Evolutionary Ecology Research, 2006, **8**: 1–21

Local adaptation to serpentine and non-serpentine soils in *Collinsia sparsiflora*

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ABSTRACT

Question: Are populations of a native California annual plant locally adapted to serpentine and non-serpentine soils?

Study species: *Collinsia sparsiflora* (Scrophulariaceae *s.l.*), which grows both on and off serpentine soils.

Field sites: We studied six populations at the McLaughlin University of California Natural Reserve, in the North Coast Range of California. All sites were within 1 km of each other, but were notably variable for a number of soil characters, including concentrations of calcium and magnesium, key factors in serpentine soils.

Methods: We conducted reciprocal transplant experiments in 2002 and 2003. The 2002 experiment involved four study populations, two serpentine and two non-serpentine. In 2003, an additional population on each soil type was included in a second reciprocal transplant study. Seeds generated in greenhouse crosses were planted reciprocally into each population, providing replication within the soil types.

Results: Both experiments demonstrated the presence of significant reciprocal adaptation to soil type. We also observed variation in phenology between transplants on the two soil types. We conclude that plants from these six populations represent two distinct, soil-specific ecotypes, raising questions about the relative role of selection and gene flow in promoting genetic differentiation in these contrasting environments.

Keywords: *Collinsia sparsiflora*, ecotypic differentiation, fitness, local adaptation, phenology, reciprocal transplant experiment, serpentine soils.

INTRODUCTION

Divergent selection in contrasting environments can result in populations that are adapted to conditions in their local habitat. Much theoretical work has focused on modelling the conditions under which local adaptation will occur. Major factors include the strength of divergent selection pressures (Haldane, 1956; Holt and Gaines, 1992; Gomulkiewicz and Holt, 1995;

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Kawecki, 1995), the extent to which important phenotypic variation is heritable (van Tienderen, 1992), and levels of gene flow between populations due to dispersal and immigration (Holt, 1996; García-Ramos and Kirkpatrick, 1997; Holt and Gomulkiewicz, 1997; Kirkpatrick and Barton, 1997; Ronce and Kirkpatrick, 2001; Kawecki and Holt, 2002; Kisdi, 2002; Lenormand, 2002). These models agree in finding that local adaptation is a likely evolutionary outcome when gene flow between divergent selection regimes is insufficient to homogenize locally adapted gene complexes.

Local adaptation is typically demonstrated by using reciprocal transplant experiments, where individuals from different source populations are moved into a range of destination sites. A population is locally adapted if controls re-planted into their home site out-perform individuals moved there from other sites. Although many studies using this design have demonstrated adaptation of plants to their home site (Waser and Price, 1985; Jordan, 1992; Bennington and McGraw, 1995; Nagy and Rice, 1997; Hämmerli and Reusch, 2002; Volis *et al.*, 2002; but see Helenurm, 1998), few have identified the critical environmental parameters leading to adaptive differentiation (e.g. Montalvo and Ellstrand, 2001; Volis *et al.*, 2002). To infer which axes of environmental variation are most important in engendering adaptive differentiation requires transplantation among multiple sites, each of which has been thoroughly characterized. For example, the classic experiments of Clausen *et al.* (1940, 1948) demonstrated the existence of high-, middle- and low-elevation ecotypes in several species of broadly distributed plants because multiple replicates of each elevation class were used.

Given the intimate association between plants and soils, it is not surprising that several well-documented cases of local adaptation in plants are associated with edaphic heterogeneity (Kruckeberg, 1951, 1954, 1967; Antonovics *et al.*, 1971; Macnair, 1987; Ernst *et al.*, 1992; Al-Hiyalay *et al.*, 1993). As sessile organisms, individual plants are restricted to the edaphic environment where they germinate, and to succeed must be able to survive and grow in that soil habitat. Soil supplies water, supports microbial mutualists and is the source of most plant nutrients, but can also harbour pathogens and contain toxins such as heavy metals, alkaline compounds, salt and environmental contaminants. These soil attributes have the potential to exert the types of diverse selective pressures that can result in local adaptation. The most extreme cases of local adaptation to soil chemistry concern plant populations growing on soils derived from mine tailings that are contaminated with heavy metals. In some of these mine sites, selection has generated strong gradients for metal tolerance over very short spatial distances and over very short periods of time (less than 200 years) (Antonovics *et al.*, 1971; Macnair, 1987; Ernst *et al.*, 1992). Rapid evolution of heavy metal tolerance has also been documented for plants growing under electricity pylons (Al-Hiyalay *et al.*, 1993). The evolution of these heavy metal-tolerant ecotypes demonstrates how quickly edaphic challenges can lead to adaptive differentiation in plant populations.

Compared with strong evidence for plant adaptation to edaphic conditions of anthropogenic origin, there are fewer experimental demonstrations that plant species have diversified genetically in response to naturally occurring soil heterogeneity. The evolution of natural soil ecotypes seems likely (Kruckeberg, 1984), and many floras include examples of sub-specific variants associated with different soil types. However, without experimentation, one cannot know whether such variants are either genetically differentiated or locally adapted.

Exposed ultramafic, serpentinized rocks weather into soils of unique chemistry and composition, which often support unique vegetation. Serpentine soils have an extremely low calcium : magnesium (Ca : Mg) ratio, usually below 0.5. They are often high in heavy metals (particularly nickel), as well as low in nutrients, and they tend to be xeric. However, it is the calcium and magnesium that appear to be the driving force in making serpentine soils inhospitable to non-tolerant plants (reviewed in Brooks, 1987). Magnesium is toxic at high concentrations. Calcium appears to play an important role in countering the negative effects of heavy metals such as nickel (Brooks, 1987), so at low calcium concentrations, the toxic effects of metals are more severe (Johnson and Proctor, 1981). While these are characteristics that are common to serpentine soils, there is a great deal of variation in their chemical composition, reflecting site-to-site variation in the parent material.

Serpentine soils occur in many regions around the world and are generally characterized by high levels of endemism and species diversity (Brooks, 1987), as well as by low frequencies of invasive species (Kruckeberg, 1984; Harrison, 1999a, 1999b). These observations are consistent with the idea that the challenging chemistry of serpentine soils exerts unusual selection pressures on the plant communities and populations that grow there. If so, differential selection on and off of serpentine soils could result in ecotypic adaptation in species that grow on both soil types. In a series of pioneering studies, Kruckeberg (1951, 1967) compared serpentine and non-serpentine populations of 21 California plant species and 18 species from Washington State by collecting seeds from both soil types and exposing them to serpentine soil in the greenhouse and/or in the field. In about 50% of the species tested, plants from non-serpentine populations performed worse than serpentine plants when grown on serpentine soils, leading Kruckeberg to conclude that these species had differentiated into serpentine and non-serpentine ecotypes.

While Kruckeberg's studies are rightly considered classics in ecological genetics, there are compelling reasons to re-visit the issue of adaptation to serpentine versus non-serpentine soils. First, it is important to test the hypothesis of adaptation using *reciprocal* transplant experiments. Otherwise, it is possible that the superior performance of serpentine plants (versus non-serpentine plants) observed in serpentine soils might also be observed on non-serpentine soils. In other words, plants from serpentine populations may show greater general vigour. Second, Kruckeberg's studies were mostly conducted in the greenhouse, and each study used only a single sample of serpentine soil at a time, making it impossible to compare effects of transplantation within versus between soil types. Third, the use of field-collected seeds can confound effects of genetic differentiation with those of varying maternal environment (Roach and Wulff, 1987). Finally, although serpentine habitats are of great importance to plant conservation, to our knowledge there are no other peer-reviewed studies which used replicated reciprocal transplant experiments in the field to demonstrate intra-specific adaptation to serpentine versus non-serpentine soils [for an example of a reciprocal transplant experiment across soil types that did not find local adaptation to serpentine versus non-serpentine grassland soils, see Jurjavcic *et al.* (2002)].

In this study, we conducted two reciprocal transplant experiments across multiple plant populations to determine whether soil-specific ecotypes have evolved in *Collinsia sparsiflora* (the Spinster's Blue Eyed Mary), a native California annual that grows on both serpentine and non-serpentine soils. At the McLaughlin Natural Reserve, where this work was conducted, transitions between serpentine and non-serpentine soils occur over fine spatial scales, from tens to hundreds of metres. Because of this local edaphic heterogeneity, populations of plants that may be adapted to contrasting soil types are separated by distances within the flight range of many pollinators. Ultimately, we hope to use this system to understand how differential selection and gene flow interact in promoting or inhibiting adaptive differentiation in response to soil type. In this paper, we address the following question: Is there local adaptation to serpentine versus non-serpentine soils in *Collinsia* *sparsiflora* at the McLaughlin Reserve? Because reciprocal transplants were conducted between more than one site of each soil type, we can distinguish between adaptation to soil type *per se* versus adaptation to other characteristics of specific home sites.

METHODS

Study species

The genus *Collinsia* (Scrophulariaceae *s.l.*) (Olmstead *et al.*, 2001) is listed as being potentially *Bodenvag* (occurring on and off of serpentine soils) by Kruckeberg (1984). Our study species, *Collinsia sparsiflora*, is a winter annual that germinates after the first rains in October or November, flowers in February or March, and then dies when the rains end in May or June.

Preliminary characterization of the study sites

Serpentine soils are derived from ultramafic (high in iron and magnesium) or serpentine parent material. The upheaving processes that result in serpentine rocks being exposed are very disjointed, and therefore islands of serpentine are formed, with potentially sharp boundaries between serpentine and non-serpentine soils. In California, mountains of the Coast Range and the Sierra Nevada are peppered with serpentine substrates.

This work was conducted at the McLaughlin University of California Natural Reserve in the North Coast Range of California in Napa, Lake and Yolo counties (reserve website: http://nrs.ucdavis.edu/mclaughlin.html). The reserve is situated on a minor fault line, along which gold was discovered and extracted by the Homestake Mining Company. As a result of that fault line, the diversity of the soil types at the reserve is remarkable, reflecting a fine-scaled mosaic of serpentine, volcanic and valley sediment parent materials. In 1999, we chose four study sites where *Collinsia sparsiflora* occurs naturally for the first reciprocal transplant experiment. In 2002, while the first experiment was progressing, two more populations were identified and included in the second reciprocal transplant. Based on local vegetation, we tentatively identified three of these sites as serpentine (S1, S2 and S3) and three as non-serpentine (NS1, NS2 and NS3) (Table 1). Sites initially characterized as non-serpentine have many more grasses and much greater vegetation cover, including a partial over-story of deciduous oaks. In contrast, our three serpentine sites are much more open, with some areas of bare soil and rock. Only one of those sites (S3) had any oak canopy, which created variation in shading across study plots. There are also changes in species composition between the serpentine and non-serpentine sites. Species that grow on the non-serpentine sites, but which are not found on serpentine soils, include *Dodecatheon hendersonii*, *Torilis* spp. and *Nemophila menziesii. Gilia tricolor* and *Linanthus dichotomus* occurred on serpentine soils, but not at the non-serpentine sites.

Reciprocal transplant experiments to test for adaptation to soil type

Experiment 1: 2002

In the spring of 1999, seeds were collected from each of four *C. sparsiflora* populations (S1, S2, NS1 and NS2), returned to the greenhouse and planted into their native soil. They were

Table 1. Characteristics of the three serpentine (S) and three non-serpentine (NS) *Collinsia sparsiflora* study sites

	Population code					
	S1	S2	S3	NS1	NS2	NS3
Distance from NS2	129 m	74 m	215 m	951 m	0 m	892 m
Aspect	north	east	north	north-west	north	north
2001 LAI	0.220	0.154	NA	1.052	1.083	NA
Trees present?	no	no	few	yes	yes	yes
Site name	Saddle Ridge	Mercury Meadow	Knoxville Woods	Gold Pit	Mercury Woods	Clover Valley

Note: Distance from the most central site was estimated from GPS coordinates. Aspect is approximate. Leaf area index (LAI), an indicator of overhead canopy, is the average of a series of measurements taken 2 cm above ground level along a transect through four of the study sites in 2001. Trees were deciduous oaks, which were found on all three non-serpentine sites and only one of the serpentine sites. NA = not available.

sub-irrigated with water containing ample amounts of supplemental nitrogen, phosphorus, potassium, calcium and magnesium. Within each source population, crosses were performed to produce seeds for the field-transplant experiment.

The seeds from these crosses were planted out in a reciprocal transplant experiment in the winter of 2000. At each of the two serpentine and non-serpentine destination sites, seeds from all four source populations were planted into a randomized block design, with five blocks (100 × 50 cm) per destination site. For transplantation, soaked seeds were placed into small (~0.7 cm) plastic collars containing the native soil for that destination site. Each block was protected by a cattle-exclusion cage with an approximately 5 × 5 cm mesh size. The cages also had the effect of reducing large mammal herbivory throughout the growing season. Deer and hares were present on the reserve and could have potentially consumed the plants; however, the potential impact of trampling by cattle outweighed our concerns about the possible reduction in natural herbivory caused by the cages.

Once the plants began to initiate flower buds, the cattle-exclusion cages were covered with fine-mesh cloth, making them into pollinator-exclusion cages, which prevented pollen movement from experimental blocks into the local populations. We had previously found genetic differences between *C. sparsiflora* populations occupying serpentine and non-serpentine sites at McLaughlin (J.W. Wright *et al.*, unpublished data), so we did not want to disrupt the natural population structure of these sites through genetic contamination. Tests were conducted on field plants to determine the effect of pollinator exclusion on fruit set. Because *C. sparsiflora* is fully capable of self-pollination, fruit set was statistically indistinguishable for plants placed under pollinator exclusion bags and those left open to pollinators (J.W. Wright, unpublished data).

In the winter of 2000, there was very low emergence of transplanted seeds; only 121 seedlings (out of 1749 planted seeds) emerged in 2000 (7% emergence). The seed collars were left in place and in the fall of 2001, 700 seedlings emerged across the four sites (representing 40% of seeds planted). Only data from the 2001–2002 cohort, which flowered in 2002, will be considered in this paper.

Results of this first transplant experiment were consistent with our hypothesis that *C. sparsiflora* had differentiated into serpentine and non-serpentine ecotypes (see Results). However, because experimental seeds had been collected from plants grown on their native soil in the greenhouse, there was a possibility that maternal environmental effects had contributed to the apparent pattern of local adaptation to soil type. Also, we sought to increase replication of both serpentine and non-serpentine sites. Therefore, a second reciprocal transplant experiment was conducted to address the limitations of the first, and to determine if the pattern of adaptation to soil type would be robust across years.

Experiment 2: 2003

In the spring of 2001, seeds were collected from six sites, three serpentine and three non-serpentine. These seeds were returned to the greenhouse and grown up in greenhouse soil, a 1 : 1 : 1 mix of ProMix® (Premier Horticulture Ltd, Dorval, Canada), Turface® (Profile Products LLC, Buffalo Grove, IL) and sand. Crosses were performed within each population to produce experimental seeds for transplanting, as for the first experiment. To maximize germination, these seeds were planted into the field in June 2002, so that they would experience summer conditions and germinate with the naturally occurring seeds in the fall. Seeds were glued to toothpicks using a tiny drop of water-soluble glue, and these toothpicks were inserted into 30 × 50 cm plots. There were again five replicate blocks at each site. Cattle were removed from the reserve in the spring of 2002, and therefore cattle-exclusion cages were not required for the second experiment. However, to reduce the potential for genetic contamination, we again covered each of our experimental blocks with a pollinator exclusion cage as plants came into flower. Altogether, 46.5% of the seeds planted emerged in 2003, for a total of 1340 seedlings.

Field data collection

Data collection procedures were similar in both 2002 and 2003. We measured three phenological attributes for each transplant: emergence date, flowering date and the date the senescent plant was collected (death date). We then determined each plant's lifetime production of flowers and fruits. As fruits matured, they were removed just before dehiscence to prevent genetic contamination of the local population through seed introductions.

Characterization of soils within experimental sites

At the end of the 2001–2002 growing season, soil samples were collected at a depth of 5–20 cm from immediately next to each of the blocks used in the first experiment. At the two sites used only in 2003 (S3 and NS3), soil was collected in a similar manner from five locations within each site. Samples were sent to A&L Western Agricultural Laboratories, Inc. for analysis (Modesto, CA; soil test suite S3C). Sulphate and all nutrient cations were extracted using neutral 1 M ammonium acetate: potassium (ppm), magnesium (ppm), calcium (ppm) and sodium (ppm). Soil pH and hydrogen concentration (in meq · 100 g^{-1}) were measured on soil solutions. Nitrogen (as NO_3; ppm) was extracted using a potassium chloride extraction and cadmium reduction of nitrate to ammonium. Zinc (ppm), manganese (ppm), iron (ppm), copper (ppm) and boron (ppm) were extracted with barium chloride. Concentrations of soluble salts (mmhos · cm^{-1}), cobalt (ppm) and nickel (ppm) were measured, and texture analysis revealed fractional percentages of sand, silt and clay.

Data analysis

Characterizing soil heterogeneity

All analyses were conducted in SAS version 8.02 (SAS Institute, Cary, NC). To summarize potentially important axes of soil variation, soil data from each experimental block were subjected to principal components analysis (PROC PRINCOMP). We plotted the first two principal components to characterize patterns of soil variation within and among the six study sites. Analysis of principal components verified our initial categorization of the six *C. sparsiflora* population sites as serpentine or non-serpentine (see Results).

Analysing phenological responses

Phenology data were analysed using PROC LIFETEST, which compares the timing of phenological events for individuals from defined categories, and determines if temporal patterns vary among those categories (Fox, 2001). We used this method to examine emergence date, flowering date and survivorship for *C. sparisflora* transplants in 2002 and 2003. Two independent analyses were done for each of the three phenological characters, the first comparing survivorship curves for plants experiencing the two destination soil types (serpentine and non-serpentine). The second analysis tested for effects of source soil type within each of the two destination soil types. For hypothesis-testing, PROC LIFETEST generates chi-squared values using a Wilcoxon test.

Testing for specific home site advantage

We did a preliminary analysis to ensure that any apparent adaptation to serpentine versus non-serpentine soil type was not being driven by adaptation to specific home site attributes. Consider a serpentine destination site for the 2002 experiment, into which we planted seeds from four source populations. Seeds from both non-serpentine source sites are non-native to the destination; in contrast, half of the seeds from serpentine sites originated in that specific destination. It was therefore important to determine if genotypes were adapted to specific home sites, within either the serpentine or non-serpentine soil type category. We tested for specific home-site advantage using a general mixed-model analysis (PROC MIXED). The model was designed to test for differences in performance between plants growing on their source soil type at a given site, some of which were ‘at home’ on that specific site, while the rest were alien to that site. For example, plants from NS1 growing at NS1 were considered ‘home’ plants, and they were compared with the ‘away’ plants from NS2 growing at NS1. Plants from S1 or S2 growing at NS1 would be excluded, as they are from a different soil type. We calculated relative fruit and flower number as standard deviation units, by subtracting each individual value by the site mean, and dividing by the standard deviation for each site. The model included the fixed effects of transplant type (‘home’ vs. ‘away’), destination soil type (serpentine vs. non-serpentine), and their interaction. The random effects of site within destination soil type, transplant type × site within destination soil type, and block within site were included in the model, but are not discussed further here. PROC MIXED assumes that the random factors are normally distributed, and with a low number of random factors (2) testing for the statistical significance of random effects is fraught with peril (Littell *et al.*, 1996, p. 139). Therefore, we present only the tests for significance of fixed effects. We used the Kenwardroger method to determine the degrees of freedom for the analysis, which takes into consideration variation within treatment groups (SAS Institute Inc., 1999). As described in the Results section, we found no specific home-site advantage for either year, so while subsequent general mixed-model analyses included the random effect of site, we did not distinguish between sites of origin, except by their soil type categories.

Testing for local adaptation – categorical performance measures

For each of the two transplant experiments, categorical analysis was used to determine the relationship between source soil type, destination soil type and survival to flowering or fruiting using the CATMOD procedure (Christensen, 1997). Each transplant was placed into the appropriate category for source soil (non-serpentine or serpentine), destination soil (non-serpentine or serpentine) and survival to flowering/fruiting (yes or no). Categorical analysis was then used to determine if there was any association between these three factors. We used a model-building technique, in which each possible model is compared with the complete model, which contains all main effects and interactions, including the three-way interaction of source soil × destination soil × flowering (or fruiting) success. Local adaptation is demonstrated by a significant three-way interaction, indicating a relationship between all three main effects (destination soil type, source soil type and survival to flowering or fruiting) and thereby showing local advantage of plants growing on their home soil type. The complete model will always be the one that best describes the data, so the null hypothesis being tested is that each model we construct describes the data as well as the complete model. If the null hypothesis is not rejected, then the simpler model describes the data as well as the complete model. If the null hypothesis is rejected for all of the possible reduced models, then the complete model is the model that best describes the data, and the three-way interaction of source soil × destination soil × flowering, found only in the complete model, can be considered significant.

Testing for local adaptation – continuous performance measures

To test for adaptation to serpentine versus non-serpentine soils across four study sites in 2002 and all six sites in 2003, continuously distributed fitness characters were analysed using a general mixed-model analysis (GMM) and the MIXED procedure in SAS. Source soil type and destination soil type were considered fixed effects. The random effects of site within destination soil type and block within site, as well as the necessary interactions, were also included in the model. We report the significance of fixed effects here, since they were the focus of our investigation. A Levene's test (Levene, 1960) showed that there was significant heterogeneity of variances among blocks (i.e. for the effect of block nested within destination site) in both 2002 and 2003. To reduce the effects of that heterogeneity on hypothesis-testing, we conducted a weighted GMM, weighting each observation by the inverse of the variance of the residuals for the block into which that individual was planted (Neter *et al.*, 1996). The results generated by the weighted GMM were similar to those generated by an unweighted test, but significance levels were generally lower using the weighted GMM, except for flower number in 2003, where both the weighted and unweighted tests gave strongly significant results, but the unweighted GMM gave a slightly higher level of significance for the interaction between origin and destination soil type.

Local adaptation is demonstrated when individuals perform relatively better in their native environment, compared with individuals transplanted into those conditions from other environments. Since destination sites undoubtedly vary in overall quality, it is relative performance within sites that is of primary interest. For this reason, we tested for local adaptation by analysing the influence of source and destination soil types on relative fitness within sites. For each destination site, means and standard deviations were calculated for each measure of performance (lifetime fruit or flower production). Subsequently, individual phenotype values were subtracted from the mean for that destination site and then divided by the standard deviation. This results in each plant's phenotype being expressed in units of standard deviation from the local mean (Sokal and Rohlf, 1995).

RESULTS

Principal components analysis of 22 soil attributes shows that our serpentine study sites differ consistently from our non-serpentine sites for a number of soil attributes. Serpentine and non-serpentine sites segregate with respect to principal component 1 (PC1), which explains 33.3% of the sampled variation in soil attributes (Fig. 1). Non-serpentine sites have positive values for PC1 (indicating soils with high levels of calcium and nitrogen, and low levels of magnesium), while serpentine sites have, in general, negative values for PC1 (Table 2, Fig. 1). These results support our preliminary classification of soil types within these study populations, with serpentine sites having Ca : Mg ratios much less than 1, and non-serpentine sites having Ca : Mg ratios greater than 1. PC2, with heavy loadings of calcium, copper, sodium, potassium, nickel and sand content, explains an additional 25% of the variation among soil samples (Table 2). Despite substantial variation for PC2 among locations, serpentine and non-serpentine sites did not segregate consistently along this second axis of soil heterogeneity.

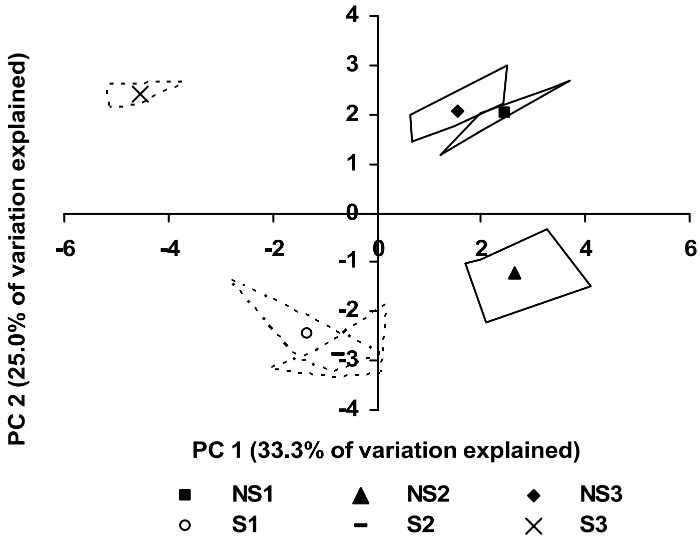


Fig. 1. Two principal components (PCs) describe over 50% of variation in soil attributes among the six study populations of *C. sparsiflora* in 2002. The symbols show the mean PC scores for each of the six population sites; the polygons around each symbol enclose the PC scores for the five experimental blocks at each site. Non-serpentine sites (NS) are shown as solid symbols and lines. Serpentine sites (S) are shown as open symbols and dashed lines. Soil attribute loadings onto PC1 and PC2 are given in Table 2.

Table 2. Principal components analysis of soil variation, and mean values for soil attributes in six serpentine and non-serpentine study sites in 2002

			Non-serpentine		Serpentine	
Soil attribute	PC1	PC2	Mean	*s*	Mean	*s*
Organic matter (%)	0.1964	0.0757	5.53	2.56	3.17	0.82
Phosphorous (Olsen)	0.1939	0.1697	24.53	10.32	12.67	3.89
Potassium (ppm)	0.1259	0.2868	384.20	125.88	239.27	100.82
Magnesium (ppm)	−0.3254	0.1076	673.87	273.81	3044.07	1981.08
Calcium (ppm)	0.1783	0.3205	2152.20	740.71	724.53	430.59
Sodium (ppm)	0.0387	0.3569	17.80	3.67	14.60	3.11
pH	−0.3102	−0.0245	6.35	0.20	6.89	0.30
Hydrogen ($\text{meq} \cdot 100\ \text{g}^{-1}$)	0.2942	0.1204	1.85	0.66	0.36	0.57
Cation exchange capacity ($\text{meq} \cdot 100\ \text{g}^{-1}$)	−0.2747	0.2433	19.16	2.66	29.70	17.95
Nitrogen (as NO_3) (ppm)	0.1068	0.1107	22.53	39.68	8.60	1.72
Sulpher (as SO_4) (ppm)	0.0236	−0.2115	3.40	2.92	4.27	3.26
Zinc (ppm)	0.2978	0.1283	1.09	0.31	0.49	0.30
Manganese (ppm)	0.2735	0.0425	19.60	7.17	10.00	3.00
Iron (ppm)	0.2804	−0.0231	28.60	5.67	21.13	6.35
Copper (ppm)	−0.0966	0.3179	0.96	0.32	0.94	0.58
Boron (ppm)	0.2065	0.0827	0.86	0.54	0.29	0.13
Salts ($\text{mmhos} \cdot \text{cm}^{-1}$)	0.0868	−0.2037	0.17	0.06	0.18	0.04
% Sand	0.1779	−0.2919	56.60	10.67	53.00	16.88
% Silt	0.2436	0.1236	28.67	7.16	18.87	4.22
% Clay	−0.2934	0.2063	14.73	4.06	28.40	18.42
Cobalt (ppm)	0.1403	−0.2680	0.61	0.52	0.58	0.34
Nickel (ppm)	−0.0140	−0.3567	11.94	14.22	26.45	15.71

Note: Soil attribute loadings for the first two principal components, which together account for 58.3% of sampled soil variation, are shown. Means and standard deviations (*s*) within soil type are based on samples from five blocks within each of three sites ($n = 15$ for each soil type).

Seasonal schedules for emergence and flowering were driven mostly by the destination environment, and showed consistent patterns of variation between serpentine and non-serpentine sites. In 2002, transplanted seeds placed into serpentine sites emerged as seedlings earlier than those in non-serpentine sites, regardless of the seed source (Fig. 2A, Table 3A). This accelerated phenology in serpentine destinations was also seen for flowering and senescence in both years (Fig. 2A, B). Transplants commenced flowering almost 2 weeks earlier in serpentine destinations than in non-serpentine sites nearby. There is also some evidence for genetically based differences in flowering time between serpentine and non-serpentine populations. In non-serpentine destinations in 2003, transplants from non-serpentine origins began flowering significantly earlier than their counterparts from serpentine sites of origin (Table 3B). The same trend was seen in the 2002 experiment, but was not statistically significant (Table 3A).

In contrast to emergence and flowering schedules, patterns of survival over time were strongly influenced by whether the soil type in a transplant's population of origin matched that in its destination site. In serpentine destinations in both years, transplants from non-

Table 3. Failure-time analyses for the impact of destination soil type and source soil type within destination soil type on emergence date, flowering date and survivorship of *C. sparsiflora* transplants at four sites in 2002 (A) and at six sites in 2003 (B)

Phenological trait	Prediction factor	χ^2	d.f.	*P*
A. 2002				
Emergence date	Destination soil type	364.45	1	**<0.0001**
	Source soil type within NS destination soil	0.03	1	0.8699
	Source soil type within S destination soil	0.56	1	0.4526
Flowering date	Destination soil type	116.31	1	**<0.0001**
	Source soil type within NS destination soil	2.55	1	0.1102
	Source soil type within S destination soil	1.23	1	0.2667
Death date	Destination soil type	232.07	1	**<0.0001**
	Source soil type within NS destination soil	0.15	1	0.6992
	Source soil type within S destination soil	17.02	1	**<0.0001**
B. 2003				
Emergence date	Destination soil type	4.27	1	0.0389
	Source soil type within NS destination soil	0.53	1	0.4647
	Source soil type within S destination soil	5.47	1	0.0194
Flowering date	Destination soil type	78.47	1	**<0.0001**
	Source soil type within NS destination soil	6.41	1	**0.0114**
	Source soil type within S destination soil	3.59	1	0.0583
Death date	Destination soil type	98.38	1	**<0.0001**
	Source soil type within NS destination soil	22.97	1	**<0.0001**
	Source soil type within S destination soil	71.12	1	**<0.0001**

Note: Each row in the table represents a separate analysis. Significance values are shown for the Wilcoxon test (PROC LIFETEST in SAS). *P*-values shown in **bold** remained significant after applying the sequential Bonferroni correction for three tests within each phenological attribute. NS = non-serpentine, S = serpentine.

serpentine sites died significantly earlier than their serpentine counterparts (Fig. 2, Table 3). Conversely, transplants from non-serpentine originations had a survival advantage in non-serpentine transplant sites; this difference was significant in 2003, but not in 2002 (Table 3). These patterns of survival are consistent with ecotypic adaptation in response to both serpentine and non-serpentine soil types. They are also consistent with the recorded patterns of precipitation in the region during both growing seasons (Table 4). In 2002, there was limited rainfall during the end of the growing season, particularly in April, leading to a rapid rate of senescence in our populations. In 2003, April was much wetter than in the previous year, resulting in more gradual senescence during the second transplant experiment.

Despite the substantial variation in soil characters among sites characterized either as serpentine or non-serpentine (Fig. 1), we found no evidence for specific adaptation to home sites within either soil type (Fig. 3, Table 5). General mixed-model analyses show that, within either serpentine or non-serpentine destinations, transplants returned to their specific home site did not significantly out-perform transplants originating from different sites of the same soil type. This lack of specific home-site adaptation was observed in both

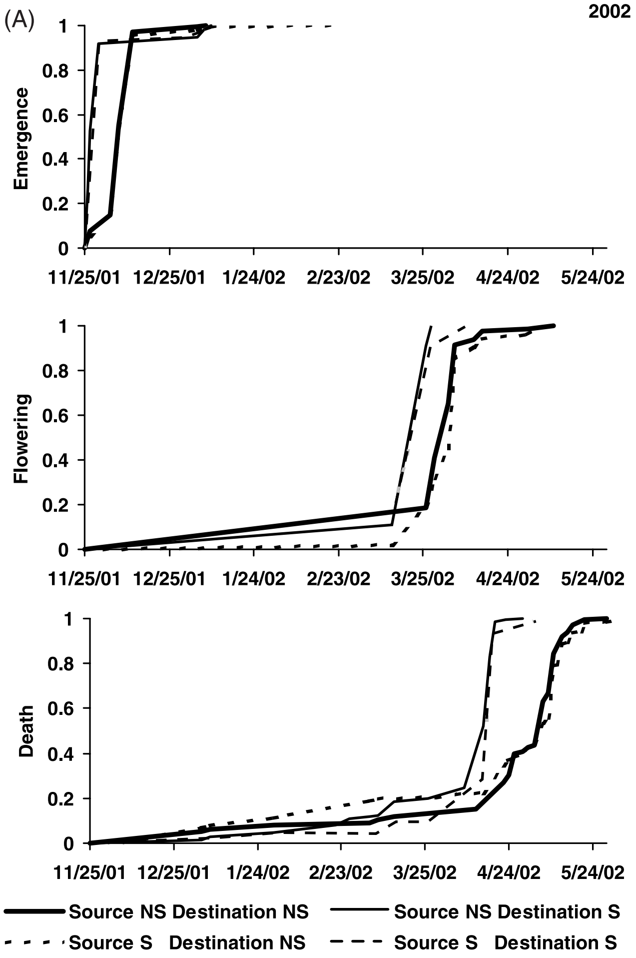


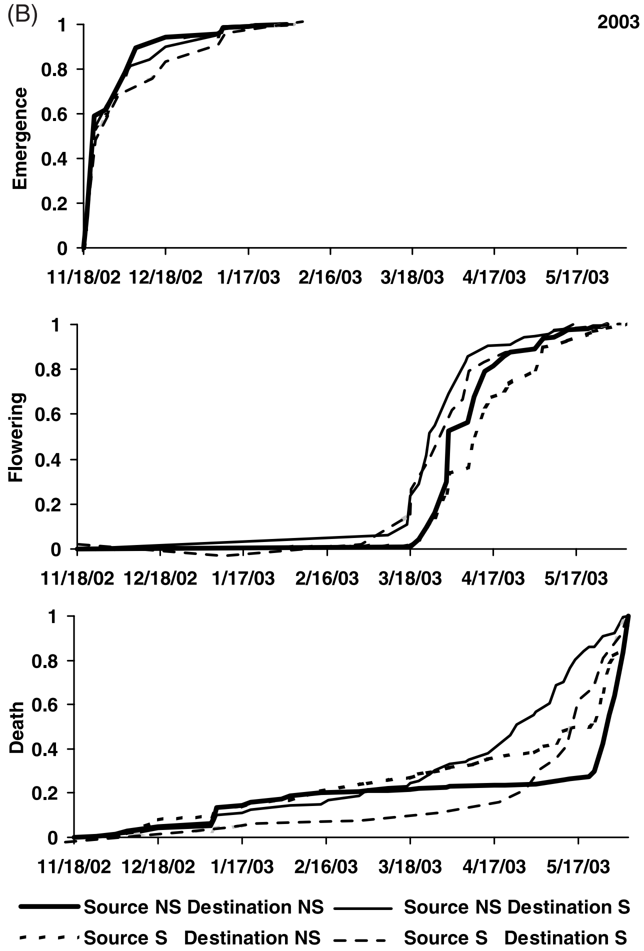
Fig. 2. Phenology of transplants with respect to emergence date, flowering date and survivorship in 2002 (A) and 2003 (B). The y-axes indicate the proportion of plants having emerged, flowered or senesced. Regular lines indicate transplants on serpentine soils (S); bold lines indicate plants on non-

Table 4. Monthly precipitation in inches for north-central California during the *Collinsia sparsiflora* growing seasons in 2002 and 2003

	November	December	January	February	March	April	May
2001–2002	7.32	10.11	4.18	2.64	4.01	1.22	1.34
2002–2003	4.56	15.44	4.15	2.31	4.26	6.83	1.78

Note: Each value represents the mean of monthly precipitation totals for all US weather stations reporting within the north-central region of California (Division 2) during the months shown.

Source: US Divisional Surface Data, made available by the National Climatic Data Center of the US National Oceanic and Atmospheric Administration.



serpentine soils (NS). Solid lines show transplants from non-serpentine soils; broken lines show transplants from serpentine soils.

2002 and 2003 (Table 5A, B). We conclude that any fitness reductions associated with moving transplants to different soil types are not simply due to adaptation to specific home-site conditions, and so we did not distinguish specific home sites in subsequent analyses.

Transplants grown in their native soil type were more likely to produce fruits and flowers than transplants moved from the other soil type in both 2002 and 2003 (Fig. 4A, B). By testing all possible categorical models for interactions between transplant success and soil type, we were able to show that the complete model was significantly better at describing interactions than any of the sub-models for both years of data (Table 6). Only the complete model includes the three-way interaction between source soil type, destination soil type and survival to produce flowers or fruits. The form of that interaction (Fig. 4) demonstrates ecotypic adaptation to serpentine and non-serpentine soils in *C. sparsiflora*.

For lifetime fruit production, general mixed-model analysis revealed significant reciprocal adaptation to the two soil types in 2003, while the data in 2002 revealed a strong trend

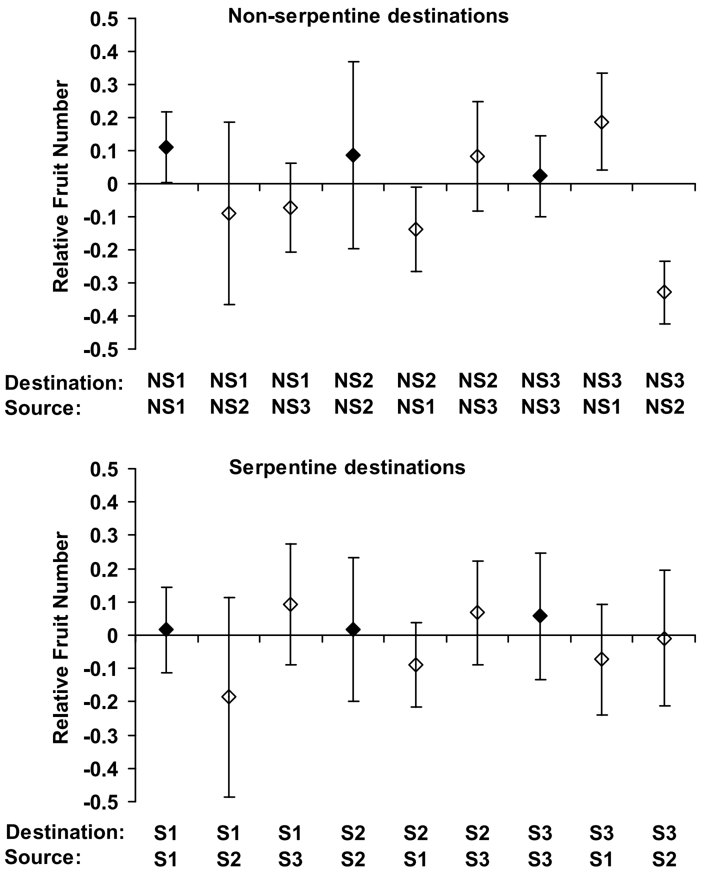


Fig. 3. Transplants growing in their native soil type do not produce more fruit if they grow on their home site. The solid diamonds show the average relative fruit number for transplants growing in the home site, while the open diamonds show averages for transplants from the other two sites within the same soil type. Relative fruit number was calculated as standard deviation units from the site mean for only the transplants growing in the native soil type. Error bars show one standard error. Data for 2003.

towards local adaptation. The interaction between source soil type and destination soil type was marginally significant for lifetime fruit and flower production for plants growing in 2002, but strongly significant for plants growing in 2003 (Table 7). Overall, the pattern of the interaction indicates adaptation to home soil type. Transplants produced more fruits in their home soil type, relative to the transplants from the alternative soil type (Fig. 5). When averaged across the two ecotypes, lifetime reproductive output did not differ between serpentine and non-serpentine soils.

DISCUSSION

Serpentine soils have long been a focus for botanical studies, in large part because they harbour distinctive plant communities containing many endemic species (Kruckeberg, 1984; Brooks, 1987). In California, where most grassland communities have been taken over by

Table 5. Tests for adaptation to specific home site with respect to fruit number (A) and flower number (B) for both 2002 and 2003

Source of variation	Num. df	Den. df	F	P
A. Relative fruit number				
2002 ($n = 357$)				
Transplant type	1	3.32	0.27	0.6371
Soil type	1	9.13	0.22	0.6499
Transplant type × soil type	1	3.32	0.27	0.6365
2003 ($n = 697$)				
Transplant type	1	693	0.83	0.3622
Soil type	1	30.2	0.01	0.9227
Transplant type × soil type	1	693	0.1	0.7499
B. Relative flower number				
2002 ($n = 357$)				
Transplant type	1	3.01	0	0.9951
Soil type	1	10.1	0.17	0.6917
Transplant type × soil type	1	3.01	0	0.9510
2003 ($n = 697$)				
Transplant type	1	6.83	1.29	0.2938
Soil type	1	15.5	0.08	0.7861
Transplant type × soil type	1	6.83	1.21	0.3081

Note: General mixed-model analyses, conducted only for transplants growing on their 'native' soil type, were used to test for the effects of transplant type ('home' vs. 'away') on plant fitness. Only fixed effects are shown here. The full model included the random effects of destination site within destination soil type, block within destination site, and all interactions. The variation source transplant type is either 'home' for plants growing at their home site (e.g. transplants from NS1 growing at NS1) or 'away' for plants not growing on their home site, but growing on their home soil type (e.g. transplants from NS2 growing at NS1). A significant F-value for transplant type would indicate that plants perform differently when grown on their home site.

invasive annuals of Mediterranean origin, serpentine grasslands continue to support a largely native plant community (Harrison, 1999a, 1999b). For species like *Collinsia sparsiflora*, which sustain populations in both grassland types, our results indicate that divergent selection pressures on and off serpentine have led to the evolution of substantial ecotypic variation.

We used reciprocal transplant experiments across six study sites to demonstrate that *C. sparsiflora* is locally adapted to both serpentine and non-serpentine soil types. Transplants grown on their native soil type in the field survived longer and produced more fruits and flowers than individuals transplanted from the alternative soil type. Because our experiments were replicated across multiple populations and across sites with variable soil characteristics, we were able to demonstrate adaptive differentiation with respect to consistent differences between serpentine and non-serpentine soils within our study area. Moreover, this ecotypic differentiation occurs at fine spatial scales. We detected significant local adaptation even where the transition from serpentine to non-serpentine soil types occurs over distances as short as 75 m (e.g. sites S2 and NS2).

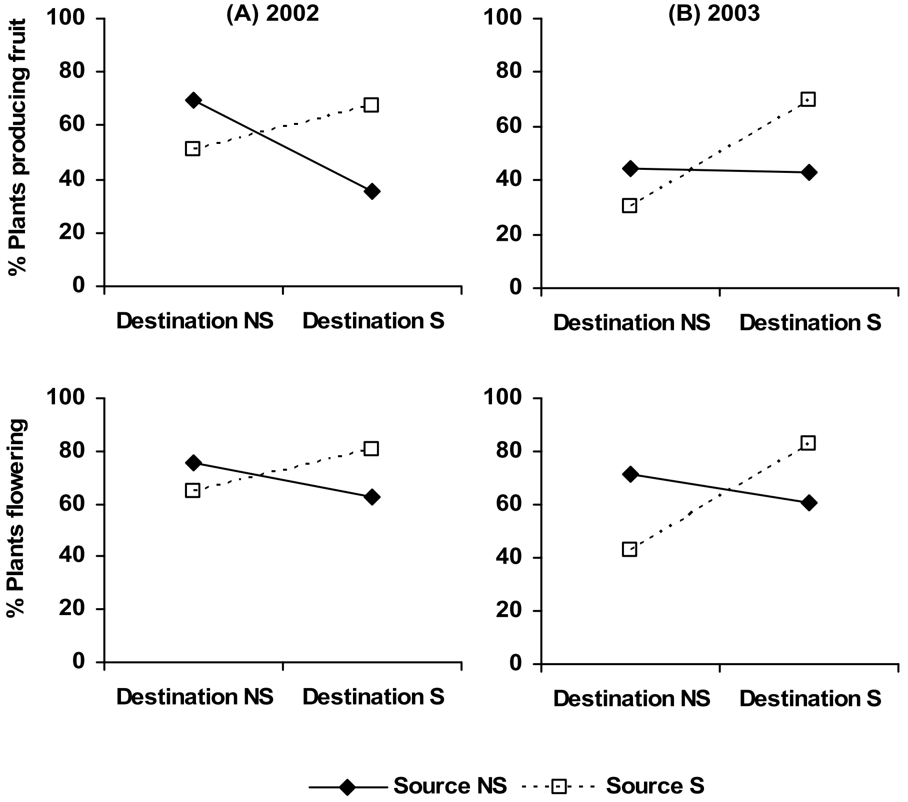


Fig. 4. Percentage of transplants producing fruits or flowers in 2002 (A) and 2003 (B) for each combination of source and destination soil type, serpentine (S) and non-serpentine (NS), in accordance with the analysis. Percentages shown are for all plants within each soil type, pooled across sites ($n = 700$ in 2002 and $n = 1315$ in 2003).

Our work was inspired by the classic studies of Kruckeberg (1951, 1954, 1967, 1984), who described a number of plant species which appeared to have formed distinct serpentine ecotypes in California. Kruckeberg (1967) found his strongest support for ecotypic differentiation in greenhouse studies, in which he compared the performance of seedlings from serpentine and non-serpentine sites when grown on serpentine soils in the greenhouse. Our studies suggest that Kruckeberg may have underestimated the ubiquity of ecotypic differentiation between serpentine and non-serpentine soils. In a greenhouse experiment using *C. sparsiflora* seeds collected from our study populations, we found that plants from our non-serpentine sites grew successfully on serpentine soils (J.W. Wright *et al.*, unpublished data). In other words, had Kruckeberg done his greenhouse experiments with our study populations, he would most likely have concluded that there was no ecotypic differentiation.

Other authors have failed to find differences between plants from serpentine and non-serpentine populations in controlled environments. Westerbergh (1994) found no genetic differences between serpentine and non-serpentine plants of *Silene dioica* growing in serpentine soil in the greenhouse. Using nutrient solutions, she also concluded that there was no difference in nickel tolerance between serpentine and non-serpentine populations. Shaw (1991) grew

Table 6. Categorical test for inter-dependence of soil type variation (serpentine vs. non-serpentine) and survival to flowering in 2002 (A) and 2003 (B) (survival to fruiting gave very similar results)

Model	Likelihood ratio (χ^2)	d.f.	P	Reject H_0?
A. 2002				
A. 1 + 2 + 3	19.22	4	0.0007	Yes
B. 1 + 2 + 3 + 4	18.90	3	0.0003	Yes
C. 1 + 2 + 3 + 5	17.54	3	0.0005	Yes
D. 1 + 2 + 3 + 6	19.18	3	0.0003	Yes
E. 1 + 2 + 3 + 4 + 5	17.21	2	0.0002	Yes
F. 1 + 2 + 3 + 4 + 6	18.85	2	<0.0001	Yes
G. 1 + 2 + 3 + 5 + 6	45.28	2	<0.0001	Yes
H. 1 + 2 + 3 + 4 + 5 + 6	17.18	1	<0.0001	Yes
B. 2003				
A. 1 + 2 + 3	107.47	4	<0.0001	Yes
B. 1 + 2 + 3 + 4	98.78	3	<0.0001	Yes
C. 1 + 2 + 3 + 5	107.29	3	<0.0001	Yes
D. 1 + 2 + 3 + 6	97.06	3	<0.0001	Yes
E. 1 + 2 + 3 + 4 + 5	98.60	2	<0.0001	Yes
F. 1 + 2 + 3 + 4 + 6	88.37	2	<0.0001	Yes
G. 1 + 2 + 3 + 5 + 6	96.88	2	<0.0001	Yes
H. 1 + 2 + 3 + 4 + 5 + 6	88.35	1	<0.0001	Yes

Note: Factors included in the complete model for interdependence are numbered as follows: source soil type (1) + destination soil type (2) + flowering (3) + source soil × destination soil (4) + source soil × flowering (5) + destination soil × flowering (6) + source soil × destination soil × flowering (7). PROC CATMOD was used to compare each of the eight possible sub-models to the complete model. The null hypothesis for each analysis is that the sub-model describes the data as well as the complete model. As the null hypothesis is rejected for all possible sub-models, we conclude that the complete model is significantly better than all other possible models at describing the data, and that the three-way interaction of source soil × destination soil × flowering, found only in the complete model, is significant.

the moss *Funaria flavicans* from two populations on nutrient media simulating serpentine soil chemistry and found no evidence for a serpentine ecotype. Similar results were found in *Pinus virginiana* seedlings from serpentine and non-serpentine sites when grown in a sand/nutrient solution designed to simulate serpentine soils (Miller and Cumming, 2000).

The contrast between our field results and those obtained in greenhouse studies suggests that it is vitally important to study the selective pressures associated with contrasting soil types under field conditions. There are at least two reasons why greenhouse studies may fail to reveal local adaptation to serpentine versus non-serpentine soil types. First, it is difficult to replicate the chemical and physical properties of different soils in greenhouse containers. For example, the coarse texture of many serpentine soils may cause them to dry out earlier under field conditions, a pattern that can be difficult to mimic in the greenhouse. Second, adaptation to serpentine versus non-serpentine environments might result from selection by factors other than the soil properties *per se*. For example, the presence of different neighbouring species may change competitive regimes, pollinator visitation, and populations of natural enemies on and off of serpentine. That said, even some field studies have failed to identify local adaptation to soil type. Jurjavcic *et al.* (2002) conducted a reciprocal transplant experiment with the native annual grass *Vulpia microstachys* across three habitat

Table 7. Effects of serpentine and non-serpentine soils on components of lifetime fitness for *C. sparsiflora* transplants for 2002 (A) and 2003 (B)

	Source	Num. df	Den. df	*F*	*P*
A. 2002					
Relative fruit number (*n* = 700)	Source soil type	1	2.29	0.40	0.5836
	Destination soil type	1	7.20	0.03	0.8749
	Source soil × destination soil	1	2.53	8.54	0.0756
Relative flower number (*n* = 700)	Source soil type	1	3.38	0.55	0.5061
	Destination soil type	1	14.9	0.02	0.9032
	Source soil × destination soil	1	3.68	5.70	0.0810
B. 2003					
Relative fruit number (*n* = 1288)	Source soil type	1	4.85	4.34	0.0935
	Destination soil type	1	18.10	0.60	0.4473
	Source soil × destination soil	1	5.06	36.25	0.0017
Relative flower number (*n* = 1288)	Source soil type	1	9.36	0.63	0.4479
	Destination soil type	1	24.50	0.58	0.4553
	Source soil × destination soil	1	9.36	21.11	0.0012

Note: Fixed effects are shown from a weighted general mixed-model analysis for two plant fitness characters – fruit number and flower number – both expressed relative to destination site means for that trait (see Data Analysis). The random effects of source site within source soil type, destination site within destination soil type, and block within destination site were included in the analysis. Sample sizes (*n*) are given for each analysis.

types (two serpentine and one non-serpentine) and found no evidence for local adaptation to serpentine versus non-serpentine grasslands. A lack of adaptive differentiation between populations occupying different soil types might exist for several reasons, including insufficiently distinct selection pressures, a history of recent colonization and/or extensive gene flow among serpentine and non-serpentine sites.

Having determined the presence of local adaptation to both serpentine and non-serpentine soils in *C. sparsiflora*, the question then becomes, how is this local adaptation maintained at such fine spatial scales? If levels of pollen or seed movement are high, alleles from populations on contrasting soil types will be introduced into locally adapted gene pools, potentially resulting in less fit 'hybrid' individuals, or, unless disruptive selection is very strong, the breakdown of adaptive differentiation across soil types (McNeilly and Bradshaw, 1968; Macnair, 1987; Kirkpatrick and Barton, 1997).

In our populations of *Collinsia sparsiflora*, there are three factors that may limit gene flow between soil types. First, *C. sparsiflora* is highly selfing. It sets seeds readily in the greenhouse, and indeed, as in this study, under pollinator exclusion cages. This ease of selfing is likely to reduce the opportunity for gene flow through pollen movement. Second, the phenology of *C. sparsiflora* on serpentine and non-serpentine sites is noticeably different. Plants growing on serpentine soils flower much earlier that plants growing on non-serpentine soils. Although there is some overlap in flowering between serpentine and non-serpentine populations, the period of peak flowering differed by 10 days in the two soil types. This offset phenology should reduce pollen movement between the serpentine and non-serpentine populations. Finally, the relatively large seeds of *C. sparsiflora* have no special mechanism

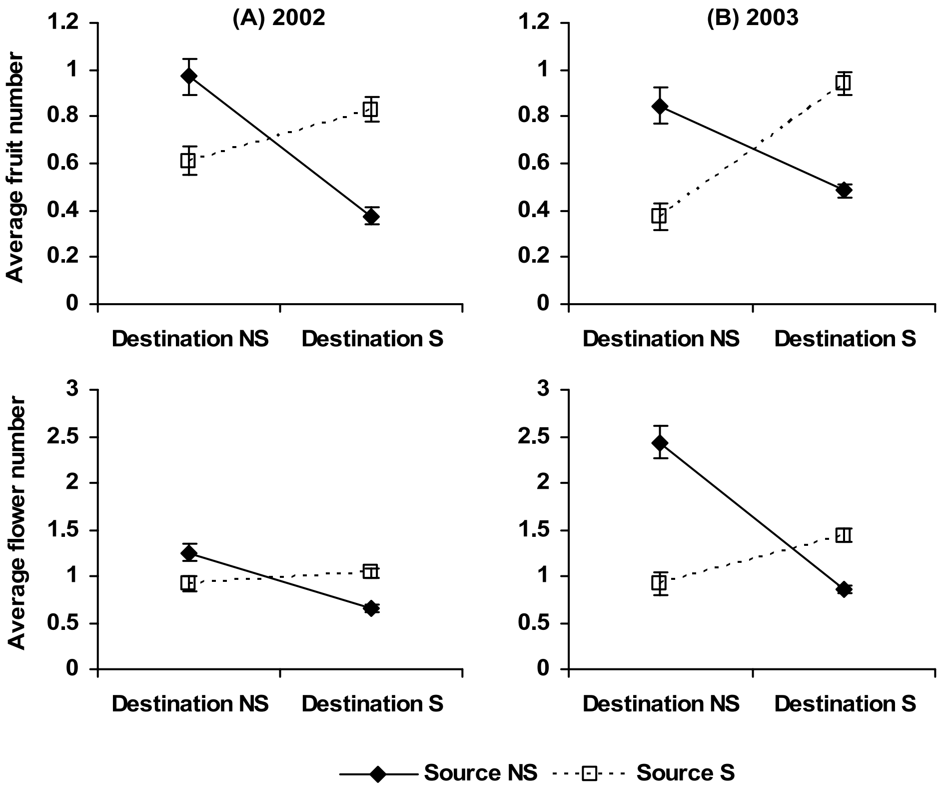


Fig. 5. Lifetime totals for fruit number and flower number for transplants in each source and destination soil type, serpentine (S) and non-serpentine (NS), in 2002 (A) and 2003 (B). Means (± standard errors) are shown for absolute fitness values to allow comparison across soil types and years. As is appropriate for investigations of local adaptation, the associated analysis was performed on relative fitness within destination sites (see Table 7).

for dispersal, but rather drop to the ground close to the maternal parent. Seed movement between soil types is probably relatively rare. We are currently working to assess the level of gene flow between our populations using molecular techniques, as well as investigating the consequences of gene flow by examining 'hybrid' fitness in the two soil types.

ACKNOWLEDGEMENTS

The authors would like to thank Jim Richards, Leonie Moyle, Mike Benard and David Ackerly for their insightful comments on early drafts of this manuscript. Eric Baack provided assistance with the categorical analysis and in the field. We are also grateful to Marina Alvarez, Walther Christen, Nancy Emery, Carson Keever, Jen Lau, Judith Lucas, Rachel Maximo, Chris Mallek, Monica Turner and Robin Waugaman for assistance in the field. This research was supported by grants from the Andrew W. Mellon and David and Lucille Packard Foundations.

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