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POPULATION DIFFERENTIATION IN *ESCHSCHOLZIA CALIFORNICA* ON THREE SOIL TYPES

A Thesis

Presented to

The Faculty of the Department of Biology

San Jose State University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Erin K. Espeland

May 2000

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ABSTRACT

POPULATION DIFFERENTIATION IN *ESCHSCHOLZIA CALIFORNICA*

ON THREE SOIL TYPES

by Erin K. Espeland

Residents of the Ben Lomond sand hills in the Santa Cruz mountains believe that the *Eschscholzia californica* (California poppy) growing there differs from other populations in the surrounding areas. To quantify these differences, populations in coastal, sand hills, and serpentine soils were compared. Phenotypic data were collected from the field in 1998 and 1999. A common garden experiment was also performed. An investigation of genetic differences between populations was conducted through microsatellite-primed PCR (Polymerase Chain Reaction). The sand hills population differs from both the coastal and the serpentine populations for the leaf characters examined. Population differentiation occurred among all three sites for at least some of the floral characters examined. Although the one primer used for the PCR does not reveal significant population differentiation, the common garden experiment indicates that many of the differences among the populations are heritable, and are not a direct response to soil type.

ACKNOWLEDGEMENTS

Thanks to my advisory team: Dr. Rod Myatt, Dr. Chris Brinegar, and Dr. Wayne Savage. Assistance was provided by Kathy Hyde, Mark Fisher, Hilair Chism, Gina Liebig, Pamela Bacon, Dr. Sulekha Anand, Carol Selter, and Peg Edwards. Other support was received from California State Parks, Dr. Tina M. Carlsen of Lawrence Livermore National Laboratory, Dr. J. Curtis Clark of CSU Polytechnic Pomona, Dr. M. Anwar Maun of the University of Western Ontario, and Dr. Bruce M. Pavlik of Mills College. The SJSU Nelson Research Fellowship and the California Native Plant Society Santa Clara Valley Dudleya - Serpentine Habitat Scholarship provided financial assistance.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

By the early part of this century, over 90 varieties of *Eschscholzia californica* had been described (Greene 1905). The 90 varieties were condensed into four by Munz and Keck (1968). They recognized coastal, central, southern, and dune varieties in California. The varieties can be identified by differing levels of leaf glaucousness and compactness as well as flower color and growth habit (annual or perennial). *Eschscholzia californica* is known for its plasticity and is now found in open, semi-disturbed habitats all over the globe. Cook (1962) performed a survey of *E. californica* over the state of California and found local differentiation in self-compatibility, flower fertility, seed production, and stamen number. He found a graded mosaic pattern in the distribution of nearly all the phenotypic characters he measured. Although his work focused on populations west of the Central Valley, *E. californica* in Santa Clara and Santa Cruz counties were not included. This study examines local differentiation in three different habitats in the south San Francisco Bay Area: a serpentine habitat, an inland sand hill habitat, and a coastal meadow habitat. The inland sand hill population, containing plants with striking red leaves with white spots, is of particular interest. Residents of the area have claimed that *E. californica* from the wild populations looks "different" when transplanted and grown beside *E. californica* grown from commercial seed. This study

looks at differentiation among the populations in three ways: field phenotypic measurement (how the plants appear in their own habitat), genetic differences identified by PCR (frequency of polymorphisms), and common garden phenotypic measurement (controlling environmental influence). This study is the first to examine *E. californica* leaf color.

Local differentiation is a much studied phenomenon because of its contribution to evolutionary and conservation theory (Kindell, Winn and Miller 1996, Mayer, Soltis and Soltis 1994, Waser and Price 1985, Montagnes and Vitt 1991, Linhart and Grant 1996). Finding how much field-observed population differences are in response to environmental factors requires some *ex situ* investigations. Common garden experiments are effective in determining the strength of the adaptation of each population to its own soil environment. By performing a common garden experiment rather than a reciprocal transplant experiment, the researcher focused purely on soil environment and controlled confounding factors such as unequal responses to differences in interspecific competition, light intensity, and predation that may have existed within each population. Red leaves have been postulated to be an environmental response to soil chemistry, to high UV intensity (Woodall and Stewart 1998, Burger and Edwards 1996, Cen and Bornman 1990), and to extreme shade (Gould et al. 1995). Red leaves are the manifestation of

anthocyanin pigments in leaf tissues. Anthocyanins are in the flavonoid group of plant phenolic compounds. Some flavonoids are produced in Apiaceae and Leguminosae in response to fungal infection (Dittrich and Kutchan 1991). Plants growing in the sand hills could form red leaves as a dynamic response to soil factors (chemistry, fungi, increased UV reflectance), and the possibility of this type of dynamic response was tested in the common garden experiment. Also, by performing a common garden experiment rather than a reciprocal transplant experiment, the researcher avoided the likelihood of polluting a possibly sensitive habitat-adapted genome (at the sand hills) with detrimental alleles.

Randomly Amplified Polymorphic DNA (RAPD) has been successfully used to show population differentiation in a number of instances (Haig 1998). RAPDs have also been used to produce relatedness maps (Kesseli, Paran and Michelmore 1992) and to identify selective breeding strategies for conservation of rare plant populations (Rossetto, Weaver and Dixon 1998). A 10-base primer of random sequence is annealed to complementary sequences in genomic DNA. By using the polymerase chain reaction (PCR), the sequences of the genome flanked by these primers are amplified so that they are numerous enough to be visible on an electrophoretic gel. By comparing the presence or absence of different sized fragments of DNA, polymorphisms within the genome can

be identified. Precise phenotypic and evolutionary information is difficult to obtain through this method (Cruzan 1998, Parker et al. 1998) because heterozygosity cannot be observed through the presence/absence method of measurement and differences in band intensity are not clear-cut enough to use any finer scoring scale than a dichotomous one (Parker et al. 1998). RAPDs hold several advantages for small studies such as this one: no prior knowledge of the species genome is required, only small amounts of DNA are needed, and the chemicals used in the process are relatively inexpensive and non-hazardous. The results generated by RAPD analysis cannot be extrapolated to fitness or any other phenotypic result, but RAPDs are a fast, inexpensive way to get a small window into possible genetic differences among populations.

The PCR technique used in this study is a variation on the RAPD technique. The primer used is not randomly selected, but is a 15- to 16-base Simple Sequence Repeat (SSR) primer composed of repetitions of 1-, 2-, 3-, or 4- nucleotide sequences. SSR-, or microsatellite-, primed PCR has been used to DNA fingerprint whiteflies (Perring et al. 1993) using multiple primers. The single SSR primer technique produces similar results to the RAPD technique (Weising et al. 1995).

The Ben Lomond sand hills is a textbook example of the geographic factors that give rise to endemism (Mayer, Soltis and Soltis 1994, Kruckeberg 1986). The intrusion

of the dry sandy soil and associated drought adapted plant communities are starkly contrasted by the damp redwood forest surrounding the sand hills. Edaphic habitat disjunction can cause parapatric speciation (Proctor and Wodell 1975, Kruckeberg1954). In the case of *E. californica*, a species that is found in open, disturbed habitats, populations in the sand hills may be isolated by large regions of dense forest habitat, eventually leading to allopatric speciation. Isolation and soil-specific adaptation can produce a great degree of population differentiation in a short period of time (Proctor and Wodell 1975). A more common example of edaphic factors causing endemism in the California flora is the presence of serpentine adapted communities throughout the state. Serpentine adaptation is not treated consistently in taxonomic terms. In some cases, a serpentine adapted group of populations is recognized as a separate subspecies (ex. *Streptanthus insignis* ssp. *lyonii*) and, in other cases, it is grouped with serpentine intolerant populations (ex. *Streptanthus glandulosus*) (Kruckeberg 1986).

Recognition of special varieties, or subspecies, of plants has proved valuable in conservation of fragile ecosystems. Most rare plant occurrences in California are in mixed chapparal, grasslands, coastal scrub, and valley-foothill woodland, yet in terms of the percent of total habitat preserved (wilderness areas, research reserves, national and state parks, wildlife refuges, and recreational sites) alpine and sub-alpine areas are

afforded the most protection, proportionally speaking (Pavlik and Skinner 1994). While the identification of a rare plant in a previously unknown area can ensure some protection (Bartel, Skinner and Knight 1994), Pavlik and Skinner (1994) recommend promoting habitat-based conservation plans, particularly in serpentinite, rocky, and sandy substrates at non-alpine elevations that harbor the highest degrees of overall endemism. The sand hills are certainly a candidate for this type of protection: California Department of Fish and Game has expressed interest in this area, and nine of the 97 species found in the sand hills are thought to be ecotypes, with eight additional species probably warranting additional taxonomic study (Lee 1996).

METHODS

Study Sites

Delineating a population of *E. californica* can be academic, as the plant is so common throughout the state. Sampling areas were defined by high *E. californica* concentration (plants generally less than 1 m apart), and also by man-made (roads, drainage ditches) and geologic (cliffs and other drastic changes in slope) barriers. It may be best to consider these "populations" as high density lobes within a greater *E. californica* metapopulation.

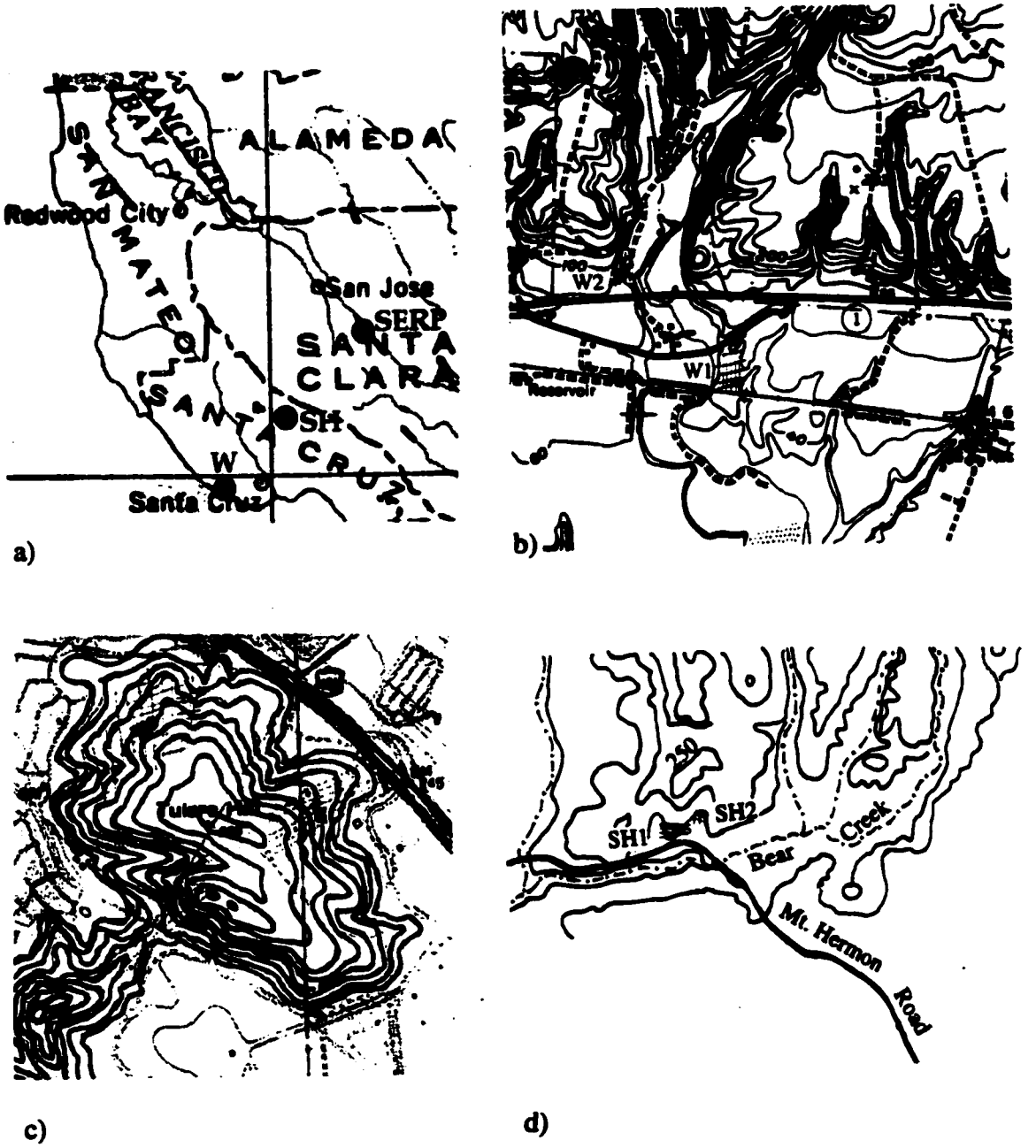

Figure 1. Maps of sites: *a*, general locations; *b*, coastal sites W1 and W2; *c*, the serpentine site (SERP); *d*, sand hills sites SH1 and SH2. In maps b, c, and d, *E. californica* sampling areas are indicated with hashmarks. Adapted from USGS 1981.

The both coastal sites were located at Wilder Ranch State Park, just north of Santa Cruz. (See Figure 1 for maps of sites.) The first site (W1) is a tabletop meadow 0.4 km southwest from the historic ranch buildings. The site is mowed every summer, late in the flowering season. *Bromus mollis*, *Brassica spp.*, and *Carduus pycnocephalus* dominate this site. The second coastal site (W2) was located 0.4 km west of the historic ranch buildings. The species composition of this south-facing community was dominated by *Bromus diandrus* and *Carduus pycnocephalus*. Although these sites are coastal, the *E. californica* at this location does not fit the description of the coastal "race" of *E. californica* (described in Munz and Keck 1968), but rather the inland variety.

The sand hills site (SH1) is located in a watershed ravine, just off Mt. Hermon Road in Scotts Valley. The property is privately owned and previously contained a sand quarry. The *E. californica* population occurred on both the south-facing and north-facing sides of the ravine, and while there was a gap in the population where the streambed occurred, the distance between plants was less than ten feet in this area. Thus, the two sides of the ravine were considered one population. The south-facing slope community contained a grassland understory (rattlesnake grass dominant) beneath scattered ponderosa pine. The north-facing slope community consisted of spring flowering annuals (*Lupinus bicolor, Gilia tenuflora, Orthocarpus purpurescens*) spread thinly over bare

sand. The second sand hills site (SH2) was on the lower sloping face of a sand quarry scar 0.2 km south of the first sand hills population. The scar bends in a semi circle from south facing to west facing slopes. SH2 flowered 5 weeks earlier than SH1, and the community consisted almost entirely of *E. californica* and *Lupinus albifrons*.

The serpentine site (SERP) is located on Tulare Hill in the Santa Teresa Hills of San Jose. The property is county owned but leased for cattle grazing. The *E. californica* population area is located on north and east facing slopes in a serpentine grassland community.

Field Data collection

In 1998, three populations were sampled for plant phenotypic characteristics. W1 was sampled on June 26; SERP was sampled on May 1; SH1 was sampled on July 1. An effort was made to sample each population when the largest numbers of plants were flowering. Between 45 and 65 plants were randomly sampled at each population. Data collected for each plant were the number of flower heads (# flowers + # buds + # capsules), leaf color (third leaf from top of longest branch), width of torus rim (Figure 2), and whether the flower on the longest branch was one-color or two-color.

Flower color was recorded by comparing the base of the petals on the flower on the tallest branch to a yellow to orange (equidistant) color chart created in Adobe

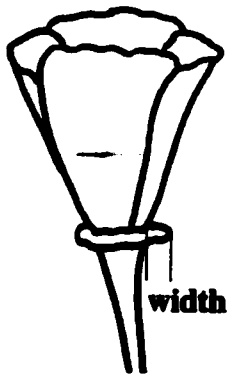

Figure 2. Diagram of torus rim width measurement.

Photoshop (Ver. 4.0). The tip of the petal was also compared to the chart. The chart contained 9 shades numbered 1 (orangest) to 9 (yellowest). Because the difference between consecutive numbers was small, and data collection could be influenced by environmental factors such as light availability, color was analyzed by taking the difference between the base and the tip of the petal. Differences greater than or equal to three were considered to be evidence of the "two-color" character in the flower.

Leaf color data were collected by observing the color characteristics of the leaf: presence of white spots, visible presence of green pigmentation, visible presence of purple pigmentation, and presence of red-tips on the leaf. Each character was noted as either present or absent for the third leaf from the top of the longest branch of each randomly selected plant. If there was doubt about whether a character was present or not, the character was marked as present. For example, if it was difficult to tell if the leaf coloration included purple in with the green, the "purple" character was marked as present. (The characters "presence of purple" and "presence of green" are not mutually exclusive.) Marking the character as present in these cases would increase the perceived presence of green leaves at the SH site and increase the perceived presence of purple leaves at the other two sites, thereby limiting Type I statistical error and reducing chances of erroneously rejecting the null hypothesis.

Stanton Cook (1962) used flower color and torus rim width in his investigations of statewide variability in *E. californica*. Leaf color was of interest because of the apparent difference between the sand hills population for this character and the other two populations.

In 1999, fifteen plants per population were sampled for the same characters to see if the differences observed among populations were consistent and if they would be present in two very different climatic years: El Niño and La Niña winters. Fewer plants were sampled to see if the differences would show up under lower statistical power. Floral characteristics (# flower heads, torus rim length, capsule length, and flower color) were measured over time at sites W1, SH1, and SERP to make sure that differences observed in 1998 were not due to artifacts of the timing of sampling. Fifteen plants were sampled in each population every two weeks. While torus rim length was recorded as discrete data in 1998 (either 1, 2, or 3 mm), in 1999 the use of calipers allowed this measurement to be recorded as continuous data. The same sampling scheme was used for each data collection. Although it is unlikely that the same plants were measured on each sampling date, the plants were located in roughly the same areas. Sampling dates were April 1, April 15, April 28, May 12, May 27, and June 10.

By monitoring the populations so closely, a more accurate estimate of flowering peak was obtained. Phenotypic characteristics were collected at the estimated peak of flowering for W1 (April 15), W2 (May 13), SH1 (May 27), SH2 (April 22), and SERP (April 28). Leaf color was collected as presence/absence of each color character recorded in 1998, except for red-tipped leaves. Instead of just looking at a single leaf of the plant, all of the leaves of the plant were examined for these characters in 1999. Other phenotypic characters measured on the 30 randomly selected plants from each population were height and percent cover in the 0.1 m^2 area centered around the plant. Floral characteristics were collected for W2 and SH2 at this time.

Common garden experiment

Seeds were collected from W1 and SH1 in July of 1998 from 30 randomly selected plants. The seeds, identified by parent plant, were stored in paper envelopes next to a single-paned window in Pacifica, CA until February of 1999, at which time the seeds were placed in 10 cm x 10 cm pots filled with soil from the W1 and SH1 population areas. A total of 210 seeds from 11 plants from SH1 and 210 seeds from 6 plants from W1 were used in the experiment.

Soil was collected from locations near, but not within the SH1 and W1 populations. This limited the possibility of contamination with seed from population

seed banks. Soil was collected within a week of seed potting to minimize biotic changes in the soil that might occur as a result of storage method. Neither SERP soil nor SERP seed was used in the common garden experiment because of the lack of differentiation of this population from the W1 population in the 1998 results.

A reciprocal planting design allowed for seed from each population to be planted in its own and the other's soil (Table 1). Each pot was labeled with a 3-digit randomly generated number to prevent bias when measuring plant characters. Recorded measurements were matched to the plant parent population only at the completion of the experiment.

Table 1. Design of the common garden experiment.

Soil source	Seed source	# pots	# seeds/pot
SH1	SH1	16	9
W1	SH1	16	9
SH1	W1	11	6
W1	W1	11	6

A wick-based watering system was used, where the pots were placed on moist quilt batting dipped in tap water. This kept the flow of water to the base of the pots consistent, and pots were able to take up as much water as was transpired or evaporated. No extra fertilization was used.

The pots were placed on the rooftop of Duncan Hall at SJSU for the months of February through April. In late April, the rooftop was so hot and sunny that the watering system was unable to keep up with the pots' water needs without daily attention. The pots were moved to a northwest-facing patio in Pacifica for the remainder of the experiment (see Figure 3 for climatic data).

Germination and leaf color were monitored on a weekly basis. Pots (planted with as many as 9 seeds, Table 1) were thinned to one plant per pot, where the plant growing closest to the center of the pot was kept. In cases where several plants were equidistant from the center of the pot, the largest plant was kept. When plants flowered, torus rim width and flower color data were collected.

Genetic Analyses

Leaves were collected from the three populations over three consecutive dates (July 1 - July 3, 1998). Three or four healthy leaves were collected from each of 30 randomly selected plants and placed in paper envelopes. The paper envelopes were

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immediately placed into a cooler containing dry ice. Leaves were lyophilized in a freeze drier within 4 hours of collection. Each envelope was labeled with a 5-digit randomly generated number to prevent bias when interpreting gel results.

Total DNA was extracted using the PureGene DNA isolation kit (Gentra Systems, Minneapolis, MN). In an attempt to standardize the amount of DNA recovered, 20-25 mg of leaf material were used for each sample and the final DNA preparation was dissolved in 15 ul TE buffer. RNA was not removed during the preparation. The primer $(GTG)_5$ was synthesized by Operon Technologies (Alameda, CA). Final PCR (25 ul) reactions consisted of 1 ul of plant DNA, 1 ul of primer (10 uM in water), and 23 ul of premade PCR Master Mix purchased from Advanced Biotechnologies Ltd. (Surrey, U.K.). This Master Mix contains 0.625 units Taq DNA polymerase, 75 mM Tris-HCl (pH 8.3), 20 mM $(NH_4)_2SO_4$, 2.5 mM $MgCl_2$, 0.01% Tween 20, and 0.2 mM each dATP, dCTP, dGTP, and dTTP per 25 ul reaction.

PCR was conducted in an MJ Research (Watertown, MA) PT 100 thermal cycler (with heated lid) using an initial DNA denaturation step of 93 °C for 1 minute followed by 40 cycles of 93 °C for 30 seconds, 58 °C for 1 minute, and 72 °C for 2 minutes. The reactions were held at 72 °C for 6 minutes as a final extension step.

Five ul of 6x DNA dye (Amresco, Solon, OH: Type I: 0.25% bromophenol blue, 0.25% xylene cyanol FF, 40% (w/v) sucrose in water) were added before the amplified product was loaded into a 6 cm x 10 cm 2.5% agarose gel in TAE buffer. Electrophoresis was at 150 volts for 4 hours. Gels were stained for 30 minutes in 1 ug/ml ethidium bromide and then destained in water for 10 minutes. The results were digitally recorded using a Gel Doc 1000 image analyzer (BioRad, Hercules, CA).

Gels were scored visually by determining the presence or absence of each band. Bands analyzed were the five that had the easiest to read results, amplified consistently, and there was rarely a question if a band was present or absent (Figure 4). On the uncommon occasion that it was unclear if a band was present or absent, the band was scored as present. A second researcher also scored the gels, and on points of difference, the band was scored as present. After scoring, each plant sample was identified by population.

Data Analysis

The majority of statistical tests were performed in SAS (Ver. 6.0) using the general linear model. G-fit tests performed in Microsoft Excel (Ver 7.0) were used to determine the differences among populations for frequencies of discrete characters (Sprinthall 1987). Discrete character frequencies were normalized before analysis. The

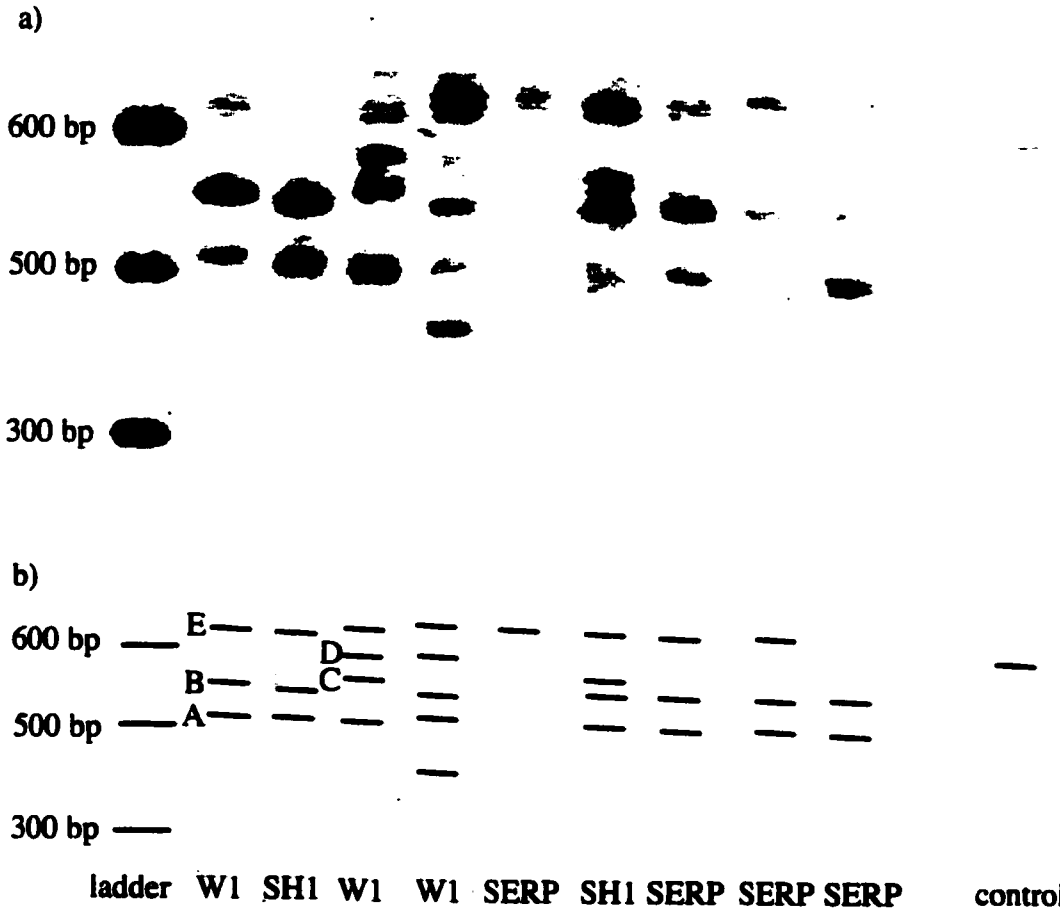

Figure 4. An example of genetic data: *a*, photo of electrophoretic gel; *b*, haplotypes recorded from gel, with bands A, B, C, and D labeled.

Bonferroni correction was used in deciding the critical value (starting from alpha = 0.05) to minimize the risk of coming upon a chance difference between populations because of the number of characters being compared. A repeated measures MANOVA (SYSTAT Ver. 5.2.1) was used to define differences over time in 1999 data for torus rim width and flower number per plant. All percentage data were arcsine transformed prior to analysis.

RESULTS

Field data collection

In both 1998 and 1999, purple leaves were much more prevalent in SH populations than in either the SERP or W populations (Figure 5). Green leaves were more commonly absent from SH than from the other two sites (Figure 6). White-spotted leaves were more common in SH than in either of the other two sites (Figure 7). No differences were found in the distribution of the red-tipped leaf character.

In an effort to determine if the presence of the purple leaf characteristic was related to low cover in the 0.1 m^2 surrounding the plant, a t-test was performed on data collected in 1999. No relationship was found between the percent cover and the presence or absence of the purple-leaved character ($p = 0.65$).

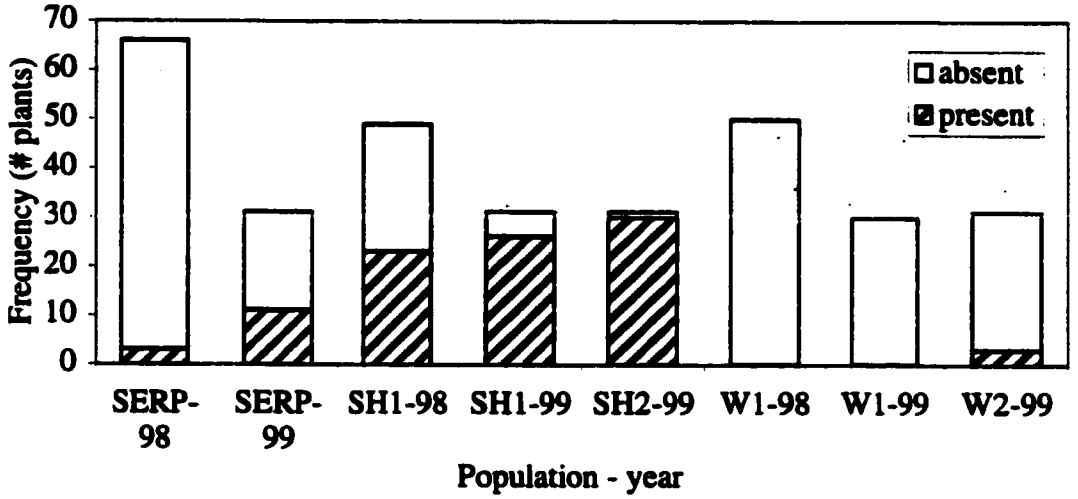

Figure 5. Presence/absence of purple leaves by population: 1998 and 1999.

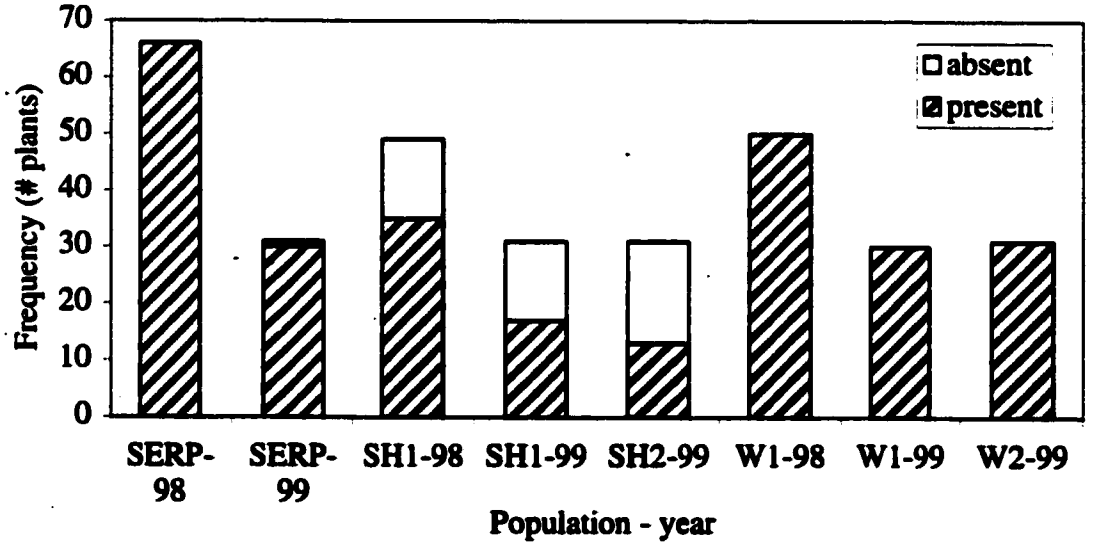

Figure 6. Presence/absence of green leaves by population: 1998 and 1999.

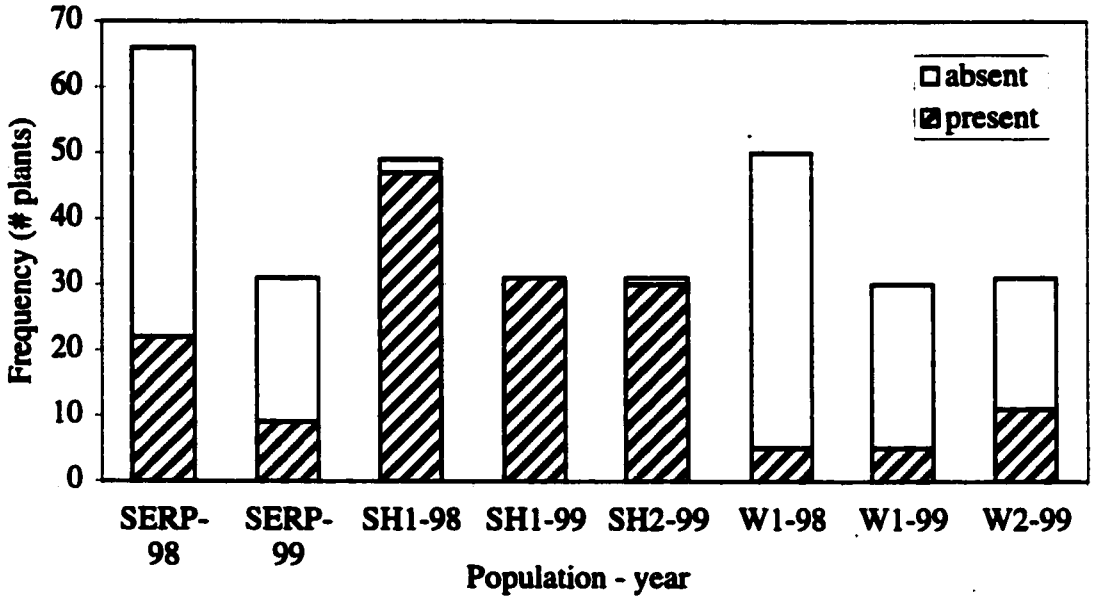

Figure 7. Presence/absence of white-spotted leaves by population: 1998 and 1999.

Differences in floral characteristics were found between populations in 1998 (Table 2). The torus rim was much narrower in the SH populations than in the W and SERP populations ($p < 0.01$). The two-color flower character was less prevalent in the SH population in 1998 than in the other two populations ($p < 0.01$). In 1998, SH sites also tended to have more flower heads than the other two site types ($p = 0.01$).

Floral measurements were taken over time in 1999 to test the validity of a single-date sampling scheme in 1998. If the characters changed over time, differences observed in 1998 could be an artifact of the sampling technique. No differences were found in torus rim width over time ($p = 0.14$). There was a statistical interaction between measurement date and population site for the bicolor flower character ($p < 0.002$): the number of two-color flower heads increased over time in the SERP population ($p < 0.01$), but remained about the same in the other two populations, although the dip in the W population in week two is not a random effect ($p < 0.01$, Figure 8). The number of flower heads per plant increased over time in all populations, but increased more slowly in the SERP population versus the other two populations (Figure 9).

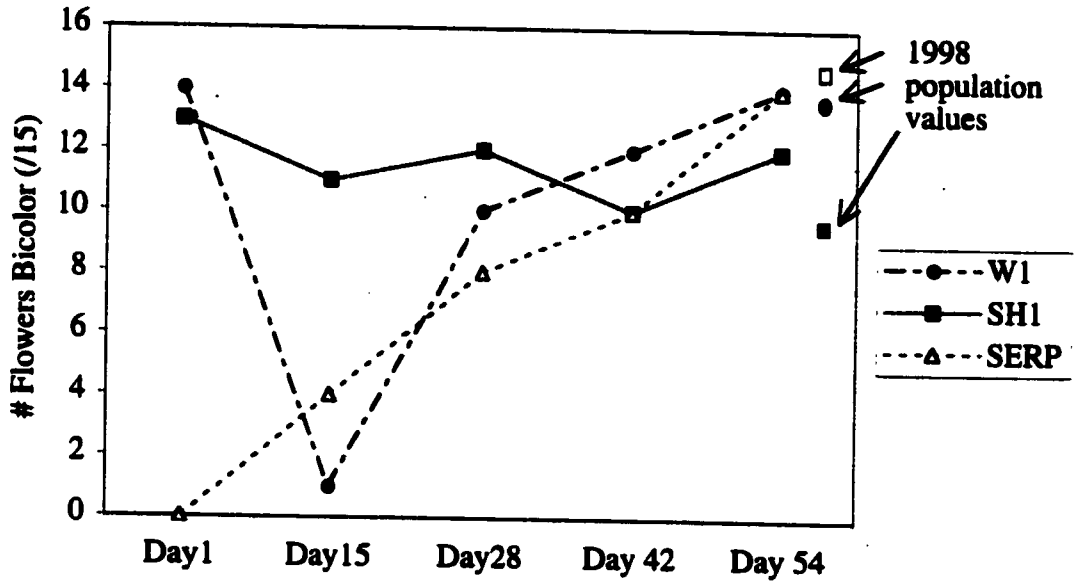

Figure 8. Average number of bicolor flowers over time by population: 1999.

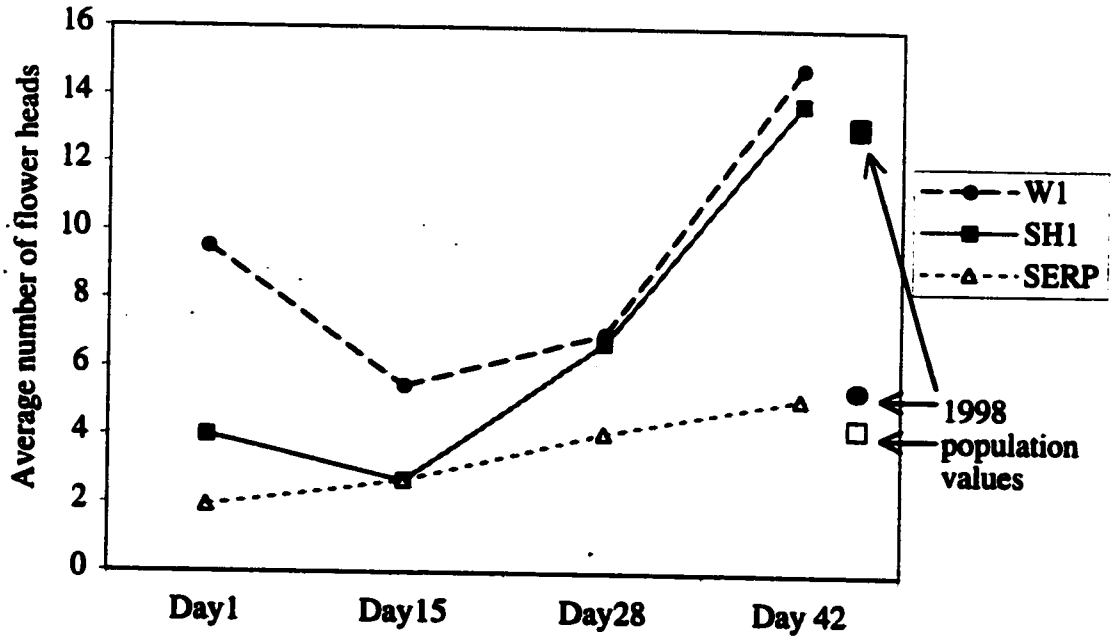

Figure 9. Average number of flower heads over time by population: 1999.

Table 2. Floral Characteristics for 1998 and 1999.

		W1	W2	SH1	SH2	SERP
# flower heads	1998	5.90±5.05	-	12.78±15.39	-	4.15±3.27
	1999*	9.22±4.12	8.40±6.98	6.80±4.96	7.00±6.31	3.45±1.42
% two-color	1998	92.0	-	65.3	-	95.5
	1999*	68.0	73.3	77.3	73.3	48.0
Torus rim (mm)	1998	1.42±0.7	-	1.04±0.2	-	1.41+0.6
	1999*	2.83±0.76	2.97±1.07	1.25±0.55	0.99±0.58	1.62±0.66
N	1998	50	-	49	-	66
N	1999*	75	16	77	15	77

* all 1999 collection dates lumped

Since torus rim width did not change over time in 1999, all collection dates were lumped to confirm the differences among populations for this character. The W1 and W2 populations were not different from each other ($p = 0.55$), and the SH populations were also not different from each other ($p = 0.11$). But when classed by soil type, the W populations were different from SH populations and each was different from the SERP population ($p < 0.0001$). No differences in capsule length were observed among

populations ($p = 0.7$). There were not enough observations for each time point to run a change over time analysis for capsule length.

Common garden experiment

Leaf phenotype remained constant throughout the experiment: once true leaves emerged, they did not change color over time. All SH progeny had purple leaves, regardless of the soil type in which they were grown (Figure 10). Two W progeny had purple leaves when grown in SH soil, but this distribution was not very different from W in the field. Green leaves were more often absent from SH progeny, again regardless of soil type (Figure 11). All SH progeny had white-spotted leaves, regardless of soil type, where this character was only sometimes present in W progeny (Figure 12).

Soil type was the primary indicator of plant height ($p < 0.002$). SH plants averaged 3.2 cm in their own soil and 5.7 cm in W soil, where W plants averaged 3.6 cm in SH soil, and 5.8 cm in their own soil. Plants were significantly shorter in SH soil than in W soil, and within-population differences in plant height were not significant when separated by soil type.

All plants planted in W soil flowered, regardless of parent type, but only 7 of the 11 SH plants flowered in their own soil, and only one of the 7 W plants flowered in SH soil. Torus rim width (Table 3) is influenced by parent population ($p < 0.001$), individual

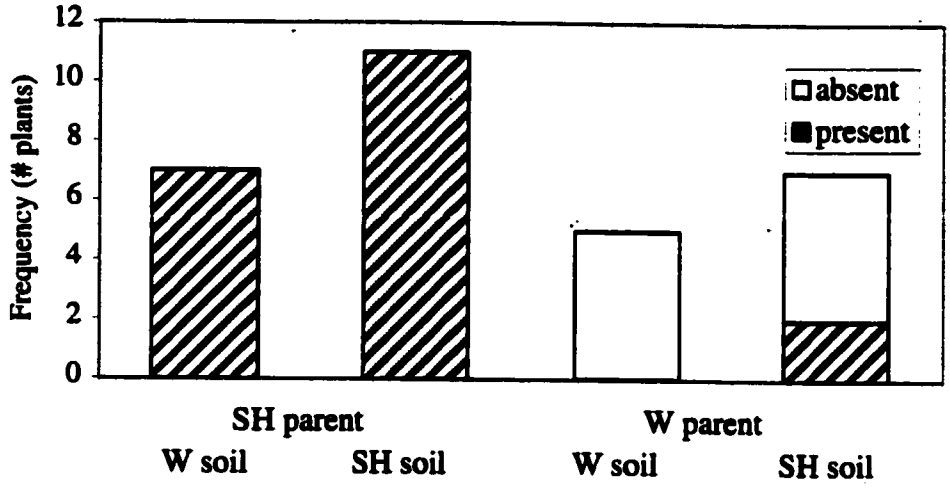

Figure 10. Presence/absence of purple leaves in common garden.

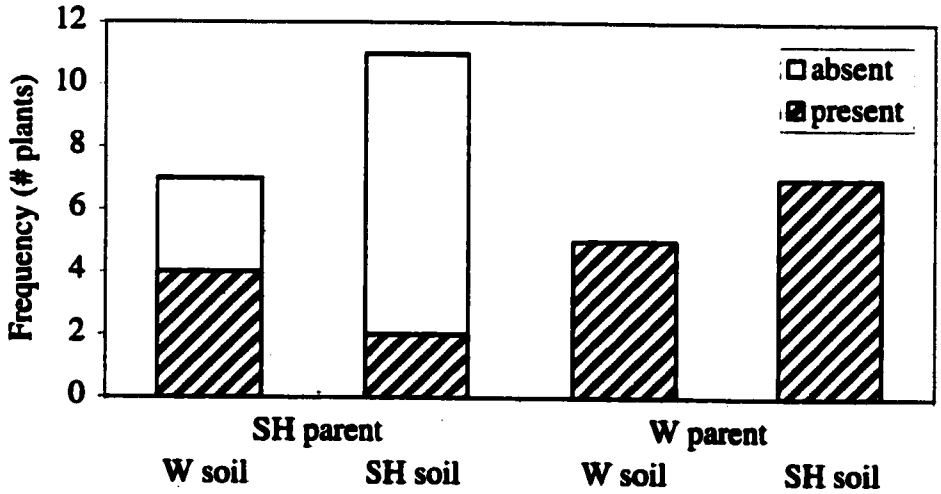

Figure 11. Presence/absence of green leaves in common garden.

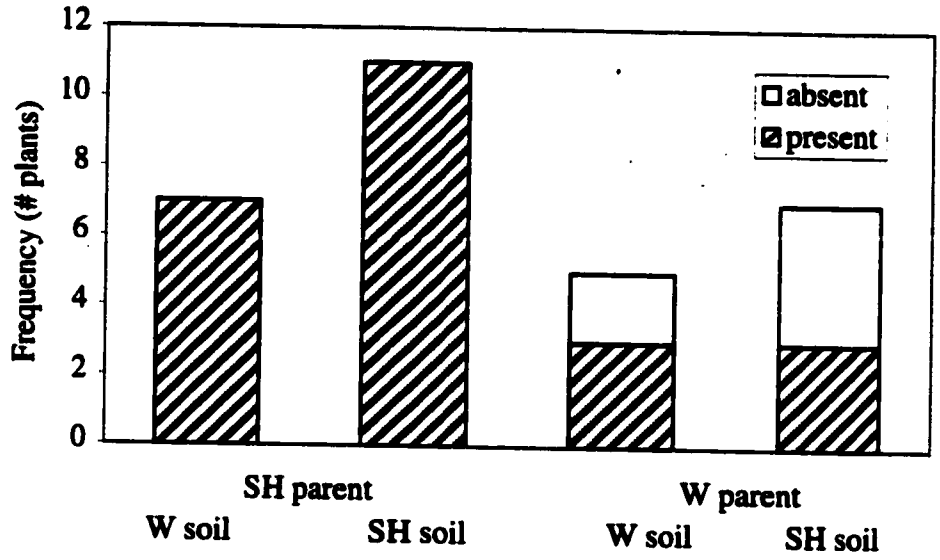

Figure 12. Presence/absence of white spotted leaves in common garden.

parent plant ($p < 0.001$), and soil type ($p < 0.0162$): rim width for SH plants was less than for W plants in both soil types, however, torus rims in general were smaller in SH soil compared to W soil.

Table 3. Torus rim width in common garden experiment by parent population and soil type.

Parent population	Soil type	Torus rim width (mm)	N
SH	W	1.07	7
SH	SH	0.54	7
W	W	2.91	5
W	SH	2.00	1

Genetic analysis

No significant differences were found among the populations for the five bands examined (Table 4). Differences in band frequencies among populations for each band were not significantly different than frequency distributions based on chance alone.

Table 4. Percent frequency of banding type in each population.

Population	Band A	B	C	D	E	N
W1	94.7%	84.2%	78.9%	63.2%	89.5%	30
SH1	100.0%	80.0%	63.3%	50.0%	83.3%	25
SERP	92.0%	76.0%	48.0%	48.0%	68.0%	19

DISCUSSION

Data gathered from analysis of the PCR show that, while there may be genetic differences among populations, the insignificant differences illuminated by this particular primer are hardly enough to make any conclusions about the three populations. If overall polymorphism had been low in the sand hills population (a band frequency of 100% or 0% indicates monomorphism) versus the other two, it may have indicated a bottleneck in the history of that population (Leberg 1992). The inability to link genetic differentiation to phenotypic differentiation is fairly common, however (Jasienski, Ayala and Bazzaz 1997, Knapp and Rice 1998, Linhart and Grant 1996).

The differences observed between the populations in terms of the two-color characteristic and the number of flower heads in 1998 were artifacts of the single-date sampling scheme. As shown from the 1999 data, the populations do differ in their expression of these traits, but with the single-date sampling scheme in 1998, lack of difference could just as easily been observed. This change in some traits over time should serve as a cautionary note to other researchers who plan to sample populations only once per year. The change in flower color and number of flower heads over time is not a uni-directionally expressed trait: the type of change over time varies among populations. It is difficult to say what affects this change over time in some populations but not others. Different types of drying patterns and different soil chemistries at the populations sites could be responsible, or these differences could be due to differential responses among the populations to the same environmental factors. Beatty (1936) found that flower color in *E. californica* is genetically determined, and casual field observations indicated that the change in flower color over time in the populations was due to different plants reaching the flowering stage, and not by single plants enduring a flower color change over the season. Pots in the common garden experiment were not allowed to dry; so the question of the differential response of plants to soil drying cannot be answered by common garden data. The dip in the number of bicolor flowers at the W1 site on day 15

was probably not due to random effects, and did not reflect a climatic event (for example, rain) that could have disrupted the linear seasonal progression of drying soil. The dip may have been due to a one-colored subpopulation coming in to flower just prior to that date.

Torus rim width does not appear to change over the flowering season and differences observed between the populations in 1998 were confirmed in 1999. The additional difference between the serpentine population and the Wilder Ranch population observed in 1999 was probably due to the finer scale of measurement used. Because the serpentine population was not used in the common garden experiment, it is unknown whether this difference between these two populations is solely attributable to soil type.

In the common garden experiment, sand hills plants kept their purple and white coloration even when planted in Wilder Ranch soil. The potted sand environment is obviously stressful to plants from both populations, but the sand hills plants were slightly better able to overcome this stress and flower. It is clear that the expression of leaf coloration characters that contribute to population differentiation in *E. californica* is heritable. The lack of relationship between the purple-leaved character and surrounding amounts of cover indicates that it is probably not a UV response. In addition, differences between the populations are consistent, regardless of the soil type in which the progeny

are grown. The purple-leaved, white-spotted characteristic of sand hills plant leaves does not appear to be a direct response to soil type. It is possible, however, that these differences are not genetic. Effects of the maternal environment in which the seed ripens have been known to include everything from seed germination rates to progeny plants' tolerance to saline environments (see Rossiter 1996 for review). Although leaf color in particular has not been shown to be determined by maternal environment, seed gathered from controlled pollinations of the potted plants should be grown to determine the strength of any effects of maternal environment upon leaf phenotype.

The selective advantage of the purple-leaved character in the sand hills environment is unknown at this time. It is possible that the pigmentation of the leaves is an adaptive, rather than dynamic, response to the increased UV reflectance found at the sand hills. Berger and Edwards (1996) found that red-leaved plants suffered less damage from UV-B and UV-C radiation than related green-leaved varieties. Anthocyanins in leaves have also been linked to higher photosynthetic rates by diminishing photoinhibition (Gould et al. 1995).

Population differentiation is a relatively common phenomenon, but differentiation among populations is expected to be less strong when the flowers are large and the plants outcross as compared to small-flowered autogamous species (Linhart and Grant 1996).

Floral characteristics are less likely to be divergent among populations than vegetative ones (Slentz, Boyd and McDade 1999), as usually even the most disparate populations still have the same pollinator species, and thus floral characters tend to be uniformly selected.

The differences observed between the sand hills population and the serpentine and coastal populations definitely warrant further investigation. Given that the coastal and serpentine populations occur in fairly different climatic conditions and extremely different soil types, and yet no differences occurred between them for the leaf characters measured, indications are that something very special is occurring at the sand hills. The sand hills are host to many endemic species (Lee 1996), probably adapted to the particular edaphic environment. The demonstrated heritability of some of the phenotypic differences observed (leaf color and torus rim width), in spite of the lack of difference perceived in the PCR analysis, indicate that the sand hills population is probably genetically differentiated from the other two populations in this study. Proximate causes of this differentiation are still a mystery: is this differentiation maintained by lack of gene flow into the population, or solely by strong selective forces at the population site?

This study was designed to determine if the populations at the sand hills at the very least represent an ecotype of the *E. californica* species (*sensu* Toresson 1922 and

Kruckeberg 1951). The results indicate that a sand hills ecotype does indeed exist. Testing the interfertility of sand hills with surrounding populations and examining the possibility and efficacy of gene flow to the sand hills population will determine if subspecies status is warranted for the sand hills ecotype of *E. californica*.

Small, isolated populations are more likely to suffer bottlenecks and other drift events that may eventually lead to intrinsic reproductive isolation. *Eschscholzia californica* was only found in three of 20 sand hills locations in 1995 (Lee 1996). Fire suppression has caused a more closed community to arise in many sand hills areas. Prior to fire suppression, the open habitat-loving *E. californica* could have been more prevalent on these soils. The restricted distribution of *E. californica* upon this specific substrate increases the potential for isolation of these populations. The low self-compatibility of *E. californica* indicates that even though it is a successful colonizer, many plants are needed to begin a population (Cook 1962). This inability to self-fertilize limits the species' ability to suffer from founder effects. In all likelihood, sand hills populations were begun by more than a few individuals.

Not much is known about the insect communities of the sand hills. *Eschscholzia californica* has a fairly nonspecific pollination regime, as the pollen can be carried by wind, beetles, thrips (Thripidae), honeybees (*Apis*), bumblebees (*Bombus*), and hover

flies (Syrphidae). Various Halictidae and Melittidae have all been observed pollinating the species in other studies (Cook 1962). Although *E. californica* is generally attractive to pollinators, the insect community at the sand hills may have adapted to more specific requirements of other endemics, and thus may be different than coastal or inland pollinator communities. Specialized insect communities for serpentine flora have been observed in California (Schoenherr 1992). Even though *E. californica* is a near-obligate outbreeder, pollinated by insects and wind which can carry pollen over a considerable range, Cook (1962) found that *E. californica* populations can be differentiated in as small a scale as hundreds of feet. The balance between phenotypic plasticity and differential adaptation is not known in this species. Most of Cook's results were from field observations: only self-compatibility was tested in a common garden experiment. It is certainly possible that the species is not as plastic as we once thought (the same genes reacting differently to different environments), but instead adapts locally (different genes in different environments) to exhibit the great variety we see every day in our travels around the state and elsewhere.

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