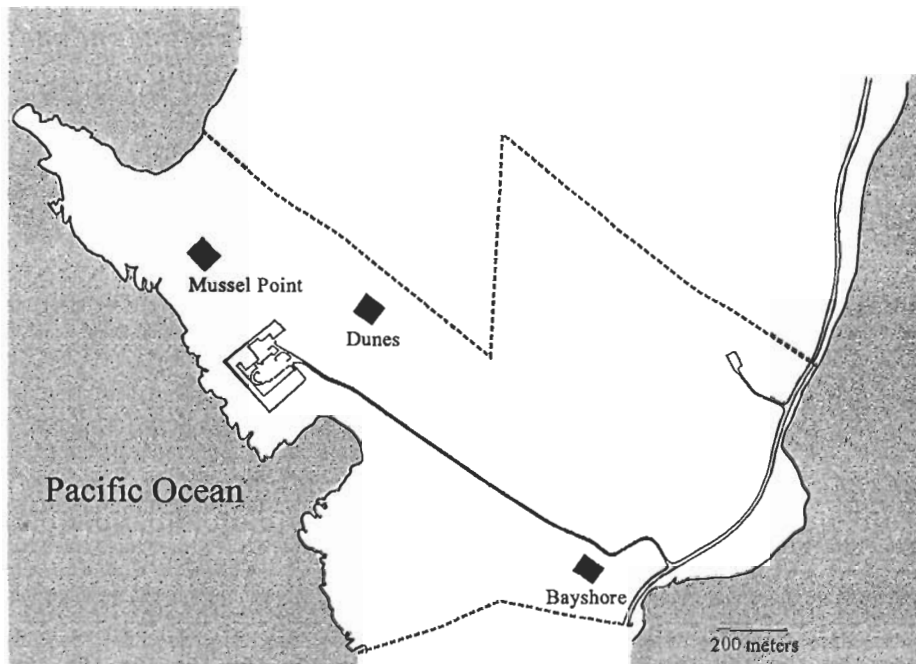
FIG. 1.   Map of the Bodega Marine Laboratory and Reserve illustrating the locations of three lupine stands: Bayshore (BS), Dunes (DUN), and Mussel Point (MP). Stippled boundaries delineate the reserve property. Solid boxes indicate the relative location of common gardens. Half-sibling/full-sibling families were made in areas adjacent to the common gardens.

such as differences in plant cover, soil organic content, nitrogen, and water availability (Bodega Marine Reserve, unpubl. data). In grasslands, lupine populations often fluctuate dramatically. In some grassland sites, lupine populations oscillate from <5% to 75% cover (Strong et al. 1995; pers. obs.). Plants recruit episodically, grow at high density, but frequently die from insect herbivory (Strong et al. 1995; Maron 1998). In other sites, both in grasslands and dunes, lupine population dynamics are more stable. Because phenotypic traits influence plant fitness, patterns of differential survival and reproduction unique to each microsite could lead to the evolution of localized genotypic structure (Antonovics and Bradshaw 1970; Schemske 1984). In turn, heritable variation in plant life-history patterns between populations can feed back to further reinforce local dynamics.

Here we ask if fine-scale variability in abiotic and biotic environments reflecting differing selective regimes may have led to local adaptation and genetically based differentiation in life-history features of lupine. Although these general issues have been addressed in many annual plant populations (Antonovics and Bradshaw 1970; Snaydon and Davies 1982), local adaptation and differentiation of adaptive traits in woody perennial plant populations has seldom been examined (but see Sork et al. 1993), especially over relatively small spatial scales. Moreover, few studies examine genetic structure for more than one plant life-history stage (but see Tonsor et al. 1993; Cabin 1996; Kalisz et al. 2001). In this paper, we explore how genetic structure, life-history traits, and local adaptation vary among bush lupine from one dune area and two grassland sites in close proximity (separated by 0.50–0.75 km). Plants from these sites are part of a more extensive, continuous population of bush lupine. From estimates of genetic structure in seeds and juvenile plants, we infer potential

levels of gene flow between these nearby sites. In turn, we ask how genetically based life-history traits expressed in lupine stands may be affected by outcrossing, migration, and selection.

## MATERIALS AND METHODS

### Study Organism and Lupine Stands

Yellow bush lupine, *Lupinus arboreus* (Fabaceae), is a perennial, nitrogen-fixing, shrub that grows along portions of the Californian coast (Hickman 1993). At our study site on the Bodega Marine Reserve (BMR) in Sonoma County, California, bush lupine is abundant and grows in adjacent grassland and dune habitats. Bush lupine seedlings emerge during the rainy season from December to March. Plants grow rapidly, attaining heights of 0.5–0.7 m within one season (Davidson and Barbour 1977). Most shrubs flower in their second spring (April–June); relatively heavy lupine seeds disperse locally via explosive dehiscence from pods in late summer. Bush lupine has a mixed mating system, with an outcrossing rate of 0.78 (Kittelson and Maron 2000). Inflorescences mature acropetally; a whorl of four to six hermaphroditic flowers open along the raceme every three days. Nectarless lupine flowers are pollinated primarily by honey bees (*Apis mellifera*) and bumblebees (*Bombus vosnesenskii*; Barbour et al. 1973; Davidson 1975). These pollinators can potentially fly long distances.

Figure 1 shows the location of the three study areas. Bayshore (BS) and Mussel Point (MP) are grassland sites. At Bayshore, cover and persistence of bush lupine is highly variable, with average lupine cover fluctuating from 0% to 60% over 10-year periods (Strong et al. 1995). Fluctuations in cover may be caused by high rates of herbivore damage

(Strong et al. 1995; Maron 1998; Maron et al. 2001). At Mussel Point, lupine cover is more extensive (45% to 75%), but does not fluctuate dramatically. The third site is the Dunes (DUN). Lupine cover is sparser here than in grassland sites, but is relatively stable (cover ranges from 20% to 45%). Plants in the dunes experience some insect herbivory, but rarely suffer from severe herbivory and stand die-off.

### Genetic Structure

Change in lupine population structure over time was examined by comparing spatial genetic structure of two age classes. We determined the extent of spatial genetic structure in seeds and juvenile plants by performing enzyme electrophoresis and examining variation in neutral markers. In 1995 we collected leaf tissue and seeds from lupine in each of the three areas. Seeds were collected from 40 bushes at each site. To estimate allele frequencies, we randomly selected only one seed per bush from our initial seed collection. If expression of any enzyme was unclear, we discarded the results from that seed and sampled another sibling. To collect leaf tissue for electrophoresis, in March 1998 we clipped newly expanding leaves from 60 plants in each subpopulation that we had previously marked as seedlings in 1996 and 1997. Leaf tissue was placed into plastic bags and refrigerated no more than 15 h. Ultimately, we used one leaflet per leaf for each juvenile plant ($n = 60$ juvenile plants in each subpopulation).

Arrays of selfed families were used to verify that all loci segregated in a Mendelian fashion. We analyzed only loci that produced identical banding patterns for both seed and leaf tissue. Consistently scoreable results were obtained for seven enzymes coding for 10 loci: alcohol dehydrogenase ($Adh_1$, $Adh_2$), esterase ($Est$), isocitrate dehydogenase ($Idh$), phosphoglucoisomerase ($Pgi_1$, $Pgi_2$), phosphoglucomutase ($Pgm$), malic enzyme ($Me$), triamino peptidase ($Tap_2$, $Tap_3$). $Adh_1$, $Adh_2$, $Est$, $Me$, $Tap_2$, and $Tap_3$ were resolved in lithium hydroxide gels (pH 8.1/8.4; Werth 1986), run for 6–7 h at 50 mA and 200 volts. $Idh$, $Pgi_1$, $Pgi_2$, and $Pgm$ were resolved in a histidine citrate gel (pH 5.7; Wendel and Weeden 1989), run for 2–3 h at 40 mA and 160 volts. For complete details on the methods of preparing, running, and staining starch gels see Kittelson (1998).

We determined the extent of genetic subdivision within and among seed and juvenile lupine stands using $F$-statistics (Wright 1968; Weir and Cockerham 1984; Cockerham and Weir 1993). Each allele and the multilocus population average were tested for conformance to Hardy-Weinberg equilibrium by using an exact test and Levene's correction for small sample size. Total allozyme variation was partitioned within and among stands following Weir and Cockerham (1984). The three $F$-statistics are interrelated:

$$1 - F_{IT} = (1 - F_{IS})(1 - F_{ST}), \qquad (1)$$

where $F_{IT}$ is the magnitude of total fixation relative to the expectation that random mating occurs in the population as a whole, $F_{IS}$ is the fixation index within subpopulations relative to the expectation of random mating within subpopulations, and $F_{ST}$ is the amount of genetic subdivision among subpopulations. $F_{ST}$ ranges between zero and one. $F_{ST}$-values

of one imply complete genetic differentiation, whereas zero values indicate no genetic differentiation. Hierarchical analysis of genetic markers provides insight into the potential role of gene flow and drift, although past effects of selection can affect values (Bossart and Prowell 1998; Whitlock and McCauley 1999). In equilibrium conditions, an $F_{ST}$ of zero implies high levels of gene flow between subpopulations (Wright 1969; Slatkin 1994). However, indirect estimates of gene flow from $F_{ST}$ require many assumptions that are not likely to be met and should be considered at best only gross estimates of migration (Bossart and Prowell 1998; Whitlock and McCauley 1999).

$F$-statistics were calculated for each locus separately and mean values were generated by averaging across all alleles and loci using a weighted method (Weir and Cockerham 1984). Ninety-five percent confidence intervals (CI) for mean $F$-statistics, generated by jackknifing across loci (Weir 1990) were plotted about the mean ($n = 120$ for seed cohort, $n = 180$ for juvenile plants). We also randomly assigned genotypes among all three areas 1000 times to test the null hypothesis that $F_{ST}$-values significantly deviated from zero (Heywood 1991). $F$-statistics and significance tests were performed using the program Genetix (available at: www.univ-mont2.fr/~genetix/genetix.htm).

### Creation of Full-Sibling and Half-Sibling Families

To examine both genetic and environmental influences on life-history traits among *L. arboreus* stands, we made full- and half-sibling families within each of the three sites, and planted these families into common gardens at each site. Beginning in April 1995, we randomly selected six pollen-donating plants (sires) to cross with five seed-producing plants (dams) within each of the three sites. We conducted crosses in a complete $6 \times 5$ factorial design. This resulted in a total of 30 full-sibling, six paternal, and five maternal half-sibling families within each site.

To prevent natural pollination, we enclosed each maternal bush with 1-$m^3$ pollinator exclosure tents made with white polyester tent-window fabric attached to a PVC frame. Plants were sprayed with the insecticide Sevin (Union Carbide Corp., Danbury, CT) to kill any insects that could transfer pollen. To ensure that fruit set resulted only from hand-pollination, we followed a multistep pollination process. First, we removed stamens and keel petals from each flower per whorl 24 h prior to stigma receptivity. After emasculation, the gynoecium was again enclosed within the wing petals. One to two days later we applied pollen from the appropriate donors using a wooden stick with a small piece of velour fabric glued on the end. One flower per whorl was emasculated, but not pollinated to serve as a control for inadvertent pollination. If that flower set seed, we discarded all seeds from that whorl. Our crossing design ensured that all seeds from the same fruit and inflorescence were full-siblings, those from different inflorescences on the same dam were maternal half-siblings, and those from different bushes that were pollinated from the same sire were paternal half-siblings. For complete methodology crossing *L. arboreus* flowers, see Kittelson (1998) and Kittelson and Maron (2000).

We collected seeds from our crosses from July to Septem-

ber 1995. As fruit development progressed, we covered individual inflorescences with bridal veil netting to prevent seed loss due to explosive dehiscence. Mesh tents were kept over all dams until after all seeds were collected. Collected seeds were counted and stored at room temperature until January 1996.

## Common Gardens

In spring 1995, we established 20 m × 20 m common-garden plots within three lupine stands: Mussel Point, Dunes, and Bayshore. We cleared common garden plots of any existing shrubs, and allowed all vegetation, except lupine, to reestablish. In January 1996, we weighed and scarified 24 randomly selected seeds from each full-sibling family. Seeds were planted into randomly assigned pots (diameter = 38 mm, height = 135 mm) arrayed in groups of 98. Racks of pots were randomly assigned to positions on greenhouse benches and watered twice daily for four weeks. Because we hand-scarified the seeds, over 99% of all seeds germinated and there were no differences among families in germination rate (P. M. Kittelson, unpubl. data). In February 1996, we transplanted these seedlings into each common garden. We subdivided each garden into three blocks and planted two randomly selected seedlings from each of the 90 full-sibling families into each of these blocks. Each garden contained a total of six seedlings from each of 90 families per subpopulation (540 seedlings/site × 3 sites = 1620 seedlings per garden). Each seedling was marked with a flag and a numbered identification tag. During weekly inspections in spring, we weeded gardens of any volunteer lupine seedlings that had germinated from the seed bank.

## Trait Measurements

In August 1996, we measured plant height, maximum canopy diameter, and a second canopy diameter at right angles to the maximum and counted the number of branches. Because branch number was highly correlated with canopy volume ($r = 0.86$, df = 1383, $P < 0.001$), we did not continue counting the number of branches on each plant. We used August canopy diameters to calculate canopy volume (height × the two canopy diameters). We also measured plant height and crown diameter at the end of August 1997. From March to August 1996 we censused plants every six weeks and recorded any plants that had died since the previous census. Starting in spring 1997, experimental plants flowered for the first time. To determine if flowering time varied within and among gardens, we monitored floral development by counting the total number of whorls on each raceme. Because the plants produce two whorls each week, this is a good estimate of the number of weeks that plants had been flowering (Kittelson and Maron 2000). To estimate the number of seed produced, we counted the number of fruits on every plant and the number of seeds in a subset of 40 randomly selected fruits. We then multiplied the total number of fruit per plant by the mean number of seeds per fruit. If plants produced less than 40 fruits, we counted the number of seeds for all the fruit produced by that plant. We also continued to monitor mortality and cause of death through 1997.

## Statistical Analyses

To determine how genetic and environmental factors affected plant traits, we performed a MANOVA using SAS (SAS Institute 1990) to explore how the correlated traits of canopy volume (1996 and 1997), floral development, and fecundity were affected by destination garden, seed origin (all fixed effects), block, and maternal and paternal identity (all random effects) and their interactions. To meet the assumptions of the MANOVA, canopy diameter and seed number were log transformed. Seed weights differed significantly among the origins (MS = 0.003, $F_2 = 17.4$, $P < 0.001$), so we controlled for maternal environmental effects on plant traits by adding seed weight as a covariate in each of the mixed models. When overall MANOVA results were significant (see Results), we report effects of dependent variables on each trait in isolation using a mixed-model ANOVA (Proc GLM; SAS Institute 1990).

In our analyses, a significant destination effect indicates that the common garden environment alters expression of plant traits. Significant origin effects indicate that genetic or maternal environmental factors influence traits among areas. Paternal effects indicate that there is additive genetic variance for particular traits, whereas maternal effects indicate either significant genetic and/or maternal environmental influences on trait values.

A significant site × origin interaction is similar to a genotype × environment interaction and provides evidence for origin-specific differentiation in response to site. In other words, the relative rank of genotypes with respect to a trait such as fecundity changes significantly across the common-garden environments. We consider local adaptation to occur if genotypes that originate from the destination site perform better than genotypes from other sites or if genotypes have the highest fitness in their native site relative to other destinations. To determine if local adaptation contributed to any of the significant site × origin interactions, we performed a second ANOVA in which we defined a new variable called "nativity." Nativity had a value of one in cases where plant origin and common garden destination were the same. Nativity had a value of zero when plant origin was different from the common-garden destination. In this second ANOVA, one degree of freedom is lost from the site × origin term. A significant nativity term provides support that local adaptation exists, and thus that lupines tend to perform best when planted in their native site.

In addition to analyzing variation in traits across all sites, we were also interested in decomposing the sources of variation in traits within each garden. We explored this by analyzing each destination garden independently with separate MANOVAs for each destination garden. When overall MANOVA results were significant, we report effects of dependent variables on each trait in isolation using a mixed-model ANOVA. In these analyses, we examined the effects of origin, block, maternal family, paternal family, and their interactions on the traits mentioned above. Data are reported for these independent within-garden analyses only if they showed different patterns relative to analyses *across* the reserve. Within-garden analyses are only reported in the text, whereas results from across BMR are further detailed in the tables.

TABLE 1. Multivariate analysis of variance (MANOVA) performed on canopy volume (1996 and 1997), floral development, and seed number to examine the effects of destination garden, seed origin (all fixed effects), block, and maternal and paternal identity (all random effects) and their interactions (SAS Institute 1990). To meet the assumptions of the MANOVA, canopy diameter and seed number were log transformed.

| Source of variation | Wilks' Lambda | F | df | P |
|---|---|---|---|---|
| Destination site | 0.354 | 148.3 | 8 | <0.0001 |
| Seed origin | 0.793 | 26.73 | 8 | <0.0001 |
| Block(site) | 0.754 | 10.51 | 24 | <0.0001 |
| Maternal family(origin) | 0.787 | 4.50 | 48 | <0.0001 |
| Paternal family(origin) | 0.875 | 1.98 | 60 | <0.0001 |
| Maternal × paternal(origin) | 0.689 | 1.42 | 240 | <0.0001 |
| Site × origin | 0.858 | 8.59 | 16 | <0.0001 |
| Site × maternal(origin) | 0.857 | 1.43 | 96 | 0.0041 |
| Site × paternal(origin) | 0.823 | 1.45 | 120 | 0.0010 |
| Site × maternal × paternal(origin) | 0.539 | 1.21 | 480 | 0.0018 |

To analyze mortality, we calculated the proportion of dead plants in each full-sibling/half-sibling family in August 1996 and 1997. Using ANOVA, we examined the impacts of origin, destination garden, block, and their interactions on mortality. Because we used the proportion of dead plants in each family, we did not have enough power to analyze effects of maternal and paternal families.

## RESULTS

### Genetic Structure

For both seeds and juvenile plants, $F_{IT}$-values were positive. Average seed $F_{IT}$ was 0.206 (95% CI = 0.178–0.234) and juvenile $F_{IT}$ was 0.212 (95% CI = 0.150–0.274). These positive $F_{IT}$-values indicate an excess number of homozygotes for the population as a whole relative to Hardy-Weinberg expectations. Within areas, values for $F_{IS}$ show that seeds and juvenile plants were 20% more homozygous on average than expected under random mating conditions (seed $F_{IS}$ = 0.204, 95% CI = 0.172–0.236; juvenile $F_{IS}$ = 0.178, 95% CI = 0.123–0.233). $F_{IT}$-values were similar between seed and juvenile cohorts, whereas $F_{IS}$ declined slightly in the juvenile cohort. $F$-statistics for all loci in seed and juvenile plants are found in the Appendix.

For seeds, the average measure of spatial subdivision was very low ($F_{ST}$ = 0.002, 95% CI = −0.005–0.009) and not significantly different from zero ($P$ = 0.25). Thus, allele frequencies in seeds do not exhibit detectable variation among sites and there is probably little population subdivision among Bayshore, Dunes, and Mussel Point. Assuming that seeds from sample areas are in Hardy-Weinberg equilibrium, the very low $F_{ST}$-values imply that gene flow among the three stands was very high and that seeds are likely to be genetically similar. In contrast, $F_{ST}$ for juvenile plants was 20 times higher ($F_{ST}$ = 0.041, 95% CI = 0.027–0.055) and was significantly different than zero ($P$ < 0.001).

### Plant Life-History Traits

The combined traits of canopy volume (1996 and 1997), floral development, and fecundity were significantly different across BMR depending on origin, destination site, and familial and other model factors (MANOVA, Table 1). Therefore, we report effects of dependent variables on each trait in isolation from individual ANOVAs. Across BMR, juvenile canopy volume was influenced by both environmental and genetic factors and their interactions (Table 2). There were significant effects of destination (environmental factors), origin, and maternal family (genetic and maternal environmental factors) and a site × origin interaction on canopy

TABLE 2. General linear model results among all gardens for juvenile *Lupinus arboreus* size, adult size, flowering time, and seed number. Dead plants were excluded from the analyses. Error degrees of freedom were 1068 for juvenile size in 1996. Mean square and *F*-values are Type III sum of the squares.

| Source | df | Juvenile size 1996 | | Adult size 1997 | | Flowering time | | Seed number | |
|---|---|---|---|---|---|---|---|---|---|
| | | MS | F | MS | F | MS | F | MS | F |
| Destination site | 2 | 114 | 59.5*** | 129 | 122*** | 719 | 309*** | 976 | 194*** |
| Seed origin | 2 | 150 | 78.1*** | 107 | 101*** | 52.6 | 22.6*** | 164 | 32.7*** |
| Block(site) | 6 | 35.8 | 18.7*** | 15.9 | 7.49*** | 31.0 | 13.4*** | 65.7 | 13.1*** |
| Maternal family(origin) | 1 | 12.7 | 10.1*** | 6.75 | 7.49*** | 13.4 | 6.99*** | 48.0 | 9.24*** |
| Paternal family(origin) | 15 | 3.00 | 2.52 | 2.84 | 1.57 | 2.28 | 1.13 | 7.90 | 1.03 |
| Maternal × paternal(origin) | 60 | 2.78 | 0.99 | 1.98 | 1.02 | 1.93 | 0.82 | 5.50 | 0.99 |
| Site × origin | 3 | 3.68 | 1.92 | 2.09 | 1.33 | 6.40 | 2.75* | 48.7 | 9.68*** |
| Nativity | 1 | 22.2 | 11.6*** | 40.4 | 25.7*** | 45.5 | 19.6*** | 182 | 36.3*** |
| Site × maternal(origin) | 24 | 1.27 | 0.45 | 0.83 | 0.43 | 2.31 | 0.98 | 4.67 | 1.09 |
| Site × paternal(origin) | 30 | 1.21 | 0.43 | 1.78 | 0.92 | 2.43 | 1.03 | 6.19 | 1.23 |
| Site × maternal × paternal(origin) | 120 | 2.83 | 1.48** | 1.95 | 1.24 | 2.36 | 1.01 | 5.04 | 1.00 |
| Seed mass | 1 | 0.31 | 0.16 | 0.04 | 0.03 | 1.02 | 0.44 | 0.94 | 0.19 |
| Error | 875 | 1.92 | | 1.57 | | 2.32 | | 5.03 | |

* $P$ < 0.05, ** $P$ < 0.01, *** $P$ < 0.001.

volume. The absence of paternal effects suggest that there may be little additive genetic variance for this trait. Also, environmental heterogeneity contributed to juvenile size within a destination garden, as indicated by a significant block effect.

Because the site × origin factor was significant for juvenile size (MS = 11.71, $F_{4,5}$ = 14.41, $P$ = 0.007), we then examined if families planted in their home site are at an advantage relative to families from other sites (i.e., nativity). When the nativity term was included, the site × destination interaction term became nonsignificant, indicating that the original significant effect was due primarily to local adaptation. For example, BS families grew bigger at Bayshore than plants from the two other origins. In the Dunes DUN families were generally the largest, and MP families grew best in their home environment.

In 1997, there was a significant effect of destination site and seed origin on traits related to adult lupine size across the BMR (Table 2). Adult size also varied depending on the site of the garden, the plant's origin, its maternal parent, and the block into which plants were assigned. There was a greater success of plants when growing in their home environment.

Flowering duration across BMR was affected by the destination garden, the origin of the plant, block, and maternal parent (Table 2). The site × origin interaction (Table 2) resulted from DUN and BS plants flowering earlier in their home environment.

Paternal effects were detected among the plants from all sites within the Mussel Point garden, indicating narrow-sense heritability for flowering time at this site (MS = 3.72, $F_{15,62}$ = 2.46, $P$ < 0.001). On average, flowering time was earliest at the Mussel Point site and the Dunes garden was most phenologically delayed.

Seed production across BMR depended on both genetic and environmental factors. Seed production differed between destination gardens, sites of seed origin, blocks, seed parent identity, and site × origin interactions (Table 2). Significant differences between maternal families existed for all destination gardens. Overall, families achieved relatively greater fecundity in their home site, indicating local adaptation among these three environments. In all gardens, MP families generally produced the fewest seeds, but MP families were more productive in their home garden. DUN families produced the most seed in the Dunes garden relative to other families. BS families were most fecund in the Mussel Point and Bayshore gardens.

Paternal families differed only when planted within the Mussel Point garden, indicating narrow-sense heritability for seed set at this site (MS = 13.3, $F_{15,60}$ = 1.87, $P$ = 0.045).

Mortality in 1996 varied due to the origin of the plant (MS = 0.348, $F_{2,261}$ = 10.7, $P$ < 0.0001), which may include both genetic and maternal environmental components. MP and DUN families suffered the highest juvenile mortality in all gardens, and BS families generally had the lowest juvenile mortality, especially in the Bayshore garden. Cumulative mortality of plants (1996–1997) varied depending on destination site and origin (Table 3). There was a significant site × origin interaction for mortality. By August 1997, BS families consistently had the lowest mortality in all gardens on average (8–33%), whereas MP families had the highest mor-

TABLE 3. Analysis of variance for cumulative mortality of families of *Lupinus arboreus* in 1997. Mean square and *F*-values are Type III sum of the squares.

| Source | df | MS | $F$ |
|---|---|---|---|
| Destination site | 2 | 0.90 | 25.2*** |
| Seed origin | 2 | 1.04 | 29.1*** |
| Site × origin | 4 | 0.126 | 3.53*** |
| Error | 2610 | | |

* $P$ < 0.05, ** $P$ < 0.01, ***$P$ < 0.001.

tality in the Dunes (36%) and DUN families suffered on average more than 45% mortality at Bayshore.

By analyzing traits across BMR and within destination gardens, the following general trends emerge for lupine life-history characteristics. Plants originating from MP were generally smaller, produced fewer seeds, and had higher mortality in each destination garden. In contrast, plants originating from BS were larger, had the lowest mortality, and generally produced more seeds than plants from other origins. Plants show evidence for local adaptation in two ways. First, DUN and BS plants were more fit in their home environment relative to plants from other origins. Second, MP families had the highest fitness in their home site relative to other destination gardens. The Mussel Point garden appears to be the best environment for growth, reproduction, and survival because all families had the highest canopy volume and fecundity there. The Dunes garden limits plant size, especially among MP families, but seed production is equal to and higher than seed production at Bayshore. Bayshore appears to significantly restrict size and fitness of both MP and DUN families.

## DISCUSSION

How the combined forces of natural selection, gene flow, and genetic drift influence the genetic structure of perennial plant populations remains largely unresolved (Sork et al. 1993; Stanton and Galen 1997). Our objectives were to determine the amount of genetic and morphological differentiation within and among stands of a perennial shrub and infer how the opposing forces of gene flow and natural selection may influence the patterns we observed.

### Genetic Structure

Lupine seeds sampled from the three areas within our study site exhibited little spatial subdivsion ($F_{ST} \approx 0$). The lack of genetic structure in *L. arboreus* seeds is not surprising given the plant's mixed mating system and the close proximity of lupine stands (~500 m). Outcrossed, insect-pollinated plants are predicted to have moderate to high gene flow (Loveless and Hamrick 1984; but see Hogbin et al. 1998). Montalvo et al. (1997) found that canyon live oak similarly lacked genetic subdivision and showed high levels of gene flow between populations more distantly separated than ours. In our system, seeds from Bayshore, the Dunes, and Mussel Point are likely to be genetically similar because of very low $F_{ST}$-values. Factors other than gene flow, such as balancing selection or the coarse resolution of allozyme markers, also may result

in the measured homogeneity in the seed stage among lupine stands (Bossart and Prowell 1998).

The majority of gene flow in *L. arboreus* is probably due to pollen flow because: (1) pollination occurs primarily by bees (Kittelson and Maron 2000); (2) relatively heavy lupine seeds fall close to maternal shrubs; (3) there are no seed-harvesting ants at our site; and (4) any mouse dispersal of seeds is likely to be over short distances due to the limited home ranges of mice (Maron and Simms 2001). Furthermore, based on mark-and-recapture studies, we seldom observe mice (the two mice at our site are *Peromyscus maniculatus* and *Reithrodontomys megalotis*) traveling between habitats (J. L. Maron and E. L. Simms, unpubl. data). Given the measured values of $F_{ST}$ for seeds, genetic drift appears to have little effect on microevolutionary processes in these subpopulations.

In contrast to the low level of genetic subdivision among lupine seeds, juvenile plants exhibited genetic structure that was 20 times higher ($F_{ST} = 0.041$) and significantly different from zero. Our $F_{ST}$-value for juvenile plants was higher than the mean $G_{ST}$ (0.02) for small-scale intrapopulational differences among two mixed-mating species cited by Loveless and Hamrick (1984). Thus, the level of genetic structure in our population of established plants is relatively pronounced, especially considering the small spatial scale of our study area.

By examining two life-history stages, we found that population genetic structure increased between the seed and juvenile stage. Similarly, spatial population genetic structure increased substantially during the life cycle of the perennials *Trillium grandiflorum* (Kalisz et al. 2001) and *Plantago lanceolata* (Tonsor et al. 1993). The rare annual *Clarkia springervillensis* also exhibited a fivefold increase in the $F_{ST}$-value for juveniles relative to the seed cohort (McCue and Holtsford 1998). Kalisz et al. (2001) hypothesize that increased differentiation in *T. grandiflorum* may be attributed to historical patterns of establishment and/or selection between juvenile and reproductive stages. For our system, the dramatic increase in genetic structure among juvenile lupine also may be explained by selective events that occur between seed set and stand establishment, even if selection is not directly acting on allozyme markers.

The slightly higher level of homozygosity within seeds ($F_{IS}$) compared to juvenile plants is likely a result of selfing or biparental inbreeding (Kittelson and Maron 2000). The fact that juvenile plants exhibit slightly higher levels of heterozygosity may be due to a slightly lower rate of natural germination and seedling survival for inbred versus outcrossed progeny (Kittelson and Maron 2000; P. M. Kittelson, unpubl. data) and mirrors results found by Tonsor et al. (1993).

### Sources of Variation in Lupine Life-History Traits

Reciprocal common-garden experiments demonstrated striking differentiation in life-history traits between lupine stands. Traits associated with size and fecundity varied substantially across BMR, among families from different origins, and among different maternal parents.

The high degree of phenotypic variation between plants of different origins might result from genetically based variation between plants of different origin and/or from a maternal environmental effect. Maternal environmental effects arise when site quality affects the ability of maternal plants to provision seeds. Variation in seed size caused by maternal environment can influence traits expressed in the next generation, such as seedling emergence time, rates of leaf development, and juvenile size (Michaels et al. 1988; Helenurm and Schaal 1996; Stanton and Galen 1997). Maternal environmental effects are often most pronounced early in the progeny's life history (Roach and Wulff 1987) and usually become less pronounced with age (Roach and Wulff 1987; but see Miao et al. 1991). Within and among destination sites, maternal identity accounted for significant variation in phenology and seed production. Although this could result from a significant maternal environmental effect, the following lines of evidence suggest that differences in traits between plants of different origins are likely genetically based. First, adding seed weight as a covariate in our analyses did not substantially alter the effect of seed origin and maternal family on measured traits. Second, seeds from Bayshore had the lowest average seed weight relative to the other two origins (0.037 g vs. 0.043 g for DUN and 0.040 g for MP), despite the fact that plants from this seed source were generally larger and more fit than plant families that had heavier seeds. Finally, an effect of maternal parent did not decline over the three years of this study, as one might expect if maternal environmental effects were responsible for differences in traits.

Effects of maternal and paternal family, indicative of genetic variation within source subpopulations, were most often detected at Mussel Point. For example, paternal effects occurred primarily within the Mussel Point garden and rarely explained the variation in viability and fecundity at other sites. Our inability to detect family effects within the other two gardens may be a result of the smaller sample sizes due to higher mortality, hence lower statistical power. In the first two years, plants at Dunes and Bayshore suffered considerably more mortality than at Mussel Point. Consequently, there were fewer representatives at these sites to test the effect of family identity on plant traits. Moreover, the total number of maternal and paternal half-sibling families that were created in each origin was relatively low, especially for confidently detecting paternal effects.

As a whole, we found substantial differentiation in adaptive traits between lupine subpopulations, despite the fact that gene flow is probably not restricted. Therefore, it seems likely that strong selection has overcome the homogenizing influence of gene flow and produced site-specific patterns in adaptive traits of lupine. The precise selective agents responsible for producing the striking local differentiation that we observed remain to be identified, but likely involve a combination of edaphic and biotic pressures unique to each area. Measured variation in lupine life-history traits may be adaptive for site-specific selective factors such as high herbivory at Bayshore or stressful edaphic factors at the Dunes or Mussel Point (Kittelson 1998; Maron 1998; Maron et al. 2001).

### Variation in Environmental Quality among Sites

Survival and reproduction of families varied among different destination gardens. As has been found for other spe-

cies (Mazer and Schick 1991), local environment influenced the expression of life-history traits. All families, regardless of origin, had the greatest fitness at the Mussel Point garden. Thus, the Mussel Point site was more favorable for growth and reproduction than other sites. Plants were smaller and more phenologically delayed at the Dunes and plant fitness was lowest at Bayshore. Within a destination garden, there was greater block-to-block heterogeneity in plant performance at grassland sites compared to the dunes. Thus, at a microgeographic scale, grassland habitats appear more finely grained than do dunes.

Greater environmental heterogeneity in grasslands may explain why MP families performed poorly even at their home site. The Mussel Point common garden was located approximately 100 m from where plants were originally crossed. This spatial separation may mean that the Mussel Point garden was not truly a "native site" for MP families.

Families from some origins expressed considerable genetic variation in phenotypic plasticity. For example, plants derived from Bayshore and the Dunes performed better at the high-quality Mussel Point destination than they did at their home garden. Source-sink arguments (Turelli 1997) suggest that adaptation to favorable sites will be more likely than adaptation to poorer sites. Stanton et al. (1997) found that *Ranunculus adoneus* seeds from high-quality sites were most fit, tending to cause gene flow from high- to low-quality sites. But our results seem to suggest the opposite; BS and DUN families were derived from sites that are least favorable, yet families from these origins were adapted to their local conditions, whereas MP families generally had the lowest relative fitness of families at their home site.

### Local Adaptation

We detected a highly significant site × origin effect on expression of plant traits in our MANOVA, indicating that the effect of planting location varied among plants of different geographic origin. In addition, our test of performance in native sites versus nonnative sites indicated that on average, families expressed local adaptation for juvenile and adult size, flowering time, and seed production. In most instances, the relative fitness of plants often was greatest for progeny from that locality. Sork et al. (1993) found evidence for local adaptation in *Quercus rubra* for resistance to folivores in spite of high levels of gene flow across microhabitats. However, other studies have detected no evidence for adaptation to microsites (Cheplick 1988; Rice and Mack 1991; Stanton and Galen 1997). Helenurm (1998) also found no local adaptation in populations of *Lupinus guadalupensis*, but showed that some populations were more fit than others, regardless of the planting site. We similarly found that some lupines (e.g., BS) had the highest fitness relative to others, regardless of planting site.

### Conclusions

Examining multiple life stages can elucidate ecological and evolutionary processes shaping spatial genetic structure. Moreover, reciprocal common-garden experiments can reveal differentiation of life-history traits and local adaptation. Based on low seed $F_{ST}$-values, it appears that gene flow is likely high among the geographically proximate Bayshore,

Dunes, and Mussel Point areas. Yet, juvenile plants from these same areas exhibited a dramatic 20-fold increase in genetic structure. Lupine from different subpopulations also exhibited distinct phenotypic differences that are caused by the interaction of both genetic and environmental factors. Common-garden experiments demonstrated that heritable, genetically based differences in plant traits exist for plants from the three sites. Lupine displayed local adaptation, as evidenced by generally larger stature and greater flower and seed production in their site of origin. Taken together, our results are consistent with the hypothesis that strong spatially varying selection regimes are responsible for the formation of genetic structure in the population of lupines we studied. Strong differentiation of life-history traits over such a small geographic area is somewhat unexpected for a perennial outcrossing species.

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## APPENDIX

Summary of $F$-statistics for *Lupinus arboreus* seeds and juvenile plants at the Bodega Marine Reserve. Mean $F$-statistics are presented for individual loci and for a weighted multilocus mean. *Me* and $Tap_3$ were fixed for the juvenile plants. Significance from zero is denoted by $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

| | Seeds | | | Juveniles | | |
|---|---|---|---|---|---|---|
| | $F_{IS}$ | $F_{IT}$ | $F_{ST}$ | $F_{IS}$ | $F_{IT}$ | $F_{ST}$ |
| $Adh_1$ | 0.282 | 0.272 | −0.013 | 0.088 | 0.124 | 0.040** |
| $Adh_2$ | −0.006 | −0.016 | −0.009 | 0.000 | 0.000 | 0.000 |
| $Est$ | 0.164 | 0.156 | −0.009 | 0.217 | 0.2472 | 0.039** |
| $Idh$ | −0.0411 | −0.047 | −0.006 | 0.204 | 0.202 | −0.002 |
| $Me$ | 0.664 | 0.656 | −0.015 | — | — | — |
| $Pgi_1$ | 0.179 | 0.233 | 0.066*** | 0.398 | 0.462 | 0.106*** |
| $Pgi_2$ | 0.246 | 0.235 | −0.015 | −0.066 | −0.038 | 0.027* |
| $Pgm$ | −0.032 | −0.043 | −0.011 | 0.091 | 0.095 | 0.004 |
| $Tap_2$ | 0.240 | 0.233 | −0.009 | 0.125 | 0.128 | 0.005 |
| $Tap_3$ | −0.024 | −0.015 | 0.006 | — | — | — |
| Multilocus mean | 0.204 | 0.206 | 0.002 | 0.178 | 0.212 | 0.041*** |