

SPATIAL DIFFERENTIATION FOR FLOWER COLOR IN THE DESERT ANNUAL *LINANTHUS PARRYAE*: WAS WRIGHT RIGHT?

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Understanding the evolutionary mechanisms that contribute to the local genetic differentiation of populations is a major goal of evolutionary biology, and debate continues regarding the relative importance of natural selection and random genetic drift to population differentiation. The desert plant *Linanthus parryae* has played a prominent role in these debates, with nearly six decades of empirical and theoretical work into the causes of spatial differentiation for flower color. Plants produce either blue or white flowers, and local populations often differ greatly in the frequencies of the two color morphs. Sewall Wright first applied his model of “isolation by distance” to investigate spatial patterns of flower color in *Linanthus*. He concluded that the distribution of flower color morphs was due to random genetic drift, and that *Linanthus* provided an example of his shifting balance theory of evolution. Our results from comprehensive field studies do not support this view. We studied an area in which flower color changed abruptly from all-blue to all-white across a shallow ravine. Allozyme markers sampled across these regions showed no evidence of spatial differentiation, reciprocal transplant experiments revealed natural selection favoring the resident morph, and soils and the dominant members of the plant community differed between regions. These results support the hypothesis that local differences in flower color are due to natural selection, not due to genetic drift.

KEY WORDS: Cline, flower color, genetic drift, isolation by distance, *Linanthus* natural selection, polymorphism, Sewall Wright, shifting balance.

The relative importance of natural selection and random genetic drift has been the subject of debate in evolutionary biology since the field of population genetics was first established. Is natural selection alone sufficient to fix new adaptive mutations, as suggested by Fisher (1930), Haldane (1932), and Mayr (1942), or does random genetic drift also play an important role? The latter view was espoused by Sewall Wright, who proposed that random genetic drift reorganizes the genetic makeup of a population, creating new variation through epistatic interactions that is then subject to natural selection (Wright 1931, 1978). Wright suggested that genetic drift might often result from restricted gene flow in species

with continuous spatial distributions. Such genetic differentiation of populations, or “isolation by distance” (Wright 1943a), was the first step in his shifting balance theory of evolution. In the shifting balance, populations traverse adaptive valleys to move from lower to higher adaptive peaks in a stepwise process initiated by random genetic drift, followed by natural selection within demes and then interdemic selection. Wright’s shifting balance theory has received considerable theoretical attention (Crow et al. 1990; Crow 1991; Barton 1992; Goodnight 1995), but remains controversial (Coyne et al. 1997; Whitlock and Phillips 2000).



Figure 1. Blue- and white-flowered plants of *Linanthus parryae* from the Mojave Desert near Pearblossom, California, USA. D. W. Schemske.

The most thorough examination of the shifting balance in nature to date was based upon studies of a flower color dimorphism in *Linanthus parryae*, an annual plant of the Mojave Desert in California. Individuals of *L. parryae* produce either white or blue flowers (Fig. 1), and flower color is controlled mainly by a single gene, with blue dominant to white (Epling et al. 1960). Most populations are nearly monomorphic, with predominantly white-flowered populations more common than blue-flowered populations; some populations maintain intermediate frequencies of the two color morphs (Epling and Dobzhansky 1942; Schemske and Bierzychudek 2001). Field observations and theoretical analyses were conducted in the mid to late 1900s by such influential evolutionary biologists as Theodosius Dobzhansky, Harlan Lewis, and Sewall Wright, with the primary goal of assessing the relative importance of genetic drift and natural selection to the spatial distribution of the color morphs. Four contributions were published on this system, three supporting genetic drift as the primary evolutionary mechanism (Epling and Dobzhansky 1942; Wright 1943b, 1978), and one concluding that natural selection was of main importance (Epling et al. 1960). Below we provide a brief overview of these studies, as they provide a historical perspective and motivation for the work described in the present article.

After conducting an extensive survey of flower color in 1941, Epling and Dobzhansky (1942) first concluded that the spatial distribution of color-morph frequencies in *L. parryae* was consistent with genetic drift. Shortly thereafter, Wright reanalyzed their data using his newly developed inbreeding coefficients (1943a) to estimate the effective size of *L. parryae* populations, and concluded that the spatial distribution of flower color was indeed consistent with a model of isolation by distance (Wright 1943b). Epling and his colleagues continued to study *L. parryae* over the next two decades. To determine whether the relative frequencies of the two

flower morphs varied temporally, as might be expected as a result of genetic drift, Epling et al. (1960) established a 0.5 miles \times 10 ft transect in an area in which both color morphs were present. They censused the numbers of the two morphs along this transect in 10 of the 15 years from 1944 to 1958, excluding dry years when *Linanthus parryae* was absent or rare. The frequencies of the two color morphs were remarkably stable over this 15-year sampling interval. These results led Epling and colleagues to reverse the earlier view held by Epling and Dobzhansky (1942) and by Wright (1943b) that genetic drift was responsible for the spatial pattern of flower color in *L. parryae*. Instead they concluded that “. . . if genetic drift has played a role, it has been of only local consequence and not persistent in its effects” (Epling et al. 1960, p. 254). Nearly two decades after the publication of their paper, Wright published a final reanalysis in which he again supported the role of drift, stating that “in the main, the changes [in allele frequencies over time] were clearly of the nature of random drift” (Wright 1978, p. 222).

Linanthus parryae has contributed substantially to the development of modern evolutionary theory. In his biography of Sewall Wright, Provine (1986, p. 485) concluded that *L. parryae* was Wright’s “. . . first and best example of isolation by distance, a theory that Wright considered to be one of his most important contributions to evolutionary biology.” Yet Wright had no direct evidence that genetic drift was responsible for the spatial pattern of flower color, and Epling and colleagues provided no direct evidence that natural selection was at work. The inferential nature of these investigations and analyses was insufficient to determine the relative importance of genetic drift and natural selection in this system.

To resolve this debate, in 1988 we embarked on a long-term study of *L. parryae*. In our first paper reporting on the results of this work, we showed that flower color in dimorphic populations is often subject to intense, temporally varying selection (Schemske and Bierzychudek 2001). This study demonstrated that Wright greatly underestimated the role of natural selection in the evolution of *L. parryae*. In the present article, we continue to examine the relative importance of selection and drift, this time focusing our attention on the evolutionary mechanisms that contribute to spatial differentiation in the frequency of the two color morphs. Specifically, we investigate the cause of a steep, local cline for flower color in *L. parryae*. We identified an area in which flower color changed abruptly across a shallow ravine (Fig. 2A), with predominantly blue-flowered plants on the west side (Fig. 2B), and predominantly white-flowered plants on the east side (Fig. 2C). Epling et al. (1960) proposed that such striking local differences in flower color were the result of “an intense local selection because the blues are concentrated in certain areas and because persisting clines of blue and white frequencies have been found” (p. 254), whereas Wright concluded that such

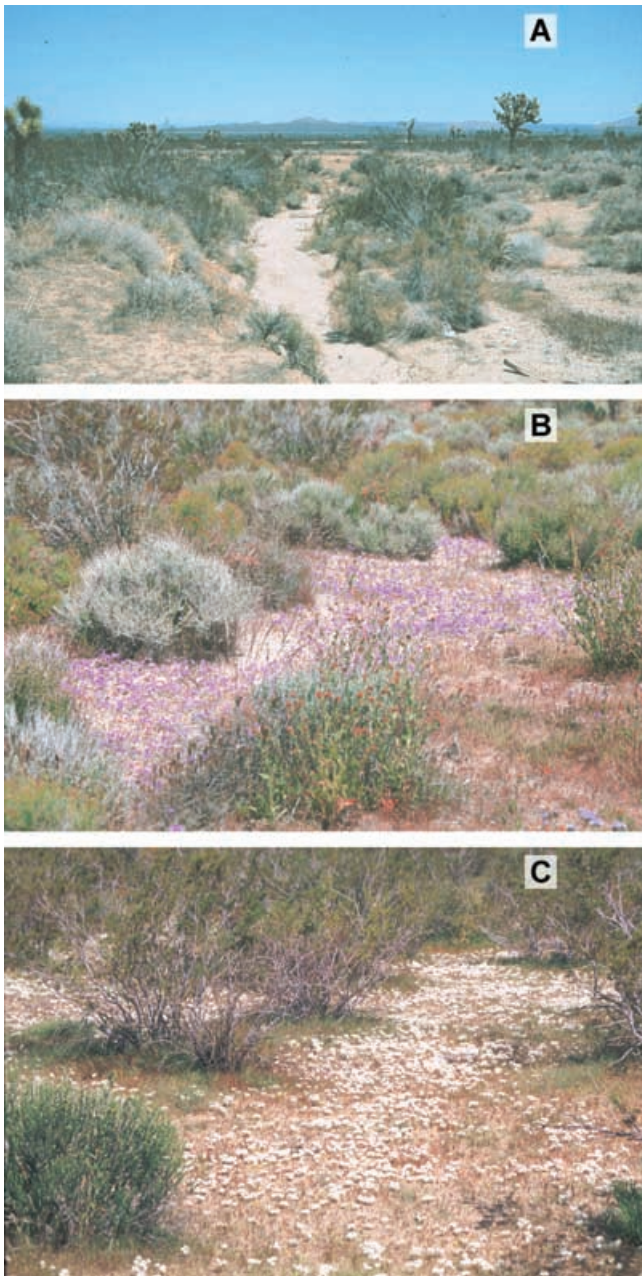


Figure 2. Shallow ravine oriented along a north–south axis in the Mojave Desert (A), which marks the transition from primarily blue-flowered plants on the west side of the ravine (B), to primarily white-flowered plants on the east side (C).

differentiation was caused by “extreme amounts of sampling drift” (Wright 1978, p. 222). Here we examine these alternative views.

We first documented the spatial pattern of flower color by extensively sampling the region in which the frequencies of the two color morphs display a sharp, local shift. We then conducted observations and experiments to test three null hypotheses, which, if rejected, would refute Wright’s conclusion that random genetic drift is the primary cause of spatial differentiation for flower color. We describe those hypotheses below.

If the spatial pattern were due primarily to random genetic drift, as envisaged by Wright, one would expect the same pattern of differentiation for other genetic characters. Thus, our first null hypothesis is that loci distributed throughout the genome should show the same clinal pattern as we observe for flower color. To test this, we quantified the spatial pattern and degree of genetic differentiation for allozyme markers.

Although Wright acknowledged that selection on flower color played a role over broad geographic regions, he believed that it was unimportant on a local scale. This motivates our second null hypothesis that there is no significant difference in the performance of blue- and white-flowered plants within the observed cline. To this end, we established experimental plots along the cline that contained seeds from both blue- and white-flowered plants. We then compared the seed production of the color morphs in two flowering seasons.

For selection to favor different colors on opposite sides of the ravine, these areas must present different selective environments. Therefore, our third null hypothesis is that there are no ecological differences between the blue- and white-flowered regions. To test this hypothesis we conducted a survey of the perennial plant community and of soil properties in the two regions.

Materials and Methods

THE STUDY SYSTEM

Linanthus parryae is a winter annual native to the Mojave Desert in California (Patterson 1993). Plants produce either blue or white self-incompatible flowers that are pollinated exclusively by a single species of Melyrid beetle, *Trichochorous* sp. (Schemske and Bierzychudek 2001). Seeds germinate after winter rains in January and February, plants flower in early to late April, and the passively dispersed seeds are shed in late May to early June. In dry years there is no seed germination (Epling et al. 1960; Schemske and Bierzychudek 2001). Seeds can remain dormant in the soil for at least seven years (Epling et al. 1960). Mean seed production per plant varies greatly between years, as a result of annual differences in spring rainfall (Schemske and Bierzychudek 2001).

We conducted this work north of the San Gabriel Mountains in Los Angeles County, CA, less than 4 km from the location originally censused by Epling and his colleagues (H. Lewis, pers. comm.). That original site was destroyed by the construction of the Pearblossom Pumping Plant. Our study area was located along Avenue V, a dirt road between 96th St. E. and 106th St. E., 4 km west of Pearblossom (elevation 900 m), midway between the towns of Pearblossom and Littlerock. This creosote bush desert includes a variety of other perennial shrubs, grasses, and ephemeral annuals. In wet years, *L. parryae* carpets the open areas among the shrubs.

This area is incised by a series of north–south desert washes or ravines created by runoff from the foothills of the San Gabriel

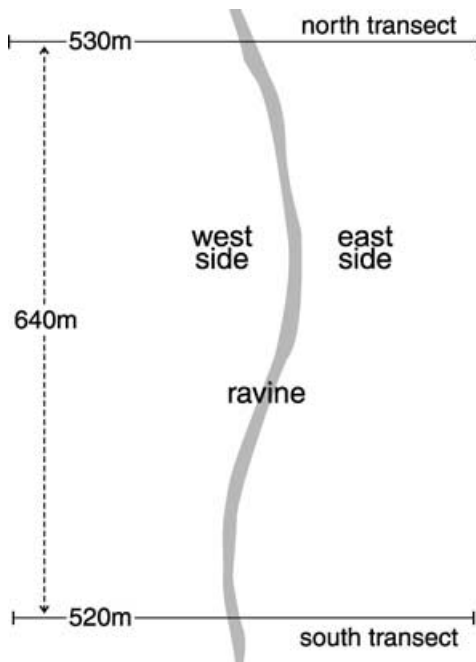


Figure 3. Study area and sampling transects.

Mountains. One such ravine bisects this area into two regions with strikingly different patterns of *L. parryae* flower color. On the west side of the ravine, nearly all the *L. parryae* have blue flowers; on the east side, nearly all flowers are white. The ravine itself is 20–30 m wide, and has sloping, sandy banks from 1 to 2 m high. No *Linanthus* grow in the unstable sand at the bottom of the ravine. On each side of the ravine for at least 300 m to the east and west, *L. parryae* plants are present and virtually monomorphic for flower color; eventually plant density declines to a few sparse individuals. This area was the focus of our study. The west side of the ravine was recently planted with orchard trees, and the *Linanthus* population there is now gone.

WHAT IS THE SPATIAL PATTERN OF FLOWER COLOR?

We obtained data on flower morph distributions by establishing two permanent, parallel transects, 640 m apart, running east–west, perpendicular to the ravine (Fig. 3). Each transect was 4-m wide and extended approximately 250 m in each direction from the edges of the ravine. We performed censuses each year from 1992 to 1998 during the period of peak flowering (mid-April). At each census, the number of blue- and white-flowered plants was determined in 2 m × 2 m subplots, giving approximately 2000 such plots each year, sampled from a total area of approximately 4000 m². To simplify data analysis and graphical representation, we pooled census data for blocks of adjacent subplots to give approximately 100 4 m × 10 m plots. For all years combined, >100,000 plants were counted.

To estimate the shape of the flower color distribution across the ravine, we fit the data to a one-dimensional model of a cline

using the “Transect, fit 1D cline” option in Analyse 1.3 (Barton and Baird 1998). Although designed principally for the analysis of genetic data, this procedure can also be applied to phenotypic traits with complete dominance (Barton and Baird 1998), as is the case for flower color in *L. parryae* (Epling et al. 1960; Wright 1978). The program fits the data using six parameters describing different aspects of cline shape. We used the Metropolis algorithm (Barton and Baird 1998) to generate combinations of the six parameters, stopping when the estimated parameter values and the log likelihood of the fit approached a constant value, typically following 2000 combinations. The census data for north and south transects were run individually, with five replicate runs for each. The estimated parameter values and log likelihoods were very similar across replicates. Following the procedure outlined in Phillips et al. (2004), we generated the likelihood surface for cline center and cline width for both north and south transects. In brief, for each parameter we began with the value giving the smallest log likelihood, then ran the cline-fitting procedure at 5-m intervals in both directions along the transect, leaving all other parameters free to vary at each transect position.

We estimated the degree of differentiation for flower color between east side and west side populations (F_{ST}) using WinBUGS version 1.4.2 (Spiegelhalter et al. 2003). WinBUGS uses a Bayesian approach to analyze a variety of complex statistical models using Monte Carlo Markov Chain techniques. We used WinBUGS code written by Kent Holsinger to estimate mean F_{ST} and 95% credible intervals (C.I.), the Bayesian equivalent of confidence intervals. Unlike other approaches for estimating F_{ST} from dominant genetic markers, this method does not require knowledge of the inbreeding coefficient and does not assume that the population is in Hardy–Weinberg equilibrium (Holsinger et al. 2002). We performed two separate west-to-east comparisons, one for each of the north and south transects.

TESTING THE HYPOTHESES

Do allozyme markers show the same spatial differentiation as flower color?

To determine whether allozyme markers showed a clinal pattern of differentiation similar to the one we observed for flower color, we collected tissue from flowering plants in mid-April 1993 from a region parallel to each permanent transect. Samples were taken from 50 m, 150 m, and 250 m to the east and west edges of the ravine. Sampling regions were located parallel to the two permanent transects described above; six were approximately 50 m south of the south transect and six were 50 m north of the north transect. From each of these 12 regions we collected at least 80 individual plants.

We froze the material at -70°C after collection, and later extracted the enzymes from the flower buds of 60 individuals from each of the 12 sampling regions using a version of

Ritland's *Mimulus* grinding buffer (K. Ritland, pers. comm.). We chose enzyme systems that were polymorphic and easily resolved: 6-phosphoglucoisomerase (6-PGD), isocitrate dehydrogenase (IDH), phosphoglucomutase (PGM), and phosphoglucoisomerase (PGI). All were assayed with 12.0% starch gels and a morpholine-citrate buffer, pH 6.1, run for approximately 9 h at a constant amperage of 50 mV (Werth 1985; Wendel and Weeden 1989; Kephart 1990). We calculated multilocus estimates of population differentiation (F_{ST}) for allozymes using HICKORY version 0.8 (Holsinger and Lewis 2003). HICKORY uses a Bayesian approach to estimate mean F_{ST} and 95% C.I. using Monte Carlo Markov Chain simulation (Holsinger et al. 2002; Holsinger and Wallace 2004). We performed two separate west-to-east comparisons, one for each of the north and south transects.

To compare the distribution of allozyme variation across the cline with that of flower color variation, we fit the frequencies of the most common allele at each locus to a one-dimensional model of a cline using the "Transect, fit 1D cline" option in Analyse 1.3 (Barton and Baird 1998), following the same procedure as we used for flower color. We carried out a separate analysis on each of the two transects.

Do blue- and white-flowered individuals have similar performance?

To quantify the performance of blue- and white-flowered individuals, in 1992 we established experimental plots containing both blue and white seeds, and compared the seed production of the color morphs in two flowering seasons. For this experiment we established six 4 m × 4 m plots on each side of the ravine, three of them 50 m north of the north transect, and three 50 m south of the south transect; all plots were separated from one another by at least 50 m. Because *L. parryae*'s spatial distribution is often heterogeneous, we did not place these plots randomly, but rather located them in areas that had medium-to-high *Linanthus* densities. Before manipulating the plots, we censused the numbers of individuals of each flower color in late April 1992. For the six plots on the west side of the ravine, the mean percentage of white-flowered plants was 1.8 (range: 0–3.7%); for the six plots on the east side, the mean percentage of white-flowered plants was 97.2 (range: 93.5–100%).

Because *L. parryae* produced extremely few seeds in 1992, we were unable to create the experimental plots by sowing them with seed produced by blue- and white-flowered individuals that year. Instead, we took advantage of *Linanthus*' long-lived seed bank to establish the treatments. We removed the top 1 cm of soil from each of the experimental plots, to recover the bulk of the seed bank (Epling and Dobzhansky 1942). Measurements of plants in both regions revealed that roots extend far below this level (mean root length = 7.2 cm), so it is unlikely that we substantially altered the soil environment experienced by plants in the plots. Then we

mixed the soil from the three experimental plots from each side of each transect, to create four pooled soil mixtures, one for each side and transect. Keeping soils from the north and south transects distinct, we added mixed soil to each experimental plot, along with its dormant seeds. Because we anticipated that our soil removal procedure probably left some seeds of the resident color in each plot, we restored soil (and seeds) to each experimental plot in a 3:1 transplant: resident ratio by volume.

In April 1993, we again censused numbers of blue- and white-flowered individuals to ascertain if transferring soil had brought about the desired effect. The mean percentage of white-flowered plants in plots on the west (blue) side of the ravine was 32.1 (range: 17.9–46.7%); that for plots on the east (white) side was 54.6 (range: 38–65.2%). However, overall plant density in 1993 was very low. Instead of measuring seed production by these few individuals, we added seeds collected from all-blue and all-white areas to each plot to increase the size of the total seed pool (650 seeds of each color per plot), and also selectively thinned some plots to bring the color ratios closer to 1:1.

By 1995 and 1996, the next years with significant *Linanthus* germination, the experimental plots appeared indistinguishable from the surrounding area, except for their mixture of *Linanthus* color morphs. In 1995, the mean percentage of white-flowered plants in plots on the west side of the ravine was 31.0% (range: 21.3–37.5%); that for plots on the east side was 55.7% (range: 40.9–65.2%). In 1996, the mean percentage of white plants in west side plots was 33.4% (range: 21.3–45.8%); that for east side plots was 54.4% (range: 31.2–75.0%). At the time of peak flowering in 1995 and 1996, we marked pairs of blue- and white-flowered plants, matching randomly chosen plants with their nearest neighbor of the opposite color. We marked plants by placing individually numbered plastic stakes next to each one. The distance between paired plants was never greater than 1 m, and typically was on the order of a few centimeters. We collected the fruits at maturity and counted the total seeds per plant. We then returned the seeds from each plant to the exact position where they were collected, still marked by its plastic stake. Seeds were returned as soon as possible after counting, usually in July of that year. We marked 464 and 465 pairs of plants in 1995 from the west and east sides, respectively. In 1996, a year of much lower plant density, we marked 65 and 53 pairs from the west and east sides, respectively. We combined the data from all plots on the same side of the ravine to create a single east–west comparison following the results of ANOVA showing no significant effect of transect or of plot nested within transect on the difference in seed number between pairs in either year. We analyzed these data using two-tailed, Wilcoxon paired-sample tests.

The two years differed substantially in rainfall patterns, with much greater precipitation in 1995 than in 1996. January precipitation, which is strongly correlated with plant density

(Schemske and Bierzychudek 2001), was 10.46 cm (1995) and 0.74 cm (1996), as compared to the median of 2.49 cm observed for the period 1941–1987 (Schemske and Bierzychudek 2001). The combined precipitation for March and April, which is strongly correlated with mean seed production (Schemske and Bierzychudek 2001), was 6.05 cm (1995) and 1.07 cm (1996), as compared to the median of 3.86 cm observed for the period 1941–1987. Thus, the two years of our experiment include one “wet” year (1995) and one “dry” year (1996), and provide the opportunity to compare absolute and relative seed production as a function of precipitation. Such a comparison is of interest given our previous findings from observational studies that the white morph is favored in wet years and the blue morph is favored in dry years (Schemske and Bierzychudek 2001).

Are the two sides of the ravine ecologically similar?

Any ecologically meaningful differences between the east and west sides of the ravine should be reflected in the species richness and/or relative abundance of plant species in the community. To test whether such differences existed, we surveyed the composition of the plant community in mid-April 1994. Every 10 m along the two permanent transects used for the census of flower color distribution, we established a perpendicular 2 m × 10 m sampling strip, with 26 strips along each of the four transect combinations (northwest, northeast, southwest, and southeast; Fig. 3). The strips ran alternately either northward or southward from the permanent transect. We subdivided each strip into 10 2 m × 2 m sections, and within each section, we estimated percent cover (to the nearest 5%) of the 21 most common species of perennial shrubs and grasses. One observer made all the estimations. The total sampling area along each transect was 1040 m². For each sampling strip we calculated the mean species richness and the mean percent cover for the 10 most common species in the total sample, defined as having mean cover >0.5%.

To further explore the possibility that the marked spatial pattern in the frequency of the flower color morphs is associated with spatially patterned ecological factors, we collected soil samples from the two sides of the ravine on 20 April 1998. We obtained five samples from each of the four transect locations (northwest, northeast, southwest, and southeast) at approximately 50-m intervals, for a total of 20 samples. At each sampling site, we pooled soil from three separate collections taken from the vicinity of *L. parryae* plants. All samples were air dried and sieved through a 2-mm mesh screen. To estimate soil-cation concentrations, samples were subjected to sodium acetate extraction and analyzed by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS; Salt 2004). In addition, we estimated pH and electrical conductivity (EC), percentage organic matter (%OM), NH₄-N, and NO₃-N, using standard methods (Page et al. 1982). Statistical analyses were conducted on 10 soil factors that are often used in comparisons

of soil composition (T. Loecke, pers. comm.), including six soil cations (Ca, K, Mg, Na, P, and S), pH, EC, %OM, and the sum of NH₄-N and NO₃-N.

We analyzed community and soil composition by MANOVA, with mean percent cover of the 10 most common plant species or the 10 soil factors as the dependent variables; and transect (north vs. south), side of ravine (west vs. east), and the transect × side interaction as model effects. An arcsine square-root transformation of percent cover was used to improve the distribution of the residuals. We then used ANOVA with transect, side of ravine and the transect × side interaction as model effects to analyze (1) the mean percent cover (arcsine transformation) for each of the 10 most common plant species, (2) the mean species richness per sampling strip, and (3) each of the 10 soil factors. All analyses were run using the FIT MODEL procedure in JMP 5.0.1a (SAS Institute, Cary, NC). For the MANOVA, we used the “Identity Matrix” option, which provides a comparison of each separate response variable.

Results

WHAT IS THE SPATIAL PATTERN OF FLOWER COLOR?

There was striking spatial differentiation in the frequency of the two color morphs, with 2.8% white-flowered plants on the west side of the ravine as compared to 97.3% white-flowered plants on the east side. Plant density on the predominantly white-flowered, east side of the ravine was approximately threefold greater than that on the predominantly blue-flowered west side (east—74,553 plants; west—25,947 plants; Appendix). As we observed in our previous study (Schemske and Bierzychudek 2001), there was substantial year-to-year variation in plant abundance, with two years of high density (1995—45,055 plants; 1998—40,282 plants), two years of intermediate density (1992—10,646 plants; 1993—3801 plants), and three years of low density (1994—9 plants; 1996—707 plants; 1997—0 plants). Nevertheless, the morph frequencies were remarkably stable. For the four years of high or intermediate plant densities, the frequency of white-flowered plants on the west side varied from 1.8 to 3.1%, whereas the frequency of whites on the east side varied from 96.4 to 97.8%. Sample sizes in the three years of low density were too small to give reliable estimates of morph frequency. The yearly totals and the percentages of each color morph are given in the Appendix.

Plant densities were greater in the north transects on both sides of the ravine, with 14,771 and 11,176 plants in the north and south transect, respectively, on the west side, and 43,844 and 30,709 plants in the north and south transects, respectively, on the east side. For the four years of high or intermediate plant densities, morph frequencies were very similar in the north and south transects. On the west side of the ravine, the mean frequency of white-flowered plants was 1.6% on the north transect and 3.5%

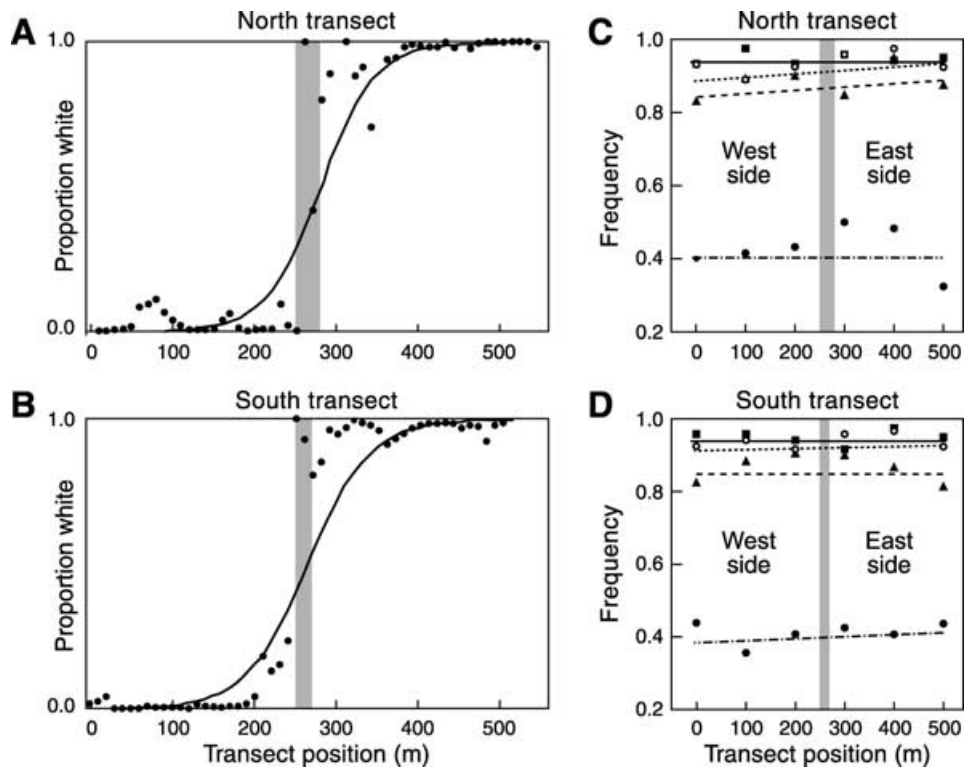


Figure 4. Spatial pattern of flower color along the north (A) and south (B) transects, and estimates of allozyme frequencies from stations located along the north (C) and south (D) transects. The vertical bar near the center of each panel marks the position of the ravine that divides the population into west and east sides (see Figs. 2A, 3). The flower color data were obtained from censuses conducted in $2\text{ m} \times 2\text{ m}$ plots from 1992 to 1998. The data shown here are for $4\text{ m} \times 10\text{ m}$ plots derived from pooling adjacent subplots, and the spatial position of each datapoint in the figure is the midpoint for each pooled plot. The data for both flower color and allozyme frequency were fit using Analyze 1.3 (Barton and Baird 1998). Allozymes sampled (C, D) are: IDH (■, solid lines), PGI (●, dashed-dotted lines), PGM (○, dotted lines) and 6-PGD (▲, dashed lines).

in the south transect, and in the east side the mean frequency of white-flowered plants was 97.6% in the north transect and 96.4% in the south (data from Appendix).

The positions and shapes of the clines for flower color were remarkably similar for the north and south transects (Fig. 4A, B). The transition from primarily blue to primarily white individuals occurs over a very short distance, and coincides precisely with the location of the ravine (Fig. 4A, B). The cline-fitting procedure gave estimates of the cline center at 285 m and 270 m for the north and south transects, respectively, and estimates of cline width of 130 m and 150 m (Fig. 5).

For the north transect, F_{ST} for flower color had a mean value of 0.58 (SD = 0.15, 95% C.I. = 0.29–0.87). For the south transect, F_{ST} for flower color had a mean value of 0.53 (SD = 0.16, 95% C.I. = 0.25–0.85). The large confidence limits reflect the fact that these estimates are based on data from a single locus.

DO ALLOZYME MARKERS SHOW THE SAME SPATIAL DIFFERENTIATION AS FLOWER COLOR?

We observed a high degree of polymorphism (four to six alleles per locus) for the allozymes we analyzed. Each of the 12 sampled

sites contained between two and six alleles for each allozyme. All but two alleles were found on both east and west sides of the ravine, and the two exceptions were alleles that were present in only two of 360 individuals sampled on the east side. For each allozyme, one allele was consistently the most common one across all sites. We used the frequency of this allele for our analysis of differentiation.

In sharp contrast to the pattern we observed for flower color, none of the four allozymes showed any evidence of clinal variation (Fig. 4C, D). The absence of a clinal pattern is demonstrated by the fact that Analyze 1.3 yielded a “cline width” for these data that was typically one or more orders of magnitude larger than the entire sampling area (data not shown). The mean F_{ST} for differentiation between west and east sides of the ravine for allozymes for the north transect was 0.11 (SD = 0.10, C.I. = 0.00–0.34); F_{ST} from the south transects was 0.04 (SD = 0.04, C.I. = 0.01–0.14). These values are much smaller than those we observed for flower color. For none of the markers did we observe a greater change in allele frequency at the ravine boundary than at other transect positions (Fig. 4C, D), as might be expected if the ravine served as a barrier to gene flow.

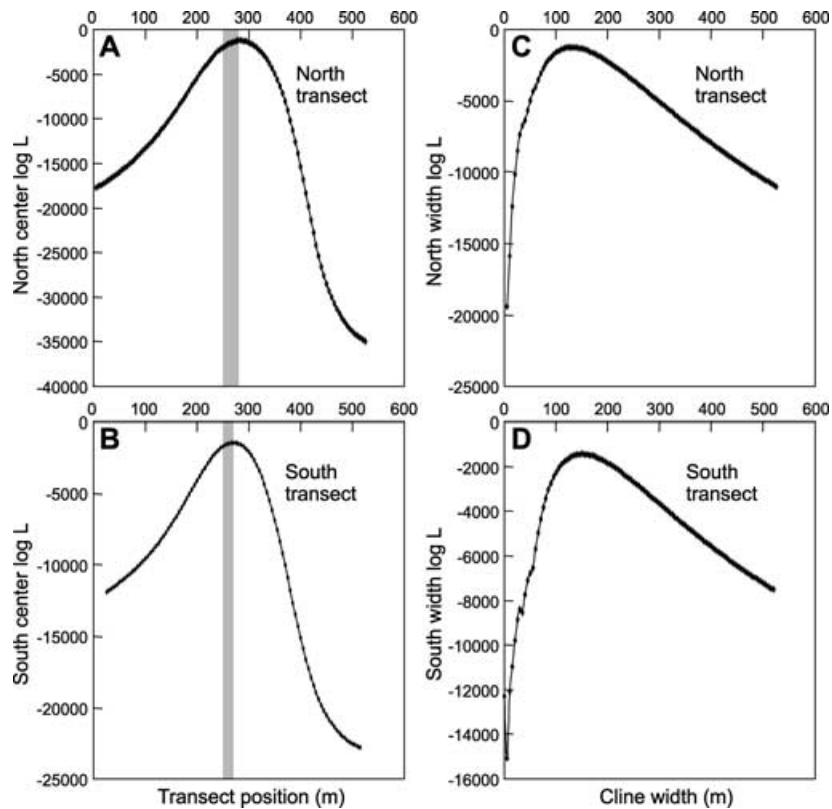


Figure 5. Log-likelihood surface for estimates of the cline center for the north (A) and south (B) transects and of cline width for the north (C) and south (D) transects, calculated using Analyze 1.3 (Barton and Baird 1998). The vertical bar near the center of the panels for cline center (A, B) marks the position of the ravine that divides the population into west and east sides (see Figs. 2A, 3).

As an additional measure of the likelihood of genetic drift, we estimated Nm , the number of migrants exchanged between populations per generation. For neutral alleles, Wright (1931) showed that $F_{ST} \approx 1 / (1 + 4Nm)$, where N is the local population size and m is the average migration rate in an “island” model (Slatkin 1985). Genetic drift will cause population differentiation only when $Nm < 1$ (Slatkin 1985). Using the multilocus estimate of F_{ST} derived for all 12 samples along the transects ($F_{ST} = 0.03$, $SD = 0.03$, 95% C.I. = 0.01–0.12), we estimate $Nm = 8.1$, providing further evidence that genetic drift is an unlikely explanation for the marked local differentiation we observed for flower color (Fig. 4A, B). This conclusion should be viewed with some caution, however, given that the assumptions for estimating Nm from F_{ST} are rarely met (Whitlock and McCauley 1999).

DO BLUE- AND WHITE-FLOWERED INDIVIDUALS HAVE SIMILAR PERFORMANCE?

Mean seed production for the reciprocal transplant experiments is illustrated in Fig. 6. In 1995, blue- and white-flowered individuals growing on the west side of the ravine (where blue plants are resident) produced nearly identical numbers of seeds per plant (blue $\bar{x} = 169.3$, $SD = 151.7$, white $\bar{x} = 169.5$, $SD = 155.9$, $n = 464$). On the east side, by contrast, the blue (nonresident) plants

produced only 77% as many seeds/plant as the white (resident) plants, a significant difference (blue $\bar{x} = 143.8$, $SD = 151.3$, white $\bar{x} = 185.9$, $SD = 176.4$, $n = 465$; Wilcoxon signed rank test, $P < 0.0001$). Additionally, plants of both flower colors produced more seeds on the side in which they were resident than on the opposite side. This difference was significant for blue-flowered plants ($P < 0.01$, $Z = 3.24$; Mann–Whitney U test), but not for white-flowered plants ($P = 0.35$, $Z = 0.94$).

In 1996, a year of much lower rainfall than 1995, *L. parryae* were much less dense and seed production levels were much lower (Fig. 6). This time, however, blue-flowered (resident) individuals growing on the west side of the ravine had 68% higher seed production than white-flowered (nonresident) individuals, a significant difference (blue $\bar{x} = 24.8$, $SD = 34.9$, white $\bar{x} = 14.7$, $SD = 30.7$, $n = 65$; Wilcoxon signed rank test, $P = 0.04$). On the east side, white-flowered (resident) individuals produced 17% more seeds/plant than nonresident blue-flowered plants (blue $\bar{x} = 21.1$, $SD = 37.2$, white $\bar{x} = 24.8$, $SD = 51.3$, $n = 53$), but this difference was not significant ($P = 0.45$; Fig. 6). Once again, plants of both flower colors produced more seeds on the side in which they were resident than on the opposite side, and this difference was significant for white-flowered plants ($P < 0.05$, $Z = 2.28$; Mann–Whitney U test), but not for blue-flowered plants ($P = 0.25$, $Z = 1.14$).

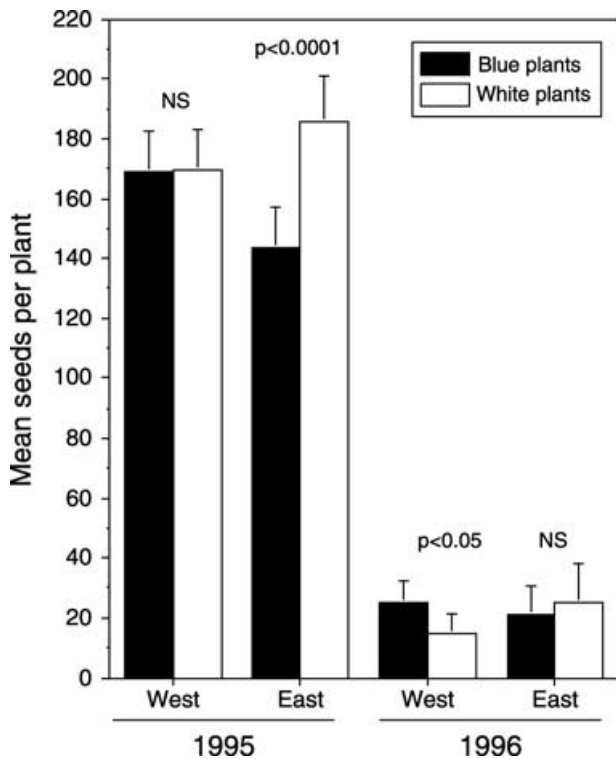


Figure 6. Mean seed production in (\pm one s.e.) 1995 and 1996 for blue- and white-flowered plants from sampling plots located on the primarily blue-flowered, west side of the ravine, and the primarily white-flowered plants, east side of the ravine (see Fig. 3).

The significant differences we observed between morphs in seed number were consistent with the observed spatial pattern of flower color. Blue-flowered plants produced significantly more seeds than whites did on the west side in 1996, and white-flowered plants produced significantly more seeds than blues did on the east side in 1995. Thus, blue- and white-flowered individuals did not have similar performance in either year, as we would expect if the observed cline were the result of genetic drift. Instead, we observed differences in reproductive success between plants with different flower colors, and the relative advantage of blue and white shifted from one side of the ravine to the other.

Is the spatial variation in selection we observed sufficient to explain this sharp, local cline in flower color? Turelli et al. (2001) found that maintaining a polymorphic population requires a negative covariance between mean seed production and relative seed number (RSN; the ratio of seed number of the two morphs). Specifically, the polymorphism is protected if the weighted average over time of RSN (weighted by the proportional contribution of that year's reproduction to the seed bank) for each morph is > 1 . If data from our reciprocal transplant experiment met these conditions, we would expect the east and west sides of the ravines to be polymorphic rather than essentially monomorphic, as we observed. Thus, we have applied this approach to our data from

the reciprocal transplant experiment at these nearly monomorphic sites.

On the east (white) side of the ravine, the weighted average RSN for the white morph for these two years was 1.29, whereas that for the blue morph was 0.77. Because the weighted average RSN was > 1 only for the white morph, conditions for maintaining the blue morph were not met on the east (white) side. This is consistent with the high frequency of white-flowered plants in this region (97.3%, Appendix). On the west (blue) side of the ravine, the weighted average RSN for the blue and white morphs were nearly identical: 0.999 for blues and 0.998 for whites. Unless this tiny difference between the blue and white morphs in this region is reflective of an actual advantage of blue over white, these data do not explain why this region is predominantly blue flowered.

The weighted average RSN is sensitive to the number of dry and wet years (Turelli et al. 2001); hence, the averages we have estimated from our reciprocal transplants would probably change if data were available from more years. To provide a rough estimate of the relative frequency of wet and dry years in the region of our nearly monomorphic populations, we used long-term weather records (1941–1998) obtained from a sampling station near our study areas (Palmdale, CA; see Schemske and Bierzychudek 2001), and data on plant performance from our long-term studies of the polymorphic Pearlblossom site 1. Here the mean combined precipitation in March and April is the best predictor of relative fitness (Schemske and Bierzychudek 2001). We observed three years of significant white advantage (1991, 1995, and 1998), where the mean March + April precipitation was 8.1 cm, and three years of significant blue advantage (1988, 1992, and 1993) with a mean precipitation of 4.5 cm. For the long-term weather data, we find that the median March + April precipitation is 3.86 cm (mean = 4.79). We obtain essentially the same value (median = 3.91) if we exclude years when winter precipitation was probably insufficient to produce flowering plants (January precipitation < 1.0 cm).

Thus, precipitation in most years is expected to favor blue-flowered plants, and our two years of reciprocal transplant data may not reflect relative performance over a longer period. This is unlikely to change our conclusion for the east (white side), where white-flowered plants outperformed blues in both years, and the difference in weighted RSN between morphs was very high. In contrast, we expect that further years of sampling would increase the estimated advantage to blue-flowered plants on the west (blue) side, as a greater proportion of dry years are normally represented than we observed in our two-year study. Because flower color is controlled mainly by a single gene, with blue dominant to white, even a small fitness advantage to blues will result in a population comprised primarily of blue-flowered plants ($> 75\%$).

In summary, the relative fitnesses estimated from our reciprocal transplant experiments support the observed spatial differences

in flower morph frequencies, particularly so for the near monomorphism of white on the east side of the ravine. However, we caution that this conclusion is based on only two years of data, and given the long-lived seed bank and the tremendous annual variation in precipitation, plant densities, and seed number, many more years of observation of reciprocally transplanted plots would be required to achieve accurate estimates of spatial differences in relative fitness.

ARE THE TWO SIDES OF THE RAVINE ECOLOGICALLY SIMILAR?

We observed substantial ecological differences between the two sides of the ravine. MANOVA (Wilks' Lambda: approximate $F_{20,267.8} = 4.29$; $P < 0.0001$) revealed that a significant fraction of the variation in percent cover of the 10 most common plant species was explained by ravine side (east vs. west) ($F_{10,91} = 11.13$; $P < 0.0001$) and by an interaction between side and transect ($F_{10,91} = 3.20$; $P < 0.01$), but there was no effect of transect (north vs. south) ($F_{10,91} = 0.79$; $P = 0.63$). ANOVA revealed that ravine side explained a significant amount of the variation ($P < 0.05$) in percent cover for nine of the 10 most common plant species; seven of these remained significant after correction by sequential Bonferroni (Fig. 7). The effect of transect was not significant for any species, and the side \times transect interaction was significant for four species (data not shown). Seven of the nine species that displayed a significant difference in abundance between the two sides of the ravine had higher percent cover on the west side. For the most part, the degree of difference between the two sides was greater for these species. For example, the mean percent cover of *Tetradymia stenolepis*, *Ericameria cooperi*, and *Salazaria mexicana* was, respectively, 9.3-, 6.1-, and 5.9-fold greater on the west side, whereas the greatest proportional difference for a species with higher abundance on the east side was the 2.2-fold greater percent cover of *Eriogonum fasciculatum*. Species richness also differed between the two sides, with a mean of 7.8 species per sampling strip ($SD = 1.94$, $N = 52$) on the west side of the ravine and 5.8 species ($SD = 1.89$, $N = 52$) on the east side. ANOVA of the mean species richness per sampling strip revealed a highly significant effect of ravine side ($F_{1,100} = 30.59$, $P < 0.0001$) and a significant side \times transect interaction ($F_{1,100} = 4.80$, $P < 0.05$), but no effect of transect ($F_{1,100} = 0.88$, $P = 0.35$).

The MANOVA conducted on 10 soil factors was significant (Wilks' Lambda: approximate $F_{30,21.2} = 1.99$; $P = 0.05$), with a significant effect of ravine side ($F_{10,7} = 8.38$; $P < 0.01$), but no effect of transect ($F_{10,7} = 1.80$; $P = 0.22$) and no side \times transect interaction ($F_{10,7} = 0.26$; $P = 0.97$). ANOVA revealed that ravine side had a significant effect ($P < 0.05$) on five of the measured soil factors; two of these remained significant after correction by sequential Bonferroni (Fig. 8).

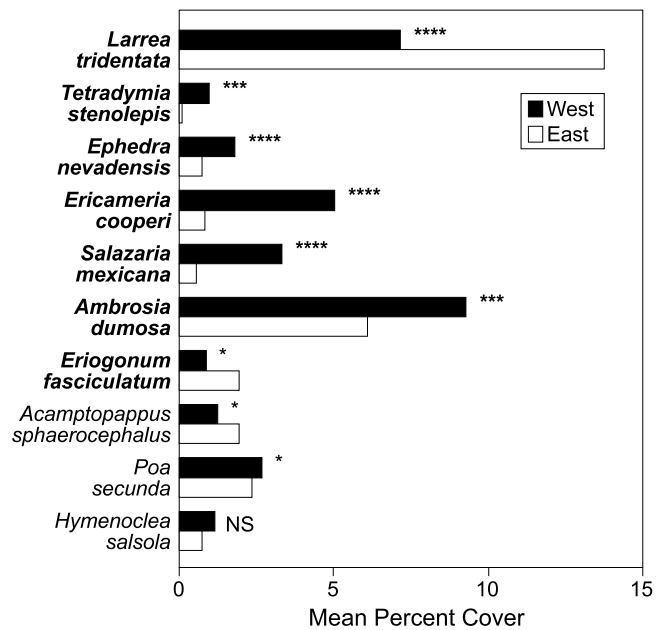


Figure 7. Mean percent cover for the 10 most common plant species on the west and east sides of the study transect (see Fig. 3). Significance levels are for the effect of ravine side (east vs. west) in an ANOVA of arcsine-transformed data with side, transect and the side \times treatment interaction. Effects for species in boldface remain significant after sequential Bonferroni correction (Rice 1989).

Discussion

Over the course of our seven-year field study, we documented a precipitous change in the frequency of flower color morphs that coincided with the location of a shallow ravine. West of the ravine, 97.2% of plants were blue flowered, whereas to the east of the ravine, 97.3% were white flowered. This pattern was not simply a local phenomenon, as the same steep cline was observed along both of our census transects, which were separated by 640 m, and the intervening areas between transects possessed essentially the same color morph frequencies as were observed within the transects (D. W. Schemske and P. Bierzychudek, pers. obs.).

There are two possible explanations for this spatial pattern of flower color. First, the ravine may pose such a strong barrier to gene flow that it maintains spatial differences in flower morph frequencies that were created initially by genetic drift. This explanation is consistent with Wright's view that local differences in the flower color of *L. parryae* were due mainly to the effects of genetic drift (Wright 1943b, 1978). Alternatively, the differences may have been both created and maintained by natural selection favoring a different flower color morph on each side of the ravine. This explanation corresponds to the conclusions eventually supported by Epling et al. (1960) in their empirical studies.

To distinguish between these possibilities, we posed three null hypotheses that, if disproven, would refute Wright's conclusion that the spatial pattern of flower color differentiation in

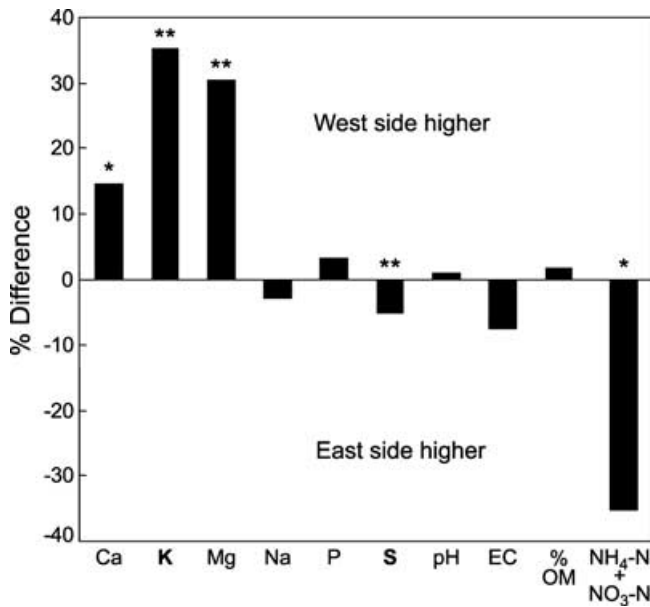


Figure 8. Differences in soil composition between the primarily blue-flowered, west side of the ravine, and the primarily white-flowered, east side of the ravine. Samples were obtained from stations located along the north and south transects, on each side of the ravine. Calculations of the percent difference are based upon the least squares means from a two factor ANOVA with transect, side and the interaction as main effects. Significance levels are for the effect of ravine side (east vs. west). Differences for those soil factors in boldface remain significant after Bonferroni correction (Rice 1989).

L. parryae was the product of isolation by distance. The first such hypothesis was that neutral markers would show the same pattern of spatial differentiation as was displayed by flower color. However, although the frequencies of the two flower color morphs showed a precipitous change that coincided with the location of a shallow ravine, allozyme frequencies were nearly uniform across the entire transect, and showed no evidence of any clinal pattern. This result makes it very unlikely that the flower color differences could possibly have originated by genetic drift. Our estimates of 130 m and 150 m for the width of the north and south clines for flower color, respectively, reveal that selection in this system operates on a very local scale. This result is consistent with the findings of Jain and Bradshaw (1966) that comprehensive studies of plant populations often reveal adaptation at the microspatial scale, and with data on cline width in natural plant populations reviewed by Endler (1977).

Our approach for using genetic markers to infer the relative roles of genetic drift and natural selection in the distribution of flower color differs from some recent analyses of spatial variation in phenotypic traits. First, we could not use the method of Spitze (1993), where the spatial differentiation of phenotypic traits (Q_{ST}) is compared to that for neutral genetic markers (F_{ST}). This ap-

proach requires estimating the heritability of the phenotypes of interest; the difficulty of germinating *L. parryae* made this impractical. One advantage of the cline-fitting method employed here is that it identifies precisely the spatial scale of genetic differentiation. Second, recent studies often use microsatellites or other DNA-based markers (AFLPs, RAPDs) for estimating population subdivision, but these techniques were not available to us at the time of the study. Allozymes have historically provided significant insights into the patterns and causes of spatial differentiation (Parker et al. 1998; Ennos 2001; McKay and Latta 2002), and given the striking differences we observed between the spatial pattern of flower color and allozymes in *L. parryae* (see Results), it is doubtful that a different methodological approach would alter our conclusions.

Our second null hypothesis was that there would be no difference in the performance of blue- and white-flowered individuals within the cline, as would be expected if selection on flower color were unimportant on a local scale. However, the results of our reciprocal transplant experiment demonstrated that blue- and white-flowered plants differed significantly in their levels of seed production when grown in a common environment. Furthermore, we documented that the magnitude and direction of this difference depended both on ravine side (east vs. west) and year. Natural selection was acting on flower color, and the direction and intensity of that selection varied not only in time, as we have previously documented (Schemske and Bierzychudek 2001), but also on a local spatial scale.

Our final null hypothesis was that the two sides of the ravine would not differ with respect to ecological factors that might serve as selective agents. We examined two aspects of the environment, and found significant differences in both. First, the soil composition on the two sides of the ravine (west vs. east) differed significantly, whereas we found no such difference in the opposite direction, over the same spatial scale (north vs. south). We have no knowledge of the history of the study area that would allow us to explain why such differences exist, but given that the ravine is part of the drainage system of the neighboring San Gabriel Mountains, it is possible that past hydrologic events, for example, periods of extreme runoff, may have resulted in persistent, local differences in soil composition. Second, the composition of the plant community differed significantly on the two sides of the ravine. The west (blue) side had greater species richness and higher percent cover of several of the most common perennial species, whereas the east (white) side had a much higher cover of creosote bush, *Larrea tridentata*. The differential response of so many plant species between these two regions is strongly suggestive of the existence of environmental differences. Whether *Linanthus* and the other plant species are responding to the differences we found in soil composition, or to some other, unmeasured factor(s) is impossible to determine at this point. However, our work does

demonstrate that important ecological factors can and do vary over this local spatial scale.

PLEIOTROPY AND MECHANISMS OF SELECTION

All these data point to the existence of strong selective forces—and not genetic drift—as responsible for maintaining the sharp cline in flower color that we have observed. However, it is unclear whether it is flower color itself that is the target of selection. In other plant species with polymorphic flower color, pollinators do discriminate between color morphs (Stanton 1987; Gigord et al. 2001; Irwin and Strauss 2005; Eckhart et al. 2006). In such cases, some local flower color polymorphisms can be explained by a combination of pleiotropy and pollinator-mediated selection (Irwin and Strauss 2005; Streisfeld and Kohn 2005). Because the beetles that pollinate *L. parryae* do not appear to discriminate between flowers of different colors (Schemske and Bierzychudek 2001), we can rule out the role of pollinators; pleiotropy must be the primary mechanism favoring one morph over the other in different environments.

Neither the flower color polymorphism, nor the sharp, local differences in flower color we find in *L. parryae* are unique within the family Polemoniaceae. Approximately 36% of the 170 species of Polemoniaceae in California possess a flower color polymorphism (from Hickman 1993). The polymorphic species represent a wide variety of life histories, pollination and mating systems, and occupy a range of habitats; thus it seems highly plausible that the pleiotropic effects of flower color on fitness are a common feature of adaptation in the family. Several polymorphic species, for example, *Leptosiphon jepsonii* and *L. bicolor*, are highly self-pollinating (Goodwillie 2000), adding further evidence that the adaptive value of flower color in this family is due to pleiotropy, and not to pollinator attraction. Furthermore, the steep cline in flower color observed in *L. parryae* is also found in a population of *Leptosiphon parviflorus*, a polymorphic member of the Polemoniaceae widespread in California grasslands. Field observations carried out at Jasper Ridge Biological Station in Northern California reveal that plants on serpentine soil are monomorphic for pink flowers, whereas those growing on neighboring, sandstone-derived soils are nearly monomorphic for white flowers, and that both color morphs are pollinated by long-tongued flies. The boundary between pink and white populations is on the order of meters, as is the case for *L. parryae*, and greenhouse experiments reveal that each morph is highly adapted to its “home” soil (D. W. Schemske, unpubl. data), a result that also parallels our findings for *L. parryae*. Taken together, these observations suggest that the association we observe between flower color and environmental factors in *L. parryae* reveals pleiotropic effects that are widespread in the family Polemoniaceae.

Pleiotropic effects of flower color are well known (Armbruster 2002), and include an association with plant vigor (Rausher

and Fry 1993), herbivore deterrence (Rausher and Fry 1993; Simms and Bucher 1996; Fineblum and Rausher 1997; Irwin et al. 2003; Frey 2004), plant secondary chemistry (Strauss et al. 2004), mating system (Fehr and Rausher 2004), and physiological traits such as heat stress amelioration (Coberly and Rausher 2003), soil ion accumulation and response to edaphic stress (Rajakaruna and Bohm 1999), or drought resistance (Warren and Mackenzie 2001). Greenhouse experiments with five unrelated species all having flower pigment polymorphisms found that the pigmented forms consistently produced more seeds than the white morphs under drought conditions, whereas the opposite result was observed under a regular watering regime (Warren and Mackenzie 2001). Likewise, in *Linanthus parryae* we also observed that the blue morph outperformed the white morph in dry years, with the reverse result in wet years (Schemske and Bierzychudek 2001).

Unfortunately, we do not know the mechanism of selection operating on flower color in *L. parryae*. It seems certain that some aspect of water use dynamics is involved, given our finding of a consistent association between rainfall level and the relative fitness of the two morphs. In our long-term study of a polymorphic site at Pearblossom (California), we found that the temporal variation in fitness of the morphs was associated with annual precipitation patterns, with an advantage to blues in dry years and to whites in wet years (Schemske and Bierzychudek 2001). In the present study of nearly monomorphic populations, the reciprocal transplant experiment demonstrated an overall advantage to the “home” morph, but the selective advantage of this morph also varied with precipitation. The advantage for blue-flowered plants on the west side of the ravine, where they are the dominant morph, occurred during the dry year of the study (1996), and the advantage for white-flowered plants on the east side of the ravine, where they are the dominant morph, occurred in the wet year (1995). It would be necessary to monitor soil moisture throughout the growing season to test the hypothesis that soil moisture is a main driver of selection on flower color. In our study of polymorphic populations, we found no difference between morphs in water-use efficiency, but our sample size was small, and the collection was made in a single year of high spring rainfall (Schemske and Bierzychudek 2001).

In a search for other possible mechanisms of selection, we conducted a preliminary study of the tissue cation composition of blue- and white-flowered plants growing on each side of the ravine. Our goal was to determine if the flower morphs differ in their uptake properties, as has been discovered in some other cases of local differentiation in plants (Kruckeberg 1954; Ramakrishnan and Singh 1966; Snaydon and Bradshaw 1969; Main 1974; Macnair and Gardner 1998; Rajakaruna et al. 2003). We analyzed the tissue cation concentrations (Ca, Cu, Fe, K, Mg, Mn, P, and S) of plants collected from both color morphs from both sides of the ravine in 1995, a year of high rainfall. In a MANOVA including flower color, side of the ravine and the interaction, we

found a marginally significant effect of flower color ($P = 0.04$). Differences in concentration for individual cations were small, and none of the separate comparisons were significant after Bonferroni correction.

To summarize, although we lack definitive evidence, the mechanism of selection on flower color in *L. parryae* is most likely a result of differential water use by the color morphs, which may also directly or indirectly affect cation uptake. Further research is needed to identify the precise mechanisms, and this should include comparative ecological and physiological studies of the many other flower color polymorphisms in the Polemoniaceae as well as detailed investigations of polymorphism within polymorphic species.

THE SIGNIFICANCE OF *LINANTHUS PARRYAE* TO EVOLUTIONARY BIOLOGY

Our results add weight to the growing list of examples of genetic structuring in plant populations that is maintained by selection, rather than drift (see Linhart and Grant 1996). However, we believe that they have broader significance. Taken together, the early studies by Epling, Dobzhansky, Wright and colleagues on the spatial pattern of flower color in *L. parryae* represent significant steps in the development of modern evolutionary theory. Since 1985, Wright's (1943a) paper describing his theory of isolation by distance has been cited over 1400 times. It was Wright's (1943b) analysis of data from *L. parryae* that initially provided the best empirical support for that theory.

Over the years, many authors have cited the example of *L. parryae* in support for the role played by random factors in population differentiation. For example, Stebbins (1950, p. 150) cited *L. parryae* as an example of isolation by distance: "In *Linanthus parryae*. . . Epling and Dobzhansky (1942) have given an example which may be explained by the past history of the population, as well as by the action of random fixation through isolation by distance. . ." Grant (1981, p. 25) wrote that the spatial distribution of the color morphs in *L. parryae* "may well have developed here as a result of restricted gene flow and neighborhood size, and without any significant role of environmental selection, although this conclusion is by no means certain." Levin (1988, p. 314) suggested that "one highly probable case for local random differentiation is described by the spatial distribution of flower polymorphism in the outcrossing annual *Linanthus parryae*." Heywood (1991, p. 351), in his review of spatial structuring of plant populations, wrote ". . .obligate outcrossing does not preclude substantial local genetic structuring due to IBD [isolation by distance], as evidenced by *Linanthus parryae*." Although Wright's conclusions have not gone completely unquestioned (see Mayr 1965; Waser and Price 1985), *L. parryae* has for the most part come to represent a prime example of the power of isolation by distance to structure populations.

Nearly 25 years after Wright's 1978 review, Schemske and Bierzychudek (2001), and Turelli et al. (2001) revisited the subject of *L. parryae*. The former paper described the results of long-term field observation of seed production in polymorphic populations. In Wright's view, natural selection for flower color in these populations should be weak or nonexistent, with no relationship to environmental factors. Instead, we found that selection was frequent, often strong, and that some regions displayed temporal variation in the favored morph that was associated with annual rainfall patterns (Schemske and Bierzychudek (2001). In our most intensively studied site, significant selection was observed in six of 11 years, with three years of blue advantage, three years of white advantage, and four years with few or no plants (Schemske and Bierzychudek 2001). Turelli et al. (2001) found that fluctuating selection was key to understanding the maintenance of the polymorphism, and specifically that white-flowered plants, despite their lower arithmetic and geometric mean fitness, had the advantage in wet years when mean seed production was high. The white morph therefore contributed most of the seeds to the seed bank, and could persist despite its overall lower fitness (Turelli et al. 2001). These results contributed to an understanding of how fluctuating selection contributes to the maintenance of polymorphisms, a subject of longstanding theoretical and empirical interest (Felsenstein 1976; Hedrick et al. 1976; Hedrick 1986; Gillespie 1991). The current article presents the results of long-term observational and experimental studies that further support the view that natural selection, not genetic drift, is the primary cause of the spatial pattern of flower color in *L. parryae*.

LINANTHUS PARRYAE AND WRIGHT'S SHIFTING BALANCE THEORY OF EVOLUTION

As chronicled by Provine in his biography of Sewall Wright, the case of *L. parryae* figured prominently in the development of Wright's view of the evolutionary process, providing for him ". . . an example of the whole shifting balance theory of evolution in action" (Provine 1986, p. 378). Wright concluded that ". . .among the continually varying genetic compositions of local populations, arrived at by random drifting of the frequencies at all other heteroallelic loci, favorable interaction systems may be arrived at which spread over large areas by interdeme selection and incidentally have some effect on the selective advantage of white over blue" (Wright 1978, p. 223). His description of the evolutionary mechanisms involved in the spatial differentiation of flower color includes each of the three phases of the shifting balance, that is, genetic drift within demes (phase I), selection within demes (phase 2), and interdeme selection (phase 3). In contrast to what Wright had envisaged for *L. parryae*, our findings of strong spatial and temporal variation in selection on flower color indicate that genetic drift is unlikely to provide the critical first step for initiating the shifting balance process.

Wright's shifting balance theory continues to serve as an important stimulus for empirical and theoretical work (e.g., Koskinen et al. 2002; Whibley et al. 2006), yet its importance in nature is a subject of debate. Bradshaw (1972, p. 38) suggested that Wright's models "... are insufficient because of their emphasis on drift and their disregard for differential selection in different populations." Whitlock and Phillips (2000, p. 348) note that the shifting balance theory "has not succeeded in predicting patterns of adaptation in nature," and Coyne et al. (1997), concluded that there is no example from nature where each phase of the shifting balance has been demonstrated. As noted by Crow (1991), even Wright broadened his view of evolutionary mechanisms in his later years and became more accepting of alternatives to his shifting balance theory. Although further research may discover examples in which Wright's shifting balance theory holds (Whitlock and Phillips 2000), our long-term studies make clear that *L. parryae* should no longer be considered an example of isolation by distance or of evolution by means of the shifting balance.

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Appendix. Summary of plant densities for blue- and white-flowered *Linanthus parryae* as determined from the two census transects (see Fig. 3) for years 1992–1998. The totals given here represent censuses conducted in approximately 2000, 2 m × 2 m plots. Entries in boldface give annual and overall totals.

Year	Transect	West side of ravine			East side of ravine		
		# blue plants	# white plants	% white plants	# blue plants	# white plants	% white plants
1992	north	2192	40	1.8	92	2797	96.8
	south	1357	26	1.2	127	4015	96.9
	total	3549	66	1.8	219	6812	96.9
1993	north	654	6	0.9	30	1062	97.3
	south	306	14	4.4	71	1658	95.9
	total	960	20	2.0	101	2720	96.4
1994	north	0	0	-	0	1	100
	south	4	0	0	0	4	100
	total	4	0	0	0	5	100
1995	north	7053	148	2.1	368	16,399	97.8
	south	5118	242	4.5	598	15,129	96.2
	total	12,171	390	3.1	966	31,528	97.0
1996	north	91	2	2.2	9	233	96.3
	south	186	29	13.5	2	155	98.7
	total	277	31	10.1	11	388	97.2
1997	north	0	0	-	0	0	-
	south	0	0	-	0	0	-
	total	0	0	-	0	0	-
1998	north	4519	66	1.4	368	22,485	98.4
	south	3751	143	3.7	325	8625	96.4
	total	8270	209	2.5	693	31,110	97.8
All years	north	14,509	262	1.8	867	42,977	98.0
	south	10,722	454	4.1	1123	29,586	96.3
	total	25,231	716	2.8	1990	72,563	97.3