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LOCAL ADAPTATION, PATTERNS OF SELECTION, AND GENE FLOW IN THE CALIFORNIAN SERPENTINE SUNFLOWER (*HELIANTHUS EXILIS*)

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Abstract.—The traditional view of the species as the fundamental unit of evolution has been challenged by observations that in heterogeneous environments, gene flow may be too restricted to overcome the effects of local selection. Whether a species evolves as a cohesive unit depends critically on the dynamic balance between homogenizing gene flow among populations and potentially disruptive local adaptation. To examine this evolutionary balance between “global” gene flow and local selection, we studied northern Californian populations of *Helianthus exilis*, the serpentine sunflower, within a mosaic of contrasting serpentine and nonserpentine areas that differ considerably in soil chemistry and water availability. Local adaptation to riparian and serpentine habitats was studied in *Helianthus exilis* along with an analysis of gene flow patterns among populations within these habitats. Local adaptation was assessed in *H. exilis* during 2002 and 2003 using reciprocal transplant experiments at multiple locations within serpentine and riparian habitats. Effects of competition and germination date on the expression of local adaptation were also examined within the reciprocal transplant experiments. Local adaptation was detected in both years at the local site level and at the level of habitat. The analysis of the transplanted populations indicated that the patterns of selection differed considerably between riparian and serpentine sites. Differential survivorship occurred in serpentine habitats, whereas selection on reproductive output predominated in riparian habitats. Local adaptation was expressed only in the absence of competition. Local adaptation in terms of survivorship was most strongly expressed in treatments with delayed seed germination. Microsatellite markers were used to quantify population genetic parameters and examine the patterns of gene flow among sampled populations. Analysis of molecular markers revealed a system of population patches that freely exchange genes with each other. Strong selection seems to maintain ecotypic variation within this endemic sunflower species, while extensive gene flow among populations prevents local speciation between serpentine and riparian ecotypes.

Key words.—Competition, ecotype, gene flow, *Helianthus exilis*, local adaptation, reciprocal transplant, reproductive hierarchy, serpentine, sunflower.

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Debate concerning the nature of the biological species has engaged evolutionists for decades (Mayr 1942; Wiley 1978; Hull 1980; Mishler and Donoghue 1982; Levin 2000). Particularly for plant species, there have been repeated challenges to the concept that the species represents a cohesive evolutionary unit comprised of populations tightly linked by gene flow (Ehrlich and Raven 1969; Levin 1979). In part because of the sessile nature of plants, it has been argued that spatially local selection when coupled with weak gene flow create a scenario where populations, and not species, are the primary units of evolutionary change (Ehrlich and Raven 1969; Levin 2000). Counter to this argument is the idea that only very small amounts of gene flow are required for globally advantageous mutations to rapidly spread across populations (Rieseberg and Burke 2001). This rapid spread of selectively advantageous mutants promotes species cohesion and the capacity to evolve (Morjan and Rieseberg 2004). Within complex environmental mosaics with patchy population structure, disruptive selection combined with restricted gene flow can foster rapid local adaptation and the formation of distinct ecotypes in plant species (see Galen et al. 1991). It has also been suggested that this patchy type of population structure may foster local speciation because of the demographic longevity and genetic structure of metapopulations (Levin 1995). On the other hand, strong selection that maintains locally adapted genotypes despite high gene flow among populations appears to be more common than

expected (McKay and Latta 2002). Under these types of conditions, although populations can diverge genetically in response to strong selection in some traits, high rates of gene flow that effectively link populations may still prevent speciation from occurring.

Populations of a plant species that occupy adjacent and contrasting soil environments present distinct advantages for the study of local adaptation because the contrasting edaphic environments can create contrasting selection pressures, while the close proximity of populations increases the opportunity for gene flow. Serpentine soils and adjacent nonserpentine habitats form an edaphic mosaic in which abiotic environmental differences change rapidly over small spatial scales (Kruckeberg 1954, 1984). Serpentine or ultramafic rocks are commonly found in the foothills of the Coast Ranges and Sierra Nevada in California and produce soils of low fertility with high levels of magnesium and the presence of heavy metals. Along with soil chemical properties, water availability is a factor that has been recognized to be crucial for plant adaptation and ecology of serpentine soils (Gardner and McNair 2000; Harrison et al. 2000a; Sambatti 2004). As a result of these extreme edaphic conditions, a unique endemic flora has evolved on serpentine soils (Kruckeberg 1984). Thus, species that occupy both serpentine and nonserpentine soils are of particular interest to the study of ecotype formation. Although gene flow among populations of plant species located across these steep soil gradients is like-

ly, there is a good probability that strong disruptive selection is also present within this edaphic mosaic.

The annual serpentine sunflower *Helianthus exilis* (Heiser 1949) was selected for study because this taxon exhibits strong phenotypic differentiation between populations found in serpentine and riparian sites (Jain et al. 1991; Sambatti 2004). A distinct advantage of this species is that it has been well studied with regard to its taxonomy (Heiser 1949; Jain et al. 1991), phylogeny (Rieseberg et al. 1988), cytogenetics (Chandler et al. 1986), morphology and physiology (Madhok and Walker 1969; Jain et al. 1991; Sambatti 2004), and ecology and demography (Wolf et al. 1999; Harrison et al. 2000b; Wolf 2001). Because of its close taxonomic relationship to *H. annuus*, microsatellite markers are readily available for *H. exilis*. Highly polymorphic, these microsatellite markers provided us with a powerful tool to study patterns of gene flow in this system as well as to estimate the relative balance between selection and gene flow among riparian and serpentine sunflower populations. *Helianthus exilis* is an obligate outcrosser with strong self-incompatibility (Wolf 2001; Harrison et al. 2000b; J. B. M. Sambatti, pers. obs.), which facilitates population genetics analyses because selfing rates do not need to be estimated. Gene flow estimates based on microsatellite polymorphisms are used as a measure of genetic cohesion between populations that can be compared to the intensity of disruptive selection at different levels: locus, quantitative trait, or individual. The larger the gene flow, the larger selection intensities are necessary to maintain ecotypic variation within a species (Lenormand 2002; McKay and Latta 2002).

To study the potential for local adaptation in serpentine sunflower, we performed reciprocal transplant experiments for two years in serpentine and riparian sites within the Inner Coast Ranges of northern California. In addition to strong abiotic edaphic differences between serpentine and riparian sites, we also suspected that competitive regimes differ markedly between the habitat types: serpentine sites exhibit low plant cover, whereas riparian sites are covered with dense vegetation. These habitat differences, when coupled with previous observations that riparian sunflowers grow taller than serpentine plants (Jain et al. 1991; Sambatti 2004), suggest that the importance of interspecific competition as a selective factor may vary significantly between habitat types. We also noted that there were distinct differences among sites in germination timing; in particular, seedlings in one of the serpentine sites emerged much later in the season than seedlings at the other sites. Despite the long tradition of reciprocal transplant designs (i.e., reciprocal common gardens) in the study of local adaptation, there have been very few studies that have included manipulation of ecological factors such as competition within the common gardens themselves. To study how competition and germination timing might affect the expression of local adaptation, we manipulated competitive background and seedling emergence time within the reciprocal transplant experiments. We felt that a better understanding of the relative importance of biotic and abiotic selective factors is likely to be gained by embedding these types of manipulations within studies of local adaptation.

MATERIALS AND METHODS

Species and Study System

Within serpentine habitat, the distribution of *H. exilis* is restricted to seasonal seeps. Although these seeps are spring fed, they typically dry up by midsummer and create a spatially patchy habitat for serpentine populations (Wolf et al. 1999; Harrison et al. 2000b; Wolf 2001). Riparian populations are found mainly along the banks of nearby streams where water is typically available year round. It has been suggested that this riparian taxon might represent a separate species (Jain et al. 1991). However, the complete interfertility of serpentine and riparian populations in artificial crosses, their close geographical proximity, and the fact that their flowering times overlap considerably makes reproductive isolation unlikely (Jain et al. 1991; J. B. M. Sambatti, pers. obs.). Thus, we conclude that the riparian and serpentine represent different habitat populations rather than different species.

Our study was conducted in the University of California Donald and Sylvia McLaughlin Natural Reserve (38°51'N, 122°24'W). A thorough description of soil, vegetation, and flora of the McLaughlin reserve can be found in UC Davis Natural Reserve System (2001). To provide site replication within the serpentine and riparian habitat types for the reciprocal transplant studies (2002 and 2003), we chose two serpentine seep sites (S1 and S2) and two riparian sites (R1 and R2). Site S1 is located along a seep that remains moist until midsummer. Gopher disturbance is low at this site although soil disturbance by runoff is common. Plants are relatively short at this site (most <0.5 m) and typically produce more than a single head per plant. Site S2 is located along a sloping serpentine seep containing occasional soil disturbances from water flow and gopher activity. In this site, plants are also short in stature but often produce only a single head per plant. The riparian sites (R1 and R2) are both located along the banks of a perennial stream (Knoxville Creek). Logistically, the sampling of these two sites was limited to the only available perennial stream in the region with sunflower populations. Vegetation, geology, and soil types vary along this stream; thus, we assume that our riparian sites were independent. The vegetation at both riparian sites is dominated by grass cover (primarily the native *Leymus triticoides* (Buckley) Pilger and *Distichlis spicata* (L.) Greene), and these sites are periodically disturbed by soil deposition (particularly R1) during winter when floods are common. Human activities along the stream may also cause periodic soil disturbances. The invasive *Tamarix parviflora* DC was present in low densities at site R1, but no *T. parviflora* individuals were located within any of the experimental plots.

Plants at both riparian sites are tall, often greater than 2 m in height, and individual plants often produce hundreds of heads. High levels of soil moisture exist at both riparian sites throughout the year (J. B. M. Sambatti, pers. obs.). The geographic distances among these reciprocal transplant sites are shown in Table 1.

Riparian populations R1 and R2 are somewhat geographically removed from most serpentine populations. To assess the effects of the correlation between geographic distance and habitat type on patterns of gene flow (see below), we

TABLE 1. Matrix of geographic distances (km) among all sites sampled for gene flow analysis. Sites R1, R2, and R3 are riparian sites; S1 and S2 are serpentine sites. Reciprocal transplant experiments were conducted at sites R1, R2, S1, and S2.

	R1	R2	R3	S1
R2	0.69			
R3	6.39	5.78		
S1	5.05	6.86	2.98	
S2	7.48	4.74	1.10	4.37

included an additional riparian population (R3) that is located in a nonperennial stream within a serpentine area close to the center of the species distribution in the region and surrounded by many serpentine populations, including populations S1 and S2.

Seeds were collected as maternal families from each of the four reciprocal transplant sites. Twenty-four families were collected from the S1 site, 33 from S2, 16 from R1, and 15 from R2. To induce germination, seeds were first surface sterilized in a 2% bleach and 1% Triton-X 100 (Perkin Elmer Life and Analytical Sciences, Inc., Boston, MA) solution for 10 min and then rinsed for 5 min with distilled water. Seeds were then scarified by cutting a small segment off the blunt end of the achene with a razor blade. Seeds were then placed on wet filter paper in a sterile petri dish and incubated for one day in the dark at room temperature. Seed coats were then removed and seeds were planted after two more days of incubation.

Four blocks were established at each site. Each block was a rectangle (80×160 cm) subdivided into two groups of four squares (40×40 cm). This series of reciprocal transplants focused on whether competition amplified or reduced the expression of local adaptation. To examine the effect of competition, one-half of the block was hoed a week before seeding to remove most above-ground vegetation, and the other half was left undisturbed. Within each of the competition treatment main plots, the four subplot treatments of seed origin were randomly assigned. The experimental design was a split block with competition as main plot treatment, seed source as a subplot treatment, and blocks as a random effect nested within sites. A 36×36 cm planting guide was used to plant seeds in a 3×3 grid within each subplot with planting locations marked by toothpicks and separated by 9 cm. Seeds were pregerminated, planted on 30 April 2002, and the maternal family origins of seeds were randomized within subplots. Emerging seedlings were identified by their close proximity to the toothpick and by the presence of a slight notch in the cotyledon resulting from the scarification procedure. After emergence, seedlings were marked with a colored wire. Survivorship was scored every other week until flowering. Reproductive plants were visited frequently so that their heads could be harvested after seed formation but before seed dispersal from the head. This frequent censusing allowed accurate determination of the total number of seeds produced by each head.

2003 Reciprocal Transplant Experiment on Effects of Emergence Time

In 2003, the field design was modified to examine the effects of emergence time on the expression of local adap-

tation. The competition treatments were replaced by an emergence time treatment in which seeds from all source populations were planted at two different dates, 12 March 2003 and 14 April 2003. At each site, vegetation was removed by hoeing from all plots within five planting blocks. Planting date (main plot treatment) was randomized within each block and seed origin (subplot treatment) was randomized within each main plot. Within each seed origin treatment subplot, 12 seeds were planted at a 7.5 cm spacing. Before planting, each seed was scarified, weighed, and germinated within 96 flat-bottom-well plates with moist filter paper placed in the bottom of each well. This allowed precision planting of the preweighed seeds into each subplot with family origin randomized with respect to planting location. Toothpicks were used to mark the location of planted seeds. Planting date, seed origin, and site were analyzed as fixed, crossed factors, and block was analyzed as a random effect nested within site. Data for survivorship and reproductive output were collected in a similar fashion to the 2002 experiment except that seed weight was used as a covariate in the analysis to help control for maternal effects.

Reciprocal Transplant Data Analysis

Data from the 2002 and 2003 field experiments were analyzed similarly. Survivorship was analyzed using the proportional hazards model (Fox 1993) in JMP (SAS 2000) for right-censored data for those plants that survived after flowering. The rationale for the right-censored data analysis procedure is that most sunflower mortality occurred early. As time passes, the assumption of a proportional death rate is more likely to be violated, because plants that survived past a certain period are more likely to remain until the end of the life cycle (Zens and Peart 2003). This is particularly true in riparian areas where water availability remained high. This caused the analysis to be biased toward a period where mortality is less important, which may lead to a distorted interpretation of death rates. Therefore, by right censoring the data after flowering time, it was possible to focus on the phase when most of the mortality occurs. It was not possible to analyze random effects in a proportional hazards model; therefore, block structure was not included in the model.

As an integrated index of fitness, we measured the mean number of seeds produced per plot. Because of high mortality in some sites, an excess of zeros in the data caused strong deviations from parametric assumptions. Our first strategy was to analyze this seed production data using a rank transformation (Conover and Iman 1981; Hora and Conover 1984). We also performed another analysis using seed output data rearranged so that seed origin and site were pooled (with proper adjustment of error degrees of freedom) into riparian and serpentine categories in order to evaluate the hypothesis that local adaptation exists at the habitat level as well as at the site level. As part of a tamarisk control program, plants of site R1 were inadvertently sprayed with herbicide in 2003 after flowering. As a result, this site was excluded from the analysis within the planting date experiment.

We applied the Fligner-Killeen test of homogeneity of variances in each analysis to test the hypothesis that reproductive variance are homogeneous across sites. This is a nonpara-

metric test based on rank data and on the absolute deviations from group medians; it tests the null hypothesis that variances are the same across groups (Conover et al. 1981). The Fligner-Killeen test has been determined by simulation studies to be very robust against distributional departures from normality (Conover et al. 1981). In estimating reproductive variance, we used the number of seeds produced per plant; zeros were included in the analysis.

Characterizing the Edaphic Environment

We expected that strong differences in the edaphic environment of serpentine and riparian sites might be important agents of selection. For example, serpentine soils are believed to possess very low $\text{Ca}^{+2}/\text{Mg}^{+2}$ ratios and reduced fertility, and contain higher concentrations of heavy metals. In addition, serpentine seeps usually dry up by midsummer whereas riparian soils can remain moist until autumn. To characterize the chemical composition of soils at our riparian and serpentine sites, we performed a detailed soil composition analysis. We also measured predawn water potentials of a subsample of plants in each site to estimate levels of soil water availability for populations in both habitats during the summer (see below). Observations at all sites indicated that soils were saturated with water at germination time. Thus, variation in predawn water potential in the summer presumably reflects differences in rates of soil water depletion after germination.

Five soil samples were randomly taken from each site within a single day in late summer 2003 and analyzed for Ca, Mg, Na, H, K, S, P (ppm), organic matter (%), pH, and cation exchange capacity (CEC) in milli-equivalents/100 g of soil. Data was transformed when necessary to satisfy parametric assumptions of multivariate analysis of variance (MANOVA). Organic matter (OM) was log-transformed and P, Ca, and Mg were Box-Cox transformed. A MANOVA was performed in JMP (SAS 2000) on nutrient variables, organic matter, and pH with sites as the independent fixed variable. Levels of Mg were highly correlated with CEC ($r = 0.90$) and negatively correlated with K concentrations ($r = -0.81$). Because Mg is an important element in characterizing serpentine soils, it was retained in the analysis, while CEC and K were removed from the model to reduce collinearity (Scheiner 1993).

At each of the sites, predawn xylem pressure potentials (MPa) were measured with a pressure chamber (PMS Instruments, Corvallis, OR) to provide estimates of soil water availability (Ritchie and Hinckley 1975; Hinckley et al. 1978; Richter 1997). Although recent work has questioned the assumption of equilibrium between plant and soil water potentials for some species (Donovan et al. 1999, 2001), we assumed a correlation between soil water potential and xylem water potential. At each riparian or serpentine site, three plants were randomly selected near each of the four blocks of the reciprocal transplant experiment between 0100 and 0600 h on 29 August 2002 ($n = 12$ for each site). Xylem pressure potential data were square-root transformed to satisfy parametric analysis assumptions and analyzed with ANOVA (JMP; SAS 2000). For analysis, block was considered to be a random factor and was nested within site.

Gene Flow Analysis

Gene flow patterns were estimated with microsatellite markers. A list of *H. annuus* primers was obtained from Steve Knapp's laboratory (Oregon State University, Corvallis, OR; see Tang et al. 2002, 2003; Yu et al. 2003). A subset of this list, optimized for the weed *H. bolanderi*, was also obtained from Keith Gardner (L. Rieseberg's laboratory, Indiana University, Lafayette, IN). Further optimization was obtained using a small parent-offspring family to verify microsatellite heritability (J. B. M. Sambatti, unpubl. data). Ten microsatellites were selected based on the degree of polymorphism, quality of amplification, and heritability. Given the phylogenetic relatedness between *H. exilis* and *H. annuus* (Rieseberg et al. 1988), we assumed that microsatellite loci that are unlinked in *H. annuus* are also unlinked in *H. exilis*.

In the summer of 2001, young leaf samples were taken along field transects established in all sites from randomly selected mature plants. For most populations, a minimum of 60 individuals were sampled. Samples were placed in labeled paper coin envelopes and placed into sealed plastic bags containing silica gel. Samples in bags were taken to the laboratory, left to dry for one week at room temperature, and then frozen within the bags at -20°C .

DNA was extracted using a Qiagen (Valencia, CA) DNeasy kit for plants with only two-thirds of the recommended amount of tissue, to increase purity. Extraction rates were measured with a fluorometer. DNA quality was tested in a 2% agarose gel. Concentrations for polymerase chain reaction (PCR) were standardized at 8 ng/ μl and Promega (Madison WI) Taq polymerase was used for all PCR amplifications. Samples were amplified, run in a 2% agarose gel, then visualized with ethidium bromide staining under UV light.

PCR conditions in the thermal cycler (MJ Research, Waltham, MA) were: 3 min at 95°C ; repeat the following cycle 10 times (30 sec at 94°C ; annealing temperature 10°C at 30 sec, reducing 1°C per cycle [touch down]; 45 sec at 72°C); repeat the following cycle 30 times (30 sec at 94°C , 30 sec at annealing temperature, 45 sec at 72°C); 20 min at 72°C ; store at 4°C . The microsatellites, their labeling dyes, and annealing temperatures were: ORS 371 (VIC), 57°C ; ORS 331 (NED), 57°C ; ORS 70 (FAM), 57°C ; ORS 240 (NED), 48°C ; ORS 442 (HEX), 54°C ; ORS 131 (FAM), 48°C ; ORS 317 (VIC), 52°C ; ORS 3 (NED), 55°C ; ORS 229 (PET), 59°C ; and ORS 691 (PET), 50°C . For PCR details, see Appendixes 1 and 2, available online at <http://dx.doi.org/10.1554/05-479.1.s1>.

Fragment analysis was performed in an ABI 3100 (Applied Biosystems, Foster City, CA; ORS 229, ORS 3, ORS 131, ORS 317) with LIZ labeling size standard Genescan-500, BaseStation (MJ Research Waltham, MA; ORS 240) with ROX labeling the same size standard and ABI 3730 XL (Applied Biosystems) (ORS 371, ORS 331, ORS 691; ORS 70, ORS 442 in two different runs) with LIZ labeling the same size standard. Groups of two, three, or four microsatellites were multiplexed in these machines with the constraint that the fragments of the multiplexed microsatellites did not overlap in size. Before fragment analysis, samples were placed in 96-well plates and diluted 20-fold. Fragment analysis was run according to Applied Biosystems and MJ Research pro-

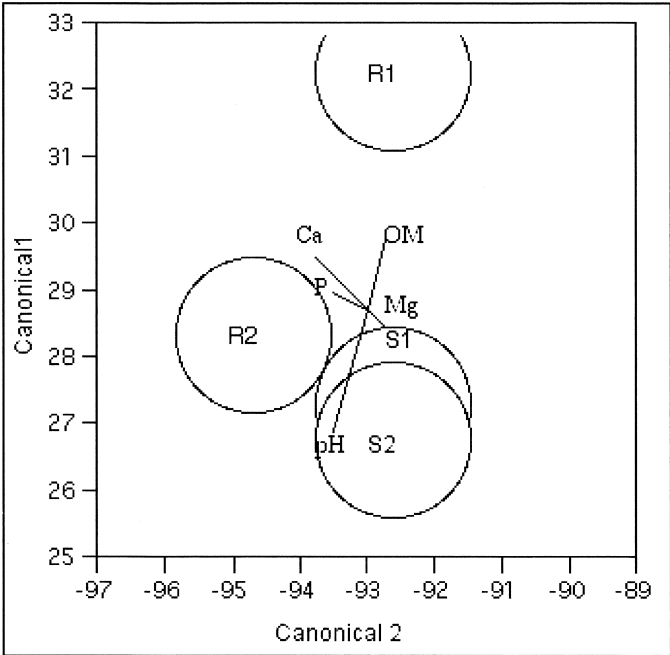


FIG. 1. Multivariate analysis of variance centroid plot of the multivariate effects of sites on the soil variables Ca, Mg, P, organic matter (OM), and pH. Circles represent the 95% confidence region. Biplot vectors show the directions of the original response variables. For example, Ca values become larger moving from serpentine to riparian soils. Ca, Mg, and P are the soil components that distinguish serpentine from riparian sites. Soils at serpentine sites (S1 and S2) have more Mg and less Ca than soils at riparian sites (R1 and R2). Soils at site R1 contained more organic matter than all other sites. Overall, serpentine sites are similar to each other.

protocols. Raw data was scored visually using Gene Cartographer (MJ Research), STRand (University of California–Davis) or GeneScan (ABI 3100) analysis software. Individuals without clear scoring were re-amplified, and only unambiguous data was used in the analysis.

Seventeen to 20 individuals per locus were randomly chosen per population to estimate bidirectional gene flow (i.e., $4N_e m$) between each population, the number of migrants per generation, and θ ($4N_e \mu$) that estimate the number of mutants per generation in a given population with effective size N_e (Hartl and Clark 1997). MIGRATE 1.7.3 (Beerli 1998, 2002; Beerli and Felsenstein 1999, 2001) was used for coalescence simulations. This program assumes an island model at equilibrium and estimates two-way migration coefficients ($4N_e m$) between pairs of populations and θ ($4N_e \mu$) for each population. Four simulations with 15, 10, 10, and 10 short chains and two long chains each were performed with different random seeds and starting F_{ST} values. Although differences between simulations were relatively small, indicating that simulations converged, a fifth simulation with a single long chain was added to obtain narrower confidence intervals.

RESULTS

Analysis of the Environment

A canonical centroid plot indicates that sites differ significantly in soil nutrients ($P < 0.0001$) but the serpentine sites S1 and S2 are very similar in soil composition (Fig. 1). Although one of the riparian sites (R2) is grouped with sites S1 and S2 in relation to pH and organic matter, soil levels of Ca, Mg, and P clearly cluster the riparian sites together. Sites S1 and S2 show lower soil levels of Ca and slightly higher levels of Mg relative to R1 and R2; Ca^{+2}/Mg^{+2} ratios are lower in the serpentine sites. The concentration of soil P is higher in sites R1 and R2.

TABLE 2. (A) Analysis of the interactive effects of competition treatment, seed source, and planting site on survival and reproduction in the 2002 reciprocal transplant experiment. Main effects of competition treatment, planting site, seed origin, and all interactions on survivorship were tested using a proportional hazards model. Treatment effects on variance in the number of seeds per plot were tested using ANOVA of rank transformed data. (B) Results with site data aggregated within serpentine or riparian habitat type, the analysis of the interactive effects of competition treatment, seed source, and planting habitat on reproduction. Treatment effects on the number of seeds produced per plot were tested using ANOVA of rank transformed data.

(A) Survival and reproduction at the site level	Survivorship		Number of seeds per plot	
	Source	df	P > χ^2	P > F
Competition		1	0.0000	<0.0001
Site		3	0.0003	0.2843
Origin		3	0.0051	0.0003
Site \times origin		9	0.0136	0.0049
Site \times competition		3	0.0022	0.0439
Origin \times competition		3	0.3176	0.0039
Site \times origin \times competition		9	0.1284	0.0292
(B) Reproduction at the habitat level				
	Source	df	P > F	
Habitat (serpentine or riparian)		1	0.1413	
Origin (serpentine or riparian)		1	0.0005	
Competition		1	<0.0001	
Habitat \times competition		1	0.1111	
Habitat \times origin		1	0.0002	
Origin \times competition		1	0.0019	
Habitat \times origin \times competition		1	0.0306	

There were significant ($P = 0.0024$) differences in predawn water potentials among sites; differences among blocks nested within sites were also highly significant ($P < 0.001$). Plants at site S1 exhibited the strongest water stress at the end of the summer (mean ± 1 SE = -1.34 ± 0.10 MPa) followed by the other serpentine site, S2 (-0.79 ± 0.06 MPa). As expected, plants in riparian sites R1 (-0.44 ± 0.03 MPa) and R2 (-0.42 ± 0.06 MPa) exhibited less water stress than plants growing in serpentine sites.

*2002 Reciprocal Transplant:
Competition and Local Adaptation*

During 2002, competition had a strong negative effect overall on plant survivorship, while a strong site effect also indicated that survivorship patterns were different among sites (Table 2A). The site-by-competition-treatment interaction reflects high death rates at site R2 in both treatments, whereas in all other sites death rates are higher in competition treatments (Fig. 2A). The impact of competition within serpentine sites was strongest during seedling establishment. For example, after some initial negative effect on seedling establishment in site S2, competition had very little impact on survival; the curves for both competition treatments parallel each other quite closely (Fig. 2A). The influence of seed origin on survivorship occurred primarily at serpentine sites (Fig. 2B). This was confirmed with post-hoc tests in which origins were tested within each site separately (JMP; SAS 2000). In these tests, differences among origins were highly significant ($P < 0.0003$) at both serpentine sites, significant at riparian site R1 ($P = 0.03$, log-rank test; $P = 0.006$, Wilcoxon test), and nonsignificant at riparian site R2. Local adaptation in terms of survivorship was detected only at ser-

pentine sites with higher survival for the local serpentine genotypes at the end of the census period (Fig. 2B).

Analyses of reproductive output among sites revealed significant interactions in the expression of local adaptation with competitive environment (Table 2A). When reproductive output data for sites were aggregated within serpentine and riparian habitats, a similar interactive effect was detected at the habitat level (Table 2B). At both the site and habitat level, the significant three-way interaction indicates that the expression of local adaptation was dependent on the competitive treatment. Under competition, there was little indication that local sources produced more seeds than nonlocal sources. Plants from the origin R1 did not produce a single seed in any site. In serpentine sites, local plants produced more seeds than nonlocal plants in the low-competition treatment (Fig. 3).

Not reflected in the above analysis is the important difference between riparian and serpentine sites in the reproductive variance among plants (Fligner-Killeen test, $P < 0.0001$). The standard error of seed output per plant decreases from riparian to serpentine sites. The seed contribution to the next generation was more evenly distributed among reproductive plants at serpentine sites (Fig. 3). In contrast, plants of R2 origin in site R1 produced a total of 895 seeds with a single plant producing 822 of those seeds.

*Reciprocal Transplant 2003:
Emergence Time and Local Adaptation*

In the planting date experiment, all effects contributed significantly to variance in survivorship except the site-by-planting-date interaction (Table 3A). The significant three-way interaction indicates that the expression of local adaptation

TABLE 3. (A) Analysis of the interactive effects of planting date, seed source, and planting site on survival and reproduction in the 2003 reciprocal transplant experiment. Main effects of planting date, planting site, seed origin, and all interactions on survivorship were tested using a proportional hazards model. Treatment effects on variance in the number of seeds per plot were tested using ANOVA of rank-transformed data. Results with and without seed weight used as a covariate are presented. (B) Results with site data aggregated within serpentine or riparian habitat type, the analysis of the interactive effects of planting date, seed source, and planting habitat on reproduction. Treatment effects on the number of seeds produced per plot were tested using ANOVA of rank-transformed data.

(A) Survival and reproduction at the site level	Survivorship with seed weight		Survivorship without seed weight		Number of seeds per plot	
	df	$P > \chi^2$	df	$P > \chi^2$	df	$P > F$
Source						
Site	3	0.0000	3	0.0000	2	0.0230
Origin	3	0.0005	3	0.0001	2	0.0230
Planting date	1	0.0000	1	0.0000	1	0.2400
Site \times origin	9	0.0138	9	0.0129	4	0.0500
Site \times planting date	3	0.4600	3	0.4460	2	0.3000
Origin \times planting date	3	0.0020	3	0.0019	2	0.3200
Site \times origin \times planting date	9	0.0450	9	0.0544	4	0.3900
Seed weight	1	0.3000				
(B) Reproduction at the habitat level						
Source	df	$P > F$				
Habitat (serpentine or riparian)	1	0.2069				
Origin (serpentine or riparian)	1	0.1477				
Planting date	1	0.2079				
Habitat \times planting date	1	0.4929				
Origin \times planting date	1	0.7768				
Habitat \times origin	1	0.0253				
Habitat \times origin \times planting date	1	0.5926				

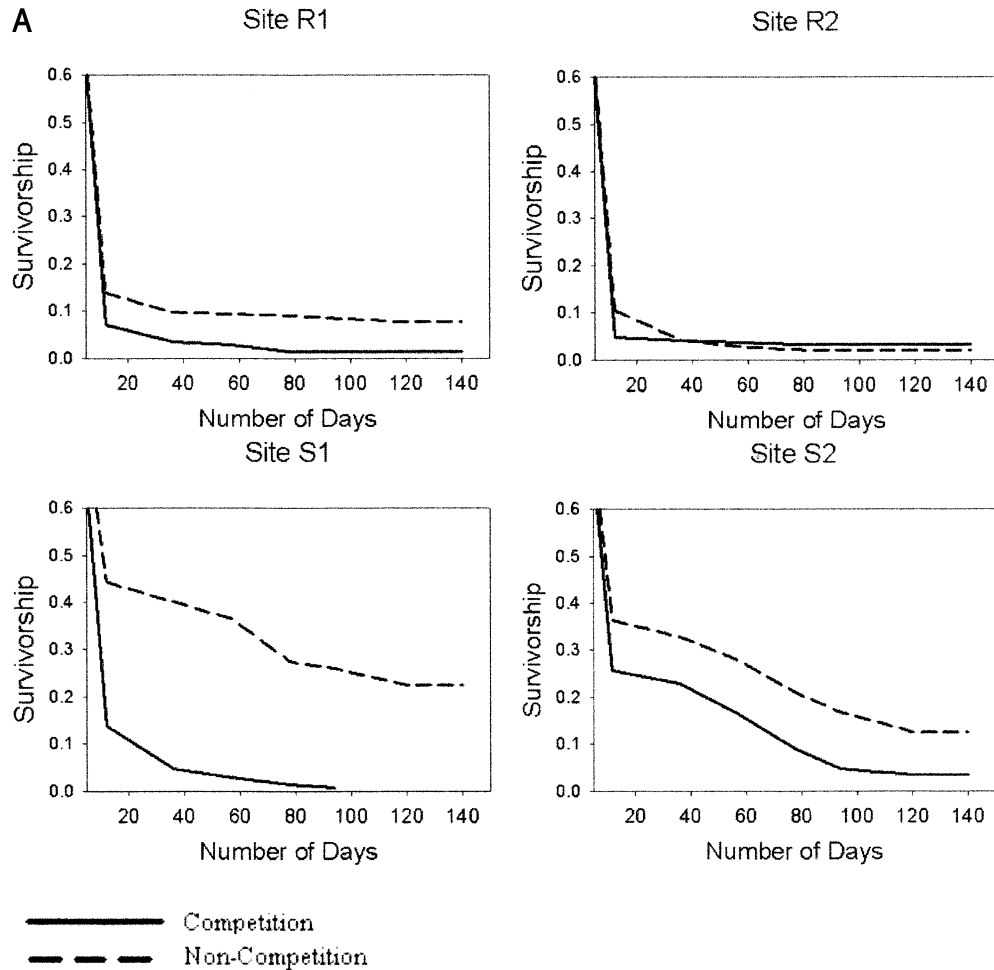


FIG. 2. (A) Survival plots of the competition treatment within each site for the 2002 experiment. Data was taken during approximately 140 days after transplant, when plants started flowering. (B) Survival of cohorts from different seed origins within each site for the 2002 reciprocal transplant experiment. Data was taken during approximately 140 days after transplant, when plants started flowering. Survivorship in riparian sites did not differ among seed sources. In serpentine sites, plants from the local seed source exhibited lower death rates.

in terms of survival was stronger in the late planting treatment; in late germinating cohorts, local genotypes died at a slower rate than nonlocal genotypes (Table 3A and Fig. 4). Higher survival of local genotypes is particularly pronounced for sites S2 and R1.

In contrast to the survival results, planting date had no effect on reproductive output (Table 3). However, local adaptation at the site level was indicated by the interactive effect of site with seed origin on seed output (Table 3A, Fig. 5). Although there was also no effect of planting date at the level of habitat, local adaptation at the habitat level was indicated by the significant interaction of seed origin with planting habitat (Table 3B).

Patterns of reproductive variance were similar to those found during the 2002 reciprocal transplant study (Fligner-Killeen test, $P < 0.0001$). The standard error of seeds produced per plant was much higher in site R2 than sites S1 and S2 (Fig. 5). At the riparian sites, the reproductive contribution to the next generation is concentrated in few plants producing

many seeds, whereas reproductive output at the serpentine sites is more evenly distributed among surviving plants.

Gene Flow Analysis

Estimated gene flow was calculated as $4N_e m$, or the number of foreign gametes present in the next generation. Migration rates were expressed as $M = 4N_e m / 4N_e \mu = m / \mu$ (Beerli 2002) and are scaled by maximum likelihood estimates of the mutation rate (μ), where N_e is the effective population size, and m is the estimated migration rate. Thus migration rates (M) represent the number of new alleles entering a population as the result of migration from other populations (m) relative to new alleles arising from within-population mutation (μ). Estimates were averaged across 10 microsatellite loci (Tables 4 and 5).

Maximum likelihood estimates of $4N_e m$ ranged from a minimum of 0.8 (from S2 to R2) to a maximum of 42.8 (from S1 to R3) with a mean value of 14.6. Population R3 received

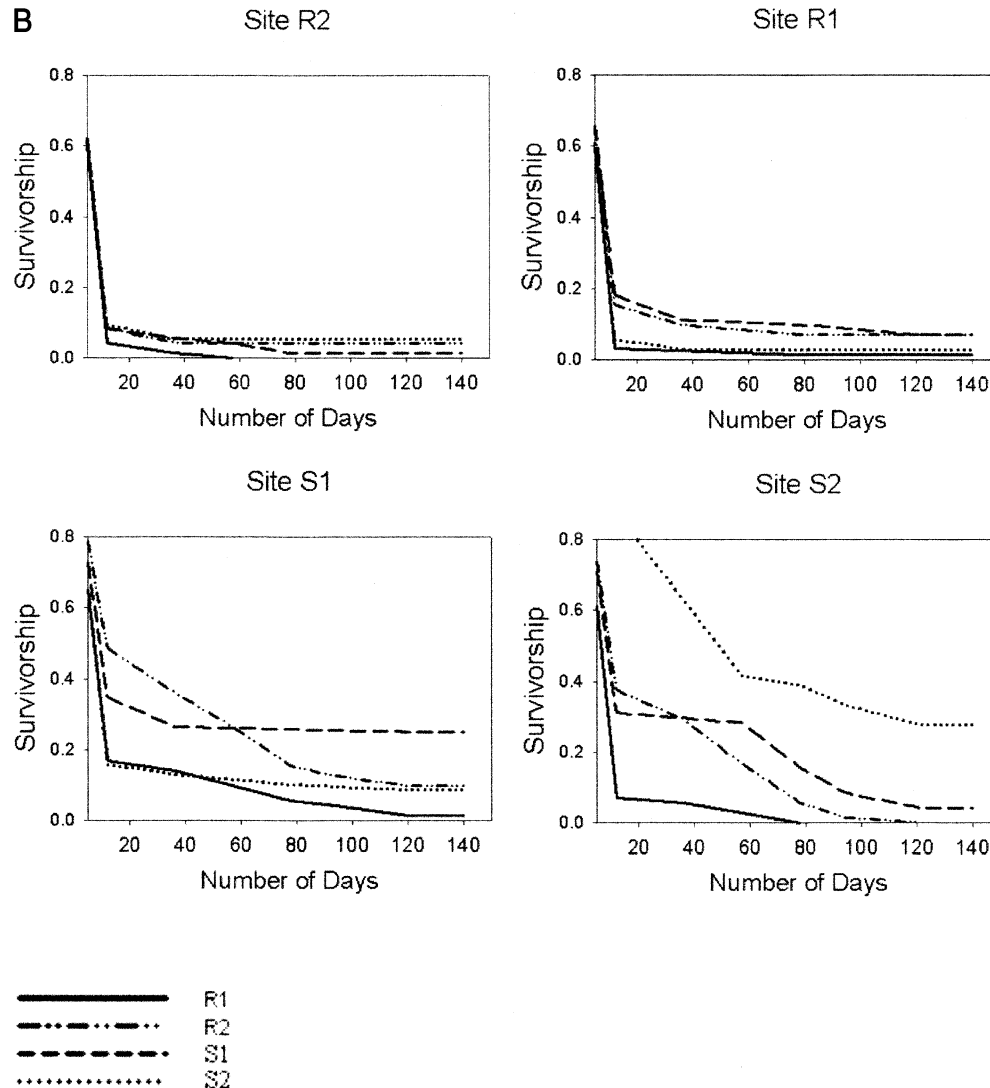


FIG. 2. Continued.

the greatest amount of gene flow (expressed as $4N_e m$) from all other populations (Table 4A). The lowest $4N_e m$ values were measured for population R2; it received few alleles from serpentine populations (i.e., the summed gene flow from populations S1 and S2 was 2.2). Serpentine populations exhibited higher values of immigration than the riparian populations R1 and R2.

Variation in maximum likelihood estimates of population mutation rates, $4N_e \mu$, (Table 4B) was between 0.83 and 7.91. Analysis of maximum likelihood estimates and their 95% confidence intervals reveals that riparian populations R1 and R2 have statistically indistinguishable $4N_e \mu$ values, and their rates are the smallest of all sampled populations (Table 4B). The same analysis also reveals that serpentine populations S1 and S2 do not differ from each other in $4N_e \mu$ (Table 4B) but have larger values than the riparian populations R1 and R2. The riparian population R3 has the highest estimated population mutation rate.

The range of M values (from 0.9 to 12.2) is smaller than the range of values of gene flow measured with $4N_e m$; the

mean value for M is 5.14. The largest values of M are found for estimate of emigration from population R1 into R2, the closest pair of populations (Table 1), and from population R3 into S1 (Table 5). The smallest values of M are found for emigration from population S2 into R2 and between populations R2 and S1 (Table 5).

Although most population pairs did not show strong asymmetry in gene flow (Table 5), there were some notable exceptions. Population R2 received relatively fewer immigrants from the serpentine population S2 than S2 received from R2. In contrast, there was a larger amount of immigration into R2 from R1 than into R1 from R2 (Table 5). Overall, the riparian population R3 acted as a “donor population” in that emigration from this population was always greater than rates of immigration into R3 (Table 5).

The mutation rate for microsatellites has been estimated experimentally to be on the order of 10^{-4} in wheat and maize (Thuillet et al. 2002; Vigouroux et al. 2002). Considering pairwise migration rates between populations (Table 5), the values of M range from 0.9 to 12.2; there was a maximum

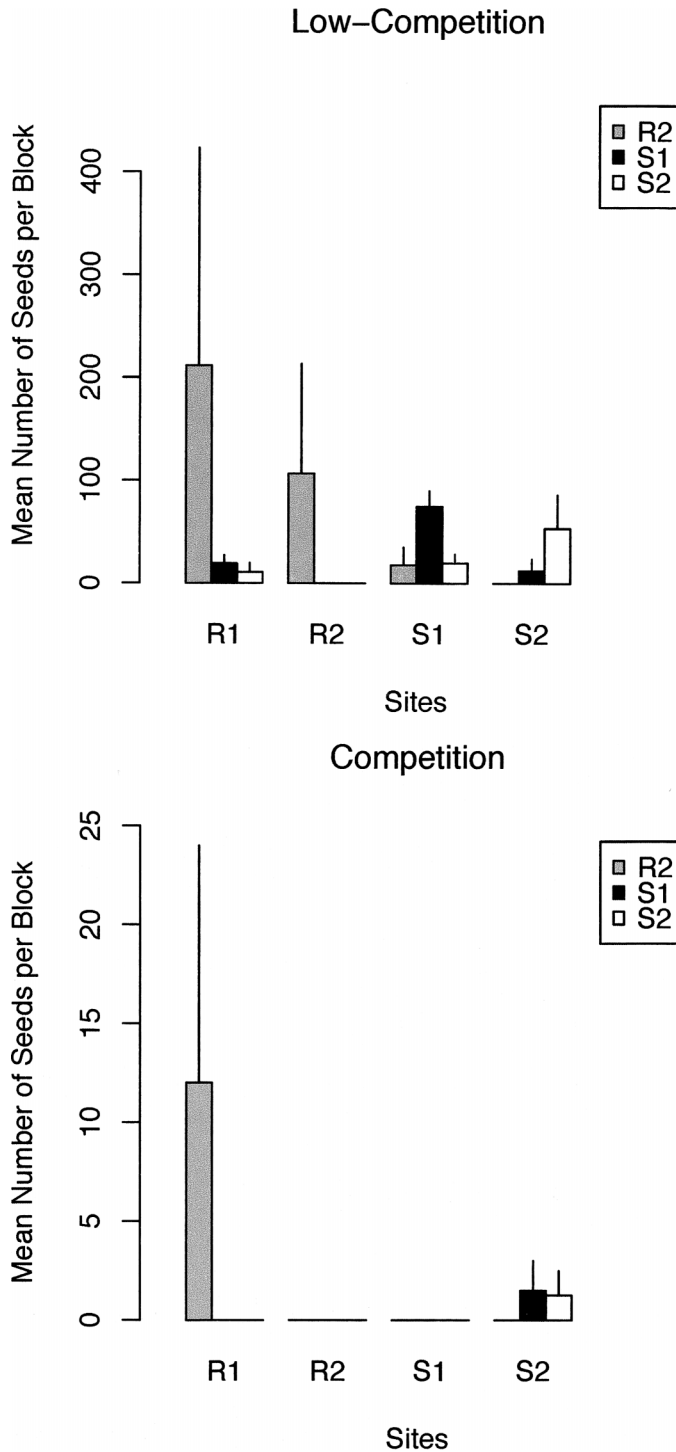


FIG. 3. Mean number of seeds per block (± 1 SE) produced per treatment combination of the 2002 reciprocal transplant experiment. Note the different vertical scales of the two graphs. Plants of R1 origin are not shown because they did not produce any seed in the period. The variance in seed output in both treatments is much greater in riparian sites, where a few plants contributed a disproportionate amount of seeds to the next generation.

of 25.6 for overall immigration (Table 5). Using 10^{-4} as a reasonable value for the microsatellite mutation rate (μ), migration rates (m) are estimated to vary between 10^{-3} and 10^{-4} .

DISCUSSION

Patterns of Local Adaptation

The results of the reciprocal transplant experiments support the hypothesis that local adaptation has occurred in *H. exilis*. In both years of the study, the significant seed-origin-by-site interactions in seed production were characterized by higher seed output from local plants; this form of crossing interaction indicates a fitness advantage for populations growing in their home site. Because we replicated planting sites within riparian and serpentine habitats, this conclusion can be extended to the habitat level. Serpentine or riparian plants generally perform better in their habitat of origin (Table 2 and 3). However, this local adaptation appears to be driven by different components of fitness depending on the habitat.

Higher survival for serpentine seed sources in serpentine sites is consistent across all experiments. This suggests that local adaptation in serpentine sites may be primarily driven by differential survivorship in response to more physically stressful conditions within serpentine habitats. As the season progresses, increasing drought stress might be an especially important cause of differential survival of seed sources in serpentine sites (Figs. 2B and 4). In particular, during the summer, the death of riparian plants in site S2 increased more rapidly than that of serpentine plants. Higher mortality in riparian source plants was also particularly pronounced in the late planting treatment in which plants had less time to grow before the onset of drought stress. Serpentine plants possess several traits likely to be adaptive to drought stress, which include larger early investment in roots, smaller leaves, higher water use efficiency, and earlier flowering time (Sambatti 2004).

The characterization of the selective environment regarding soil chemistry and soil water availability in the summer conforms with expectations that, compared to riparian sites, serpentine sites are more stressful. Serpentine sites exhibit lower Ca^{+2}/Mg^{+2} ratios, lower soil fertility as expressed by the concentration of soil P (Kruckeberg 1984), and greater soil moisture stress as the growing season progresses. Differences between sites R1 and R2 in soil chemistry are dominated by larger organic matter content in soils at site R1. This higher organic content may reflect the depositional environment of site R1; R1 is located in a flat area at the base of a hill, whereas R2 is located on a sloping hillside. Variation between serpentine sites in mean predawn water potentials estimate site differences in the amount of water is remaining in the soil. More negative values of predawn water potential observed in plants at site S1 suggest that soil water is more limited at this site than at site S2. Overall, lower soil water availability at site S1 should increase the amount of water stress experienced by plants at this site.

Although seed source had a significant effect on plant survival in serpentine habitats, site effects dominated patterns of mortality in riparian sites. Indications that environmental conditions determine patterns of mortality in riparian sites argue that expression of local adaptation results primarily

TABLE 4. (A) Maximum likelihood estimates and upper and lower 95% confidence intervals (CI) for migration expressed by $4N_e m$ for each population. Each particular $4N_e m$ value is the number of migrants per generation from populations indicated in the top row labels into populations indicated in the column labels. For example, the number of migrants per generation from population R1 into population R2 is 11, and the number of migrants per generation in the opposite direction is 6.1. The total number of migrants into a given population is the added contribution from each population. For example, in the case of population R1, the total number of migrants is 21.1 (6.1 + 3.6 + 5.8 + 5.6).

Population	Lower CI	Migration R1	Upper CI	Lower CI	Migration R2	Upper CI	Lower CI	Migration S2	Upper CI	Lower CI	Migration S1	Upper CI	Lower CI	Migration R3	Upper CI
R1				5.7	6.1	6.6	3.3	3.6	3.9	5.4	5.8	6.2	5.2	5.6	6.0
R2	10.4	11.0	11.6				0.7	0.8	1.0	1.2	1.4	1.6	4.4	4.8	5.2
S2	8.8	10.1	11.1	7.5	8.3	9.3				13.8	14.9	16.1	15.8	16.9	18.1
S1	15.4	16.8	18.1	4.5	5.2	5.9	11.8	13.0	14.1				37.9	39.8	41.6
R3	19.1	20.7	22.4	29.2	31.3	33.4	30.8	32.9	34.9	40.5	42.8	45.1			

(B) Maximum likelihood estimates and 95% confidence intervals (CI) of θ ($4N_e\mu$), the mutation rate μ scaled by the population size N_e , or the effective number of mutations per locus per generation.

Population	Upper CI	θ	Lower CI
R1	0.76	0.83	0.90
R2	0.82	0.90	0.99
S2	2.35	2.66	3.05
S1	2.96	3.36	3.83
R3	7.03	7.91	8.95

from seed source differences in reproductive output. As an example of strong seed source effects in reproduction, R2 plants were observed to produce one to two orders of magnitude more seeds than the other seed sources in riparian sites. In general, riparian plants seem to have a larger reproductive potential than serpentine plants, although the expression of this increased capacity does depend, in part, on environmental conditions.

Because field-collected seed was used in both reciprocal transplant experiments, our results on seed source effects may represent a composite effect of genotype and the seed maternal environment (Roach and Wulff 1987; Rossiter 1996). We tried to account for maternal effects in the planting date experiment by using seed weight as a covariate. Surprisingly, seed weight did not influence plant survivorship (Table 3). This indicates that the observed variation in seed size due to maternal effects was probably not important for plant survivorship. Overall, local adaptation at the site and habitat level was significant in both years of the study and persisted whether or not seed weight was included as a covariate. Although the effects of maternal environment cannot be eliminated entirely in field-collected seed, we believe that the consistency of the expression of "home site advantage" in our study argues strongly for the existence of local adaptation in *H. exilis*.

Our study suggests that disturbance in riparian areas can increase survival and reproduction significantly. In addition, in both habitats, local adaptation in terms of reproduction was only observed under reduced competition (see Fig. 3). However, it is possible that these disturbance effects are relatively transient. In fertile riparian sites, the resident vegetation, particularly grasses, will quickly regrow and compete vigorously for resources. Within riparian habitats, one might expect strong selection for increased competitive ability in riparian sunflower populations compared to serpentine plants. The morphology of riparian populations does suggest an adaptive response to competition for light. Riparian plants produce a higher number of nodes that increase plant height, have a larger leaf area and a higher relative growth rate, and

exhibit a lower investment to roots (Jain et al. 1991; Sambatti 2004). In riparian areas, nonreproductive plants are clearly those that do not grow fast enough to escape from light competition with grasses and other sunflowers. These plants were usually covered by dense vegetation and exhibited signs of severe etiolation (J. B. M. Sambatti, pers. obs).

At the population level, a striking consequence of competition in riparian sites is the production of a strong size and reproductive hierarchy among conspecifics. It is thought that more productive environments increase plant size hierarchies as a result of more intense competition for light, whereby larger plants are able to acquire a disproportionately larger share of resources by suppressing their smaller neighbors (Weiner 1985, 1990). A consequence of size hierarchies for local adaptation relates to the influence of reproductive variance on the effective population size of the population undergoing selection. In a population with a strong reproductive hierarchy, few individuals contribute most of the seeds to the next generation and the effective population size can be greatly reduced relative to the census population size (Heywood 1986; Rice 1990). The strong reproductive hierarchies observed in populations R1 and R2 may partially explain the smaller effective sizes estimated for these populations (see discussion below). Genetic drift may become a

TABLE 5. Mean values of maximum likelihoods estimates of M ($4N_e m / 4N_e \mu = m / \mu$) for each pair of population. Each particular M value is the migration rate from populations indicated in the top row labels into populations indicated in the column labels. For example, the migration rate from population R1 into population R2 is 12.2, and the migration rate in the opposite direction is 7.4. The sum of incoming M is the sum of the rows.

	R1	R2	S2	S1	R3	Sum of incoming M
R1		7.4	4.4	7.0	6.8	25.6
R2	12.2		0.9	1.5	5.3	19.9
S2	3.8	3.1		5.6	6.4	18.9
S1	5.0	1.5	3.9		11.8	22.2
R3	2.6	4.0	4.2	5.4		16.2

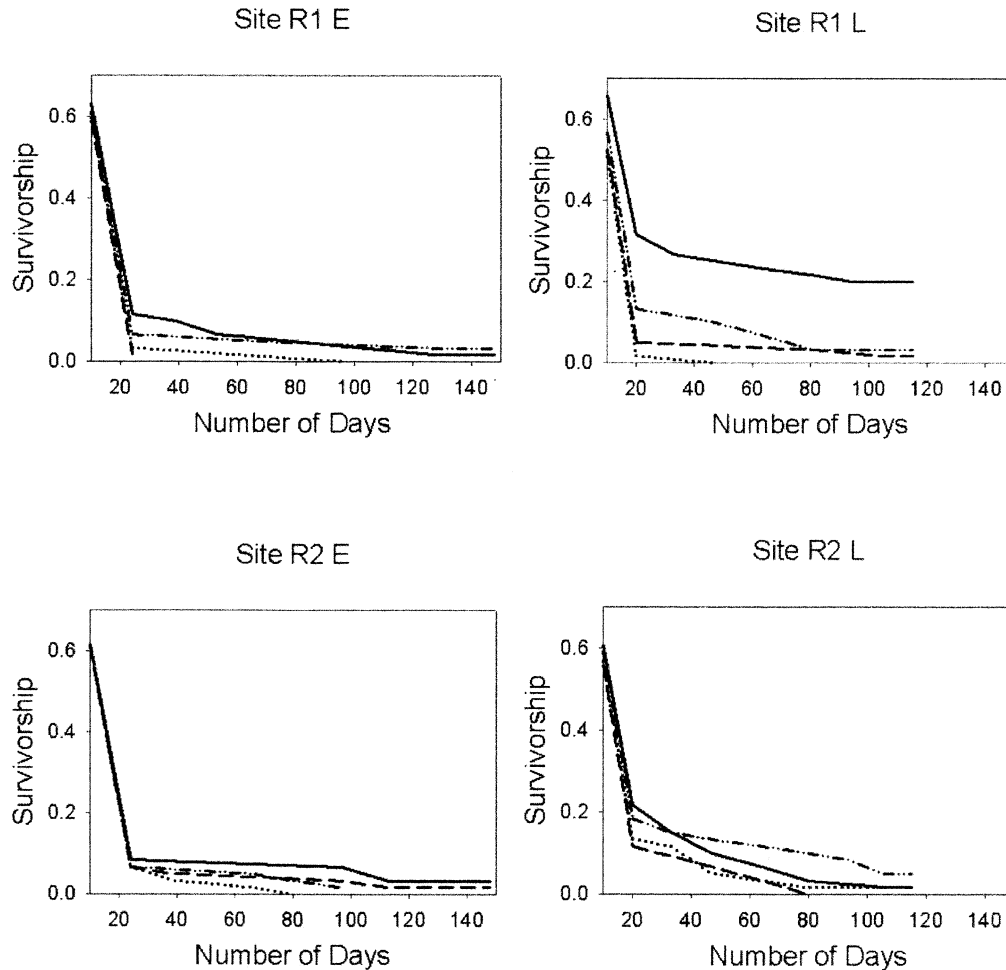


FIG. 4. Survivorship of cohorts in the planting date experiment. E and L indicate early-planted (surveys during ~140 days) and late-planted treatments (surveys during ~120 days). In general, survivorship did not differ among seed sources in early plantings. Survival was somewhat higher in local sources in the later planting treatment. The exception is site R2, where regardless of the planting date, mortality was high and dominated by site conditions.

much more important force in riparian sites compared to serpentine sites because the reproductive output is less evenly distributed among reproducing individuals in riparian sites. Consequently, for a fixed amount of additive genetic variance, the smaller the effective population size, the larger selection pressures must be to produce the same selective response (Hartl and Clark 1997).

Variation in emergence time can potentially amplify the effect of size and reproductive hierarchies and their evolutionary consequences (Fowler 1984; Weiner 1990; Miller et al. 1994; Dyer et al. 2000). In our study, early germination was observed primarily in riparian sites where competition is presumably more intense. However, our observations of higher mortality in earlier cohorts suggest a potential fitness cost to early germination. Heavy rains and flooding (which actually occurred in early spring of 2003) and seedling mortality from frost heaving are possible causes of catastrophic mortality among early-germinating seeds. Although we did not detect an effect of planting date on reproduction, the risk of early-germination might be compensated for if the amount of seeds produced by the survivors in early-germinating cohorts is much larger than those produced by plants emerging

later. We suspect that the evolution of this early-germination strategy is less likely to occur in serpentine populations because the lower fertility of serpentine soils considerably limits plant size and consequently seed output. If only a large reproductive output among survivors can compensate for the increased mortality risks associated with early emergence, the low fertility of serpentine sites should reduce the capacity for reproductive compensation.

Gene Flow among Populations

The balance between selection and gene flow determines to what extent a given trait will be differentiated between populations (Lenormand 2002; McKay and Latta 2002). In fact, cases where gene flow is strong provide the most convincing evidence that strong selection is acting to maintain ecotypic differentiation (Vekemans and Lefèbvre 1997; Brown et al. 2001; Kittelson and Maron 2001; McKay et al. 2001). Selection coefficients can effectively maintain allelic variation if, on a per locus basis, selection coefficients are larger than the estimated migration coefficient (Slatkin 1987; Brown et al. 2001). Applying this model to our data, selection

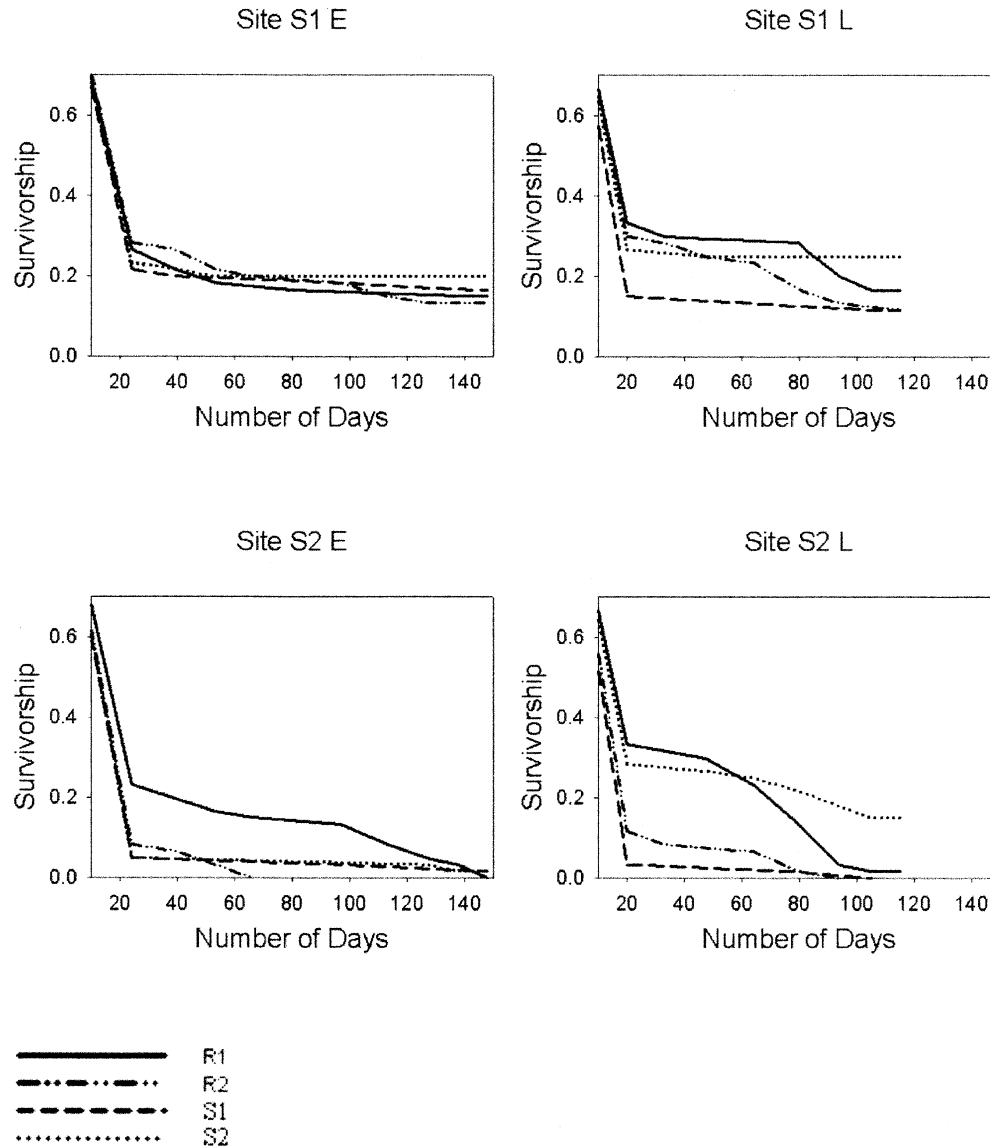


FIG. 4. Continued.

intensities on a per-locus basis must be higher than the estimated migration rates between riparian and serpentine sites (i.e., above 10^{-3}) to maintain allelic variation underlying observed ecotypic differences. Selection is predicted to be strong against foreign genotypes in sites S2, R1, and R2 where selection coefficients are close to one (Figs. 3 and 5). Because the selection coefficient at a given locus is a function of the phenotypic differences between alternative allelic genotypes (Falconer and McKay 1996), only a careful analysis of the genetic architecture of serpentine and riparian adaptation traits can determine the efficiency with which selection maintains ecotypic variation under varying rates of gene flow (see Mitchell-Olds 1995; Ungerer and Rieseberg 2003; Bradshaw and Schemske 2003).

Because the maximum likelihood estimate of the mutation rate (μ) is assumed to be the same across populations (Beerli 2002), the variation in $4N_e\mu$ among populations is expected to be almost entirely attributable to variation in their effective

population sizes (N_e). Thus, of the populations sampled, population R3 has the largest effective population size. For the reciprocal transplant populations, the serpentine populations have a larger effective population size than the riparian populations. Smaller effective population size estimates for the riparian populations R1 and R2 is consistent with our experimental results indicating that reproduction at these sites is concentrated in a few individuals.

With the same assumption of a constant mutation rate (μ) across populations, all the variation in M ($M = 4N_e m / 4N_e \mu = m/\mu$) should be due to variation in m (Table 5). The smaller variation of M (i.e., m/μ) compared to $4N_e m$ indicates that a large component of variation in $4N_e m$ is also caused by variation in effective population size (N_e). For example, population R3 was estimated to have the largest number of immigrants because it also has the largest effective population size ($4N_e \mu = 7.91$) and not necessarily because of large values for m (or M). Similarly, the larger number of immigrants

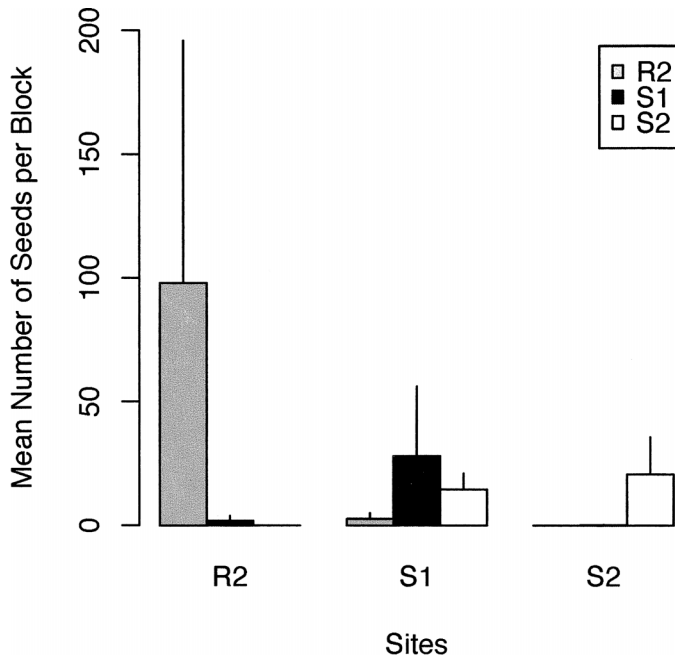


FIG. 5. Mean number of seeds per block (+1 SE) produced per origin per site in the planting dates experiment. Local adaptation is indicated at all sites by the higher reproductive output of the local seed source. Site R1 was excluded because no seeds were produced due to accidental herbicide spraying. Seed source R1 was also excluded because R1 plants did not produce any seed in any site. Variance in seed output was much greater in riparian sites, where a few plants contributed a disproportionate amount of seeds to the next generation.

into serpentine populations (S1 and S2), compared to riparian sites (R1 and R2), was also correlated with larger effective population sizes in the serpentine populations.

When values of $4N_e m$ are greater than four, it is predicted by theory that populations behave as panmictic units (Wright 1931). In general, gene flow among the populations we examined was estimated to exceed this critical value. Thus, the sunflower populations at the McLaughlin reserve should not be envisioned as a system of isolated populations, but rather as a relatively cohesive system of population patches that freely exchange genes with each other. The primary exception is population R2, which appears to receive most of its gene flow from population R1. Populations R1 and R2 are located in riparian sites and are somewhat isolated from most serpentine populations. High rates of gene flow between R1 and R2 are expected because these are the closest pair of populations. However, the high rates of gene flow from population R3 into S1 are not so obviously explained by geographic proximity. Because the distance between these populations is intermediate in relation to the other populations (Table 1), it suggests that other currently unknown factors may also affect gene flow in this system.

Analysis of gene flow estimates involving population R3 suggests that effective population size rather than habitat type is a better predictor of rates of gene interchange among populations. Population R3 is located in the core of the distribution of this species in a serpentine area—it is serpentine and riparian—and close to several serpentine seep popula-

tions. Its larger effective population size is somewhat surprising in that its census number of individuals is much smaller than the numbers observed in populations S1 or S2 (J. B. M. Sambatti, pers. obs.). Our analysis suggests that population R3 exists as a riparian portion of a patchwork of both serpentine seep and riparian populations. Because of its central location and relatively large effective population size, population R3 would be expected to provide a greater number of migrants than smaller satellite populations.

The traditional view of speciation strongly emphasizes adaptation as a crucial first step followed by reproductive isolation (Wu 2001). Combining our gene flow estimates with experimentally estimated selection coefficients, we conclude that, despite intense and contrasting selection pressures between habitats, there does not appear to be any form of reproductive isolation between riparian and serpentine populations. Neither prezygotic nor postzygotic barriers were detected. Flowering times of both taxa overlap considerably (although serpentine plants tend to flower earlier; J. B. M. Sambatti, pers. obs.), geographic barriers are not strong, and artificial crosses were easily performed and produced fertile offspring. The absence of even prezygotic reproductive isolation among *H. exilis* populations further facilitates the cohesive effects of gene flow. *Helianthus exilis* is a species composed of locally adapted populations that diverge in many ecological attributes but remain tightly linked by gene flow. Thus, despite strong selective differences among the habitats occupied by *H. exilis*, we suggest that extensive gene flow probably reduces the chance for speciation to occur between riparian and serpentine sunflower populations. Given the empirical and theoretical evidence that sympatric or parapatric speciation is more likely when ecological specialization and the evolution of reproductive isolating traits occur simultaneously (Dickinson and Antonovics 1973; Johannesson 2001; Greenberg et al. 2003), it would be interesting to test the generality of our findings in a similar environmental mosaic but with a species that exhibits more variation in mating system and outcrossing rates.

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