Assessment of Seed Bank Buffering of Genetic Change in *Dodecahema Leptoceras* (Slender-horned Spineflower)

Nancy J. Ferguson
Norman C. Ellstrand

Department of Botany and Plant Sciences
University of California
Riverside, California 92521-0124

Prepared For:
Mary Meyer
Plant Ecologist
Department of Fish and Game
Region 5

Funded By:
United States Fish and Wildlife Service Section 6 Funds
Contract Number FG-7642R5

6/15/99

ABSTRACT

Seed bank contributions to the gene diversity of standing populations may be critical to the long-term persistence of rare, endangered, or endemic plant species. Gel electrophoresis was used to monitor genetic variation in five populations of the state- and federally-listed endangered, annual, endemic *Dodecahema leptoceras*. Genetic diversity was surveyed in two generations of above-ground plants (1998-9) and in seeds (*e.g.* seed rain) produced in 1998. In both life-history stages, gene diversity exceeds that reported for other annual or endemic plant species (Hamrick and Godt, 1989). While population extirpation and habitat fragmentation over the past 50 years have reduced and isolated existing occurrences of *D. leptoceras*, there is no evidence that smaller or more isolated occurrences differ significantly in genetic variability from larger or more contiguously located occurrences. However, total species diversity depends upon the multiple geographic locations supporting the species. The total gene diversity of germinating plants was higher than that of seed rain for all years examined. Additionally, G_{ST} estimates of genetic differentiation among populations showed less differentiation in seed rain (mean $G_{ST} = 0.13$) than among germinating plants ($G_{ST} = 0.16$ in 1998; 0.22 in 1999). Within three of the five populations, the level of divergence between 1998 seed rain and plants germinating in 1999 was greater than the population differentiation over all 5 sites in any life-history stage. We conclude that the seed bank acts to enhance genetic diversity in populations and, contrary to expectation, may have the effect of increasing differentiation between populations. While the level of gene diversity observed over all years and life-history stages is high relative to other annual or endemic plant species, how long the genetic diversity observed in germinating plants will be maintained in the absence of increased diversity in the seed rain is unknown.

INTRODUCTION

The maintenance of genetic diversity is especially important for rare, endemic taxa where either the number of populations and/or the size of the populations are typically small (Falk and Holsinger, 1991; Ellstrand and Elam, 1993). *Dodecahema leptoceras* (slender-horned spineflower), state- and federally-listed as endangered in 1987 (USFWS, 1987), currently exists in a limited number of populations which range in size from less than 100 to several thousand plants. Significant loss of populations comprising the species and increased fragmentation of the habitat over the past 50 years (Rey-Vizgirdas, 1994) raise concerns about genetic diversity. If small populations are genetically isolated from conspecifics, their effective sizes may be so small that the chance loss of genes in the transition between generations (known as genetic drift) and an increase in genetic divergence between populations may result. In addition, an increase in inbreeding in small, isolation populations may occur. For outcrossing species, such as *D. leptoceras*, inbreeding can adversely affect reproduction if matings between closely related individuals exacerbate the effects of deleterious alleles within a population. Increases on homozygosity in smaller populations can be an indication that inbreeding depression may be occurring.

Annual plants typically fluctuate dramatically in above-ground population size, and often fail to produce offspring in any given season (Epling *et al.*, 1960; McCue and Holtsford, 1998). In *D. leptoceras*, the proportion of the germinating population that survives to reproduction has been observed to range from only 6 to 52% (Ferguson *et al.*, 1996). These fluctuations, in addition to isolation, indicate that genetic drift may be significant factor for *D. leptoceras*. If genetic drift is an important factor, smaller populations within the species should contain less variation than larger populations. Also, increased isolation between populations, and possibly low dispersal of seeds under current hydrological conditions suggest that populations may be comprised more and more of closely-related plants. If this is so, we can expect to see a trend towards decreasing heterozygosity in affected populations.

The seed bank is a repository of potentially viable seed in the soil which has accumulated over multiple generations. Empirical evidence suggests that a sufficient seed bank exists for this species which restores populations demographically following years of little or no seed production. The question becomes whether or not the seed bank is sufficiently diverse to restore these populations genetically. Theoretically, seed banks may function as reservoirs of genetic memory, accumulating and storing seed genotypes over many seasons (Templeton and Levin, 1979). When above-ground plants fail to reproduce or produce very little seed, this represents a potential genetic bottleneck; *i.e.* the fraction of germinating plants that reproduce is very low and therefore their progeny represent a small fraction of the population gene pool. At those times when the reproductive population is so reduced, the seed rain will also represent a limited portion of the genetic diversity previously maintained in the population as a whole. If there is not a long-lived seed bank for this species, multiple years of low reproduction and the resultant genetic bottlenecks in seeds, will reduce the genetic diversity of affected populations and possibly of the species as a whole.

In a previous study, we found that the levels of genetic diversity in this species were remarkably high (Ferguson *et al.*, 1996). Conducted on seeds produced in 1995, the study did not answer questions about the level of genetic diversity sustained in the populations over time or about possible changes in diversity following years of little or no reproductive output. In addition to investigating the dynamics of gene diversity in this endangered species, the current study is undertaken as an indirect method of evaluating the seed bank genetic diversity in *D. leptoceras*. Given the highly endangered status of the plant and lack of success in locating seeds in soil samples (Allen, 1996, Ferguson *et al.*, 1996) the destructive sampling of soils is ill advised. Using protein electrophoresis, this study examines the genetic diversity of multiple generations and life-history stages (seeds and plants) to determine whether they differ in genetic diversity or in the population structure of this diversity. Because we cannot access the seed bank directly, we must infer the genetic diversity of the seeds in the soil by comparing the genetic diversity of seed rain

(seed bank "contributions") to that of germinating plants (seed bank "withdrawals"). We examine genetic diversity in plants germinating in 1998 and 1999 and in seeds produced from plants surviving to reproduction in 1998. We also compare these data to the 1995 seed rain study. In this way we can examine the seed bank "contributions" and "withdrawals" over multiple generations.

The goals of this study are as follows: 1) to investigate the dynamics of genetic variation in populations of *D. leptoceras* over time; 2) to compare gene diversity between populations; and 3) to infer the genetic contribution of the seed bank to above-ground populations by comparing multiple generations of seed rain to germinating plants.

METHODS

Study System

Dodecahema leptoceras (Polygonaceae) is an annual plant endemic to flood-deposited alluvium in southern California (Hickman, 1993). The species typically germinates in late-February or early-March in response to winter rains, and flowers from mid-May until summer heat and drought induce senescence. Reproductive plants produce multiple, branched infloresences bearing flowers which are borne in involucres. Though flowers are protandrous (*e.g.*, anthers dehisce prior to the stigma becoming receptive to pollen), the possibility of self-fertilization between flowers on the same plant exists. The average rate of outcrossing events for the species was estimated at 66%, but population averages ranged from 18 to 100% (Ferguson *et al.*, 1996). Surviving plants can produce from a few to 100's of involucres and each involucre can contain up to five flowers (Ferguson, 1999). If pollinated, each flower can produce one achene; a dry, hard, indehiscent fruit enclosing the seed. Neither the seed dispersal nor the longevity of seeds in the soil is known. However, abundant germination occurs following successive years with little or no seed production (pers. obs.) suggesting that seeds remain viable in the soil for a number of years.

Sampling Design

In this study spatially discrete groups of conspecific individuals are referred to by their location name as "populations" or "occurrences" (*e.g.* Dripping Springs). Distinct occurrences of *D. leptoceras* within an alluvial system may represent sub-populations which experience gene flow between locations. The total population statistics refer to species-level diversity, however they are only accurate as a measure of total species diversity in so far as they represent the totality of the distribution of the species.

This study was conducted on plant material collected from six plant populations at four different geographic locations: Dripping Springs (EO #23, DS1 and DS2), Bautista Creek (EO #17, BA1; EO # 21, BA2), San Jacinto Wash (EO #1, SJ), and the Santa Ana River Wash east of Orange St. (EO #2, OS) (Fig 1). The two sampling locations at Dripping Springs are located one to the west of Arroyo Seco Creek (DS1) and the other to the east (DS2).

Plants typically occur in randomly spaced aggregates in differing degrees of isolation from each other. I attempted to collect individuals from all clusters covering the physical extent of each population. An estimate of population size was made during each collection period (Table 1). The numbers represent direct counts in populations of less than 100 plants. In larger populations, the estimates are based on the observation of each plant collected represented 1 in X number of plants surrounding it. All collections were made in areas where *D. leptoceras* plants have been observed in the past. While adjacent, previously unoccupied areas were searched, no rigorous or systematic attempt to locate plants that might occur outside of previously occupied areas was made.

Collection procedures

One to two leaves per plant were collected at one time from germinated plants at the four study sites between March 18 and April 6, 1998. Collection sizes ranged from 50-100 individuals depending upon population size. Leaves were collected after plants have become sufficiently robust to be unharmed by the removal of leaves but not so late in the growing season that field mortality had begun to any observable level. In this way the genetic diversity of the germinating population rather than the reproductive population could be estimated. Leaves from individual plants were placed in capped, plastic tubes and on ice immediately to retard enzymatic degradation. Leaf material was extracted within 5 days of harvest.

Seeds were collected from *D. leptoceras* plants at the same locations from August 11 to 14, 1998. A single inflorescence was collected from each of approximately 100 plants at San Jacinto Wash and 50 plants at all other locations. As 1998 was a high rainfall year, most reproductive plants were multi-stemmed so that the seed collected represented a small portion of the seed produced in that year. Seeds were stored in individual manila coin envelopes. Individual involucres were opened by hand to remove seeds for gel electrophoresis.

The final collections of leaf material were done at the four sites from March 22 to April 26, 1999. Regular visits to these sites to determine the approximate time of germination were begun in mid-February but due to the unusually low precipitation this season, germination was quite delayed. Population sizes were also reduced relative to other years. Once plants were established, leaves were collected and treated as in the previous year.

Gel electrophoresis

Horizontal starch gel electrophoresis was performed on enzymes extracted from leaves and seeds. Leaf tissue was extracted using a modified Morden, Doebley, and Schertz

(1987) extraction buffer. A single leaf (or two, if leaves were very small) was ground in the extraction buffer and the extract absorbed onto 2mm x 4mm Whatman 3mm filter paper wicks for loading into gels. The sample to be analyzed was frozen overnight prior to electrophoresis as it was found that frozen material gave better results than freshly extracted material. Excess wicks were stored at -80° C in case it was necessary to repeat any electrophoresis gels. Three gel systems were employed to resolve the following ten enzymes: 6-phosphogluconate dehydrogenase (*6-Pgd*), isocitrate dehydrogenase (*Idh*), and malate dehydrogenase (*Mdh*) were assayed in a morpholine-citrate pH 8.0 gel system (MC8) at 30mA and 150 volts for 5 hours; aconitase (*Aco*), phosphoglucoisomerase (*Pgi*), and shikimic acid dehydrogenase (*Skdh*) were assayed in a morpholine-citrate pH 7.0 gel system (MC7) at 30 mA and 150 volts for 5 hours; phosphoglucose mutase (*Pgm*), superoxide dismutase (*Sod*), triose-phosphate isomerase (*Tpi*), and uridine diphosphatase (*Udp*) were assayed in lithium hydroxide pH8.3 gel (LiOH) at 75mA and 200 volts for 3.5 hours.

Seeds were extracted in the buffer cited above and an Ellstrand and Lee (1987) buffer as these buffers provided optimal resolution in different gel systems. A single seed was extracted for use in each of the three gel systems employed. As with leaf tissue, seed extract was absorbed onto filter paper wicks and frozen overnight prior to electrophoresis. Frozen seed extract proved to have no shelf-life beyond a few days, but as more seeds remained from collected plants, this was not a problem for repeating procedures as needed.

The Ellstrand & Lee buffer was used to extract seeds assayed in a LiOH gel. Phosphoglucose mutase (*Pgm*), triose-phosphate isomerase (*Tpi*), and uridine diphosphatase (*Udp*) were assayed in LiOH gel buffer. Seeds extracted in the modified Morden, Doebley, and Schertz extraction buffer were run on one of two systems: MC7 and tris-EDTA-borate pH 8.8 (TEB). The same three enzyme systems resolved in leaf tissue were scored for seed tissue on MC7. Leucine aminopeptidase (*Lap*), phosphoglucoisomerase (*Pgi*), and phosphoglucose mutase (*Pgm*) were assayed in TEB at 50mA and 150 volts for 5 hours.

Genotypes are inferred directly from enzyme banding patterns based on knowledge of the overall conservation of isozyme subunit composition and isozyme number in plants (Weeden & Wendel, 1989) and the known diploid nature of the plant (n=17; Hardham, 1989). Putative isozyme loci were identified numerically according to the relative migration of their enzymatic products on the gel; loci producing the most anodal variant received the lowest numerical designation. Samples of all plant material (seeds and leaves) collected for this study were run simultaneously in order to verify allele designations across sampling periods. Within sample periods, different populations were run concurrently on the same gel to verify allele assignments across populations. Obviously, it was not possible to test the current material with seeds sampled in 1995 and no comparisons beyond species-level diversity are made between the 1995 data and the current study.

Data Analysis

Allele frequencies were determined for each locus in all populations sampled. These frequencies are used to calculate the following measures of diversity: percent polymorphic loci (P, 99% criterion), mean number of alleles per locus (A), and the mean number of alleles per polymorphic locus (Ap). Nei's (1973) total gene diversity within the species (H_T), gene diversity within populations (H_S), gene diversity among populations (D_{ST}), and the amount of genetic differentiation among populations (G_{ST}) are determined. All gene diversity statistics reported are unbiased for sample size and population number (Nei, 1986). Population level gene diversity (h) is also figured. Indices of polymorphism and the genetic diversity statistics are calculated using GeneStat-PC 3.3 (Lewis, 1992).

Tests of significance between allele frequencies in different generations and life-history stages of *D. leptoceras* were done to determine whether factors other the genetic drift and sampling error may be contributing to the changes detected between samples. Paired life-history stages were compared in a (Nx2) contingency table; *i.e.* the number of alleles at

the locus under consideration by two life-history stages. In this way, the life-history stage accounting for the significant change can be identified. For loci invariant at one life-history stage, all three samples were compared directly in a (Nx3) contingency table. Allele frequencies across years and life-history stages are compared using the Chi-square test with Bonferroni correction. Due to the low frequency or absence of alleles in different samples, some counts were combined in order to meet the assumptions of the test (Sokal and Rohlf, 1995).

F-statistics (Wright, 1938) were calculated from electrophoretic data. F_{is} measures the magnitude of homozogosity relative to the expectation assuming random mating within subunits (populations). F_{it} estimates the magnitude of the homozygosity in the population, relative to the expectation of random mating in the population as a whole. F_{st} is a measure of population divergence analogous to G_{ST}. In general, there is strong concordance between the two parameters. F_{st} is reported here as it is possible to test whether this value is significantly different than zero. In order to directly compare the gene diversity of seed rain and subsequently germinating plants, 1998 seeds and 1999 plants from each population are compared directly using F_{st}. Single locus F_{st} estimates were averaged over all alleles and loci using the weighting scheme of Weir and Cockerham (1984). Significance tests of the null hypothesis that $F_{st} = 0$ were performed by bootstrapping loci over 1000 iterations. Single-locus *F*-statistics and *F*-statistics bootstrapped over polymorphic loci were calculated after Weir and Cockerham (1984). *F*-statistics as well as expected and observed heterozogosities per population are calculated using the Genetic Data Analysis program (Lewis and Zaykin, 1999).

A total of 13 enzyme loci from seeds and 14 from leaf samples were resolved and scored over all populations. Leaf samples from population DS2 proved impossible to score reliably as the quality of the bands was very poor and could not be improved despite repeated trials. For this population, only data from seed are available. Twelve loci were consistently resolved over all life-stages. Loci *Lap*, *Skdh*, and *Sod* were eliminated from the final analyses as they could not be resolved in both plants and seeds. While we would

prefer to use the most inclusive data set for evaluating species polymorphism and gene diversity, comparisons over different years and life-history stages would be biased by the inclusion of non-concordant loci. Therefore, all data presented is that calculated using 12 loci. Modified 1995 statistics based on 12 loci and five populations are included for comparison to species-level diversity in 1998-99.

RESULTS

Species-level variation

Dodecahema leptoceras plants in both 1998 and 1999 were polymorphic at 100% of loci and seeds at 67% of the 12 loci examined. Total gene diversity fluctuated over the sampling period, ranging from 0.09 ($\pm$ 0.05) in seeds produced in 1998 to 0.18 ($\pm$ 0.05) in plants germinating in 1999 (Table 2). Plants germinating in 1998 exhibited total gene diversity of 0.13 ($\pm$ 0.05). This range of H_T encompassed that found in the 1995 seed sample (0.14 ($\pm$ 0.06)). While gene diversity in plants in 1999 was twice that of seeds in 1998, due to high sampling variance these differences were not statistically significant. Population divergence was greater among populations of germinated plants in 1998-9 than of seeds in 1995 or in 1998.

Indices of polymorphism and allelic diversity are reported in Table 3. Percent polymorphism averaged 70% for plants in both years while seeds were 44% and 40% polymorphic in 1995 and 1998, respectively. The mean number of alleles per locus detected in this recent sampling ranged from 1.78 ($\pm$ 0.08) in seeds to 2.15 ($\pm$ 0.09) in plants in 1998. While there were no significant differences in the number of alleles per locus, the reduced value in seeds relative to germinating plants reflects the fact that seeds had fewer polymorphic loci than did germinating plants. All samples differed significantly from each other in the number of alleles per polymorphic locus. *Ap* was highest in the 1998 seed rain (2.96$\pm$ 0.18) and lowest in 1995 seed rain (2.37 $\pm$ 0.09). There was no relationship between life-history stage and *Ap* indicating that while seeds did have more

monomorphic loci, at variable loci the number of alleles was not reduced relative to germinating plants.

Population-level variation

Individual population gene diversity detected over the three sampling periods ranged from 0.07 ($\pm$ 0.04) to 0.16 ($\pm$ 0.05) (Table 4). However, due to high sampling variances, none of these differences are significant. Despite the lack of statistical significance, there was a roughly 2-fold increase in diversity in plants germinating in 1999 at all populations except DS. For all populations surveyed, the level of gene diversity in seed rain was less than that seen in either generation of germinating plants.

Allelic diversity and polymorphism in individual populations varied between populations and over sampling periods (Table 5). The largest population (SJ) was consistently the most polymorphic but apart from this, only the number of alleles per locus in the 1998 seed rain and population size were significantly correlated with census size (Spearman's r = 1.0; $p < 0.001$). If genetic drift were an important factor, smaller populations within a species should contain less variation than larger populations and such a trend has not been observed in this study.

Observed heterozygosity (H_o) was consistently lower for all populations than that expected assuming randomly mating populations (H_e) (Table 6). In 1998, all populations exhibited a high degree of homozygosity as indicated by the inbreeding coefficient. All populations except DS experienced a drop in inbreeding in 1999. The greatest reduction in homozygosity was noted at the largest population, SJ. However, despite concerns that population size may adversely affect the level of heterozygosity maintained in populations, there was no correlation between estimated population size and homozygosity measured by the inbreeding coefficient (f). Population gene diversity ($h = 0.08 \pm 0.04$) and degree of inbreeding ($f = 0.39$) in seeds sampled at DS2 in 1998 did not differ significantly from any other populations examined.

Comparison of seeds and germinating plants

In general, fewer alleles were detected at a given locus in seeds (Appendix A). Despite consistent variation in the standing plant populations, seed rain contains a greater proportion of fixed or nearly-fixed loci (also reflected in *P*%). Seeds were fixed at three loci (*Idh-1*, *Idh-2*, and *Mdh-2*) in 1995 and at four loci (*Aco*, *Idh-1*, *Mdh-2*, and *6Pgd*) in 1998. In *Idh-2* and *6Pgd*, which were monomorphic in 1995 but varied in 1998, the variable alleles occurred in only one or two of the five populations and in very low frequency suggesting that the trend in these loci is ultimately towards fixation in all populations.

F-statistics are presented graphically in Figure 2. Overall homozygosity did not change between parent and progeny generations in 1998 (F_{is} = 0.42 in plants and 0.43 in seeds; F_{it} = 0.50 in both). Plants germinating in 1999 exhibited increased heterozygosity (F_{is} and F_{it} were 0.26 and 0.40, respectively) relative to 1998 plants and seed rain. The increased heterozygosity and gene diversity in plants germinating in 1999 is reflected in the increased F_{st}. *F*-statistics indicate that the level of population divergence was significantly different than zero for all generations and life-history stages sampled. Values of F_{st} with 95% confidence intervals obtained from 1000 bootstap interations over loci are as follows: F_{st}= 0.14 (0.05 - 0.50) in plants 1998; 0.12 (0.03 - 0.16) in seeds 1998; and 0.20 (0.08 - 0.28) in plants 1999.

Single-locus heterozygosity is depicted graphically in Figure 3. The percentage of heterozygous individuals in plants was greater than that in seeds at all loci except *Pgi*. At many loci, the number of heterozygous individuals was greater in plants germinating in 1999. This was most obvious at the *Udp* loci which exhibited a striking increase in heterozygotes in 1999.

The divergence between 1998 seed rain and 1999 germinating plants within each of the five locations sampled was estimated by F_{st} (Fig. 4). The values are as follows: $F_{st} = 0.03$ at DS; 0.29 at BA1; 0.35 at BA2; 0.53 at SJ; and 0.02 at SA. F_{st} was significant in three populations (BA1, BA2, SJ). The level of genetic divergence between seed rain in one year and the plants germinating in the next at these three sites was greater than the overall level of divergence between all five populations of the species in 1998-99. Two locations, DS and SA, showed no significant divergence between seed rain and subsequently germinating plants.

Allele frequencies

A total of 15 significant differences in allele frequency between one or more sample periods were found (Table 7). Eight loci, *Aco, Mdh-1, Mdh-2, 6-Pgd, Pgi, Pgm-1, Pgm-3,* and *Udp* exhibited significant changes. The these 8 loci, *Udp*, *Pgi*, and *Pgm-1* accounted for most of the changes. Changes in gene frequency occurred in all populations sampled. The significant changes in gene frequency were grouped arbitrarily into four categories as follows: 1) a shift from low- to intermediate-frequency or detection of a novel allele in plants germinating in 1999; 2) fixation or a significant decline in frequency of alleles in seeds relative to one or both plant generations; 3) non-directed fluctuations in frequency; and 4) a progressive decline in allele frequency over time or loss of a low-frequency allele. All but two of the significant changes in allele frequency were due to increased frequency of an allele or the occurrence of a novel allele in plants germinating in 1999 and/or reductions in frequency or fixation of alleles in seeds sampled in 1998.

DISCUSSION

Species-level variation

These levels of gene diversity observed in *D. leptoceras* have been detected consistently over time. Despite tremendous fluctuations in population sizes year to year no significant differences in gene diversity are observed. However, this level of diversity detected in

this study falls below that detected in 1995. Total gene diversity detected in the previous study was 0.20 (± 0.06) (Ferguson *et al.*, 1996). This reduction in diversity is due in part to the elimination of the highly variable *Lap* locus in the current study, but more so to the exclusion of two Los Angeles County populations, Bee Canyon and Tujunga Wash. Total gene diversity was reduced by 14% with the exclusion of the Los Angeles County populations.

In addition, population divergence was much more pronounced when Bee Canyon and Tujunga Wash were included in the survey. These two Los Angeles County populations accounted for approximately 31% of genetic differences between populations detected in 1995. The greater similarity between populations in Riverside and San Bernardino Counties could be due to historical founding events or to adaptation of genotypes to divergent environmental regimes in Los Angeles County. The large drop in genetic diversity for the species when the Los Angeles County populations are excluded from the study is strong evidence that the total diversity for this species is enhanced by the Bee Canyon and Tujunga Wash populations. If local extinctions occur, any unique genetic variants found in that local population are lost as well.

Population-level variation

The large amount of within population diversity (H_S) relative to total diversity (H_T) implies that numbers within each population are sufficiently large to insure that the effect of drift is weak. The consistent level of variation (h) maintained in individual populations of *D. leptoceras* indicates this as well. Though great differences in census numbers between populations occur, there is no evidence that smaller populations are significantly less variable genetically or more homozygous than consistently large populations of *D. leptoceras*. While we cannot know the historical levels of genetic diversity in these populations, *D. leptoceras* is certainly not genetically depauperate relative to other annual or endemic species (Hamrick and Godt, 1989). If habitat alterations and reduction in population numbers are adversely affecting genetic variation, the effects have not been

manifested as yet. It is possible that the seed bank is ameliorating any effects of recent, spatial changes in gene flow, assuming that the recent habitat destruction and population extirpations do represent actual reductions in gene flow. With the contribution of many years of seed rain to the genetic diversity of above-ground populations, the actual generation time is extended beyond a single year. This is possibly retarding the appearance of effects of habitat alteration and population reduction on genetic diversity.

Comparisons of seed rain and germinating plants

Gene diversity

The genetic diversity in seed rain (progeny) is much less than that in germinating plants (parents). The parents and progeny examined in 1998 show that while gene diversity and polymorphism are reduced in the progeny, the actual levels of homozygosity are not much changed. *F*-statistics and the average inbreeding coefficient between the two generations are almost identical. If the differences between germinating plants and seed rain were due solely to mating patterns (*e.g.*, a preponderance of within plant matings or mating between closely related individuals) then changes in relative homozygosity would be evident rather than changes in the overall level of gene diversity.

Even though the seed rain does in fact represent a limited portion of the genetic diversity of above-ground plants at germination, the allelic diversity and polymorphism of the seed rain is not less than that observed for other endemic or annual plants (Hamrick and Godt, 1989). Both 1995 and 1998, the two years in which seed rain was sampled, were years with greater precipitation and higher survivorship to reproduction. It is possible that the uniformity of both seed samples is due to the more benign environmental conditions under which they were produced and that under different conditions, a more genetically diverse seed rain may be produced. However, this has not been tested.

According to theoretical models (Templeton and Levin, 1979) and empirical studies (Epling *et al.*, 1960; del Castillo, 1994) seed banks may reduce population divergence. This is most likely the reason that observed G_{ST} values are less than that for other annual or endemic species. However, the results of this study suggest another effect of the seed bank upon above-ground population structure. By re-introducing alleles from past generations, the seed bank appears to responsible for the striking divergence between seed rain in 1998 and germinating plants within three of the five populations surveyed in 1999. Within population differences between seed rain and germinating plants were greater at three sites than average population differences across all sites. We can think of no obvious explanation for the similarity between seed rain and subsequently germinating plants at Dripping Springs (DS) and Orange St. (SA). These two locations represent the extremes in geographic distribution of the tested occurrences of *D. leptoceras*. In addition, the Dripping Springs occurrence is one of the consistently larger occurrences ($\geq$500 plants) while that at Orange St. has been in decline (< 100 plants) for the past few years. We can speculate that the sites display a similar pattern for different reasons; *e.g.* the similarity between seed rain and germinating plants at SA is due to reduced population sizes and low reproduction over the past three years which have depleted the seed bank at that site while the similarity at DS may be due to more benign environmental conditions which favor homogeneity of genotypes in seed rain and at germination. However, as we have no way of knowing how much of the actual seed bank and its diversity we have sampled in examining germinating plants, explanations about observed differences in the seed bank diversity between these two locations are speculative. The trend towards very high levels of differentiation between seed rain and subsequently germinating plants while highly significant at three sites, is only local.

Allele frequencies

The many significant differences in allele frequency between sample periods suggests that forces other than drift operate in these populations. Allele frequencies differed significantly most often at the *Pgi* and *Udp* loci. Previous studies comparing soil seeds to

surface plant genetics have also found the greatest differentiation at the *Pgi* locus (Tonsor *et al.*, 1993; Cabin, 1996). Many population genetic studies have also demonstrated non-random associations between electrophoretic variation at this locus and various ecological and environmental variables, most notably high temperatures and increased risk of desiccation or water stress (reviewed in Riddoch, 1973). *Dodecahema* plants are subject to both periodic droughts and seasonal Santa Ana conditions. Occurrences of this species vary tremendously in the percentage of germinating plants surviving to reproduction within any given season (Ferguson, 1999). Although the results of this and of other studies suggest that the *Pgi* locus is under selection or closely linked to other loci under selection, electrophoretic markers are generally chosen for their resolution rather than for their ecological significance which is usually not directly known (Ennos, 1989). Therefore, selective arguments are still controversial. While there is no evidence to suggest that *Udp* is under direct selection, it may be closely linked to genes that are. The dramatic increase in heterozygous genotypes in 1999 and the shifts from low to intermediate frequencies at this locus and others, suggest that selective germination of heterozygotes occurred during 1999. General reviews have concluded that greater heterozygosity characterizes species living in variable environments (Huenneke, 1991; Hedrick, 1995). Also the germination of novel alleles when environmental condition vary is predicted for species with a persistent soil seed bank (Templeton and Levin, 1979; Levin, 1990).

The fixation in seeds of loci that vary in both generations of plants is more perplexing. The fixation of loci in seeds could be due to three factors; 1) failure to detect the allele in electrophoresis of seeds; 2) sampling error; or 3) restrictions in seed diversity due to pre-reproductive mortality, the breeding system, or to selective elimination of rare alleles. Failure to detect alleles could occur because the small size of the seeds made resolution and scoring of some enzyme systems more difficult with seeds than with leaf tissue. However, the loci in question present clear results that are easily interpreted. Failure to detect variable individuals in the population due to sampling error is also unlikely as sample sizes are quite large and the lack of allelic diversity in seeds occurs at many loci. It

is more likely that seed diversity is reduced relative to germinating plants due to differential survivorship of germinating plants to reproduction, differential fecundity, or selection against certain alleles. Apart from chance mortality prior to reproduction, some germinating plants may possess mal-adapted genotypes from past generations and do not survive until reproduction or certain genotypes could be associated with reduced pollen fitness thereby reducing diversity of the gene flow between maternal and parental plants (Ellstrand, 1992). Reproductive or survival selection against rare alleles in both heterozygous and homozygous forms may be occurring in *D. leptoceras*. Selection against embryos may eliminate more heterogeneous individuals, however, ovule abortion does not exceed 20% of all ovules formed and in most cases is less than 10% (Ferguson, 1999). Survival selection may be eliminating rare alleles in both homozygous and heterozygous form. Such a phenomenon has been noted in *Pinus taeda* where rare alleles persisted in the population only due to a fecundity advantage of heterozygous over homozygous maternal plants (Bush and Smouse, 1991). In *D. leptoceras*, rare alleles persist in the soil seed bank enhancing population persistence over the longer term.

Heterozygosity

Germinating plants are more heterozygous than seed rain due to seed bank effects. Several studies have shown that heterozygosity increased over successive life-history stages in plants (reviewed in Ennos, 1989) and there is also evidence that heterozygosity increases with seed age (reviewed in Hamrick and Godt, 1989; Cabin, 1996). The relative heterozygosity of germinated plants and the single year seed rain may be a function of the relative age of these two life-history stages. The last year of abundant seed production was 1995. Virtually no seeds were produced in 1996 (Ferguson *et al.*, 1996) nor were seeds produced in 1997 (pers. obs). Therefore plants germinating from soil seeds in 1998-9 were drawn from seeds more than 3 years of age. Seeds from other annual plants species have been known to germinate after seven (Baskin and Baskin, 1978) or even ten years (Epling *et al.*, 1960) in the soil. Also, more inbred (homozygous) seeds are less likely than outcrossed seeds to perceive germination cues, and consequently have a longer

residency in the soil or possibly experience greater mortality. Kalisz (1989) found that a higher percentage of selfed seeds remained in the soil without germinating than did seeds produced by outcrossing in the winter annual, *Collinsia verna*. The increased heterozygosity in plants germinating in 1999 is an indication of the greater propensity of heterozygotes to germinate under conditions of minimal rainfall.

Research Considerations

The novel variation and increased genetic variance introduced into the 1999 samples by the *Udp* locus was pronounced. Samples from the 1999 populations were run in other gel systems in order to confirm that the unusual allele patterns were not the result of errors in running or scoring gels. While variation at the *Udp* locus contributed a great deal to the increased population divergence in 1999, it was not the only locus nor the one contributing most substantially in the calculation of G_{ST} and F_{st}. The greater variation in *Udp* at BA1, BA2, and SJ populations also contributed to the increased divergence between seed rain (1998) and germinating plants (1999). Again, *Udp* was not the sole or most significant contributor to F_{st}, and therefore we feel confident that the observed allelic differences represent actual population trends in gene diversity and population differentiation.

Summary

1) Gene diversity in *D. leptoceras* is consistently high and equals or exceeds that found in other annual or endemic plant species. Gene diversity and population differentiation were both reduced when the Los Angeles County populations of Bee Canyon and Tujunga Wash were excluded from the survey of genetic diversity. These findings indicate that the species does not appear to be in increased danger of extinction due to genetic factors. However, the Los Angeles County populations maintain a unique proportion of the total genetic diversity for the species. Further population losses will almost certainly reduce species diversity.

2) Despite large differences in population size between locations and fluctuations within populations between years, there is no evidence that any particular population maintains significantly less genetic diversity or has experienced increases in homozygosity relative to other surveyed populations of *D. leptoceras*. It is unlikely that anything like the past hydrological conditions and habitat contiguity will occur again in the alluvial systems supporting *D. leptoceras* but whatever effects habitat fragmentation and population isolation may impose, they are not currently detectable in the genetic diversity of surveyed populations. That is not to say that if gene flow patterns have been significantly altered that the effects will not become apparent in time.

3) The seed bank appears to be critical to restoring above-ground populations of *D. leptoceras* both demographically and genetically. Diversity either within or among past generations of seed rain has facilitated the accumulation of genetic variants in the soil seed bank. In the two years of examination we have not detected levels of diversity in seed rain comparable to that found in plants germinating from the soil seed bank. If selective elimination of alleles is occurring between life-history stages (*e.g.* plant to seed) and seasonal differences in the environment are a factor, then we can expect that the gene diversity of seed rain will differ under varying environmental conditions. As we do not know the true extent of the seed bank for this species, we cannot predict temporal limits

to its genetic diversity. Greater genetic variation can be maintained in fluctuating environments in which there is some escape mechanism such as dormancy (Ellner and Hairston, 1994; Hedrick, 1995).

Conservation Implications

The high diversity of the seed bank, high mortality of adult plants in the field (Ferguson, 1999), and the limited diversity of seed rain which would serve as the seed source suggest that the artificial establishment of new populations would require a large investment of seeds over many generations before populations would be self-perpetuating. Other studies have shown that successful establishment of annual plant species is difficult, requiring large quantities of seed and many years of monitoring (Jain, 1994). It is our belief that conservation efforts should center primarily on the conservation of extant populations. Given the harsh and unpredictable environment in which *D. leptoceras* grows and the fact that the factors which facilitate establishment are as yet unknown, looking towards re-introduction as a conservation aid is impractical at this time.

Though levels of genetic variability are similar in all populations, there has been a marked decline in numbers of plants at the Orange St. site during 1998-9 relative to that observed from 1994-6. A similar reduction in population size has also been noted at the Cone Camp site during the past four years (pers. obs.). As other populations in the Santa Ana Wash were not surveyed, we cannot say whether this trend is occurring in other locations in this wash. However, both Cone Camp and Orange St. populations maintained similar levels of genetic diversity in the previous study and the Orange St. population is highly variable at this time. Non-genetic reasons for their decline should be sought.

LITERATURE CITED

Allen, E. B. 1996. Characterizing the Habitat of Slender-horned Spineflower (*Dodecahema leptoceras*). Prepared for the Calif. Dept. of Fish and Game, Long Beach, CA. 12 pages.

Barrett, S. C. H. and J. R. Kohn. 1991. Genetic and evolutionary consequences of small population size in plants. Pages 3-30 in Falk, D. A. and K. E. Holsinger, eds. **Genetics and conservation of rare plants**, Oxford University Press, New York, NY.

Baskin, J. M. and C. C. Baskin. 1978. The seed bank in a population of an endemic plant species and its ecological significance. *Biol. Conserv.* 14: 125-129.

Bush, R. M., and P. E. Smouse. 1991. The impact of electrophoretic genotype on life history traits in *Pinus taeda. Evolution* 45(3): 481-498.

Cabin, R. J. 1996. Genetic comparisons of seed bank and seedling populations of a perennial mustard , *Lesquerella fendleri. Evolution* 50: 1830-1841.

del Castillo, R. F. 1994. Factors influencing the genetic structure of *Phacelia dubia*, a species with a seed bank and large fluctuations in population size. *Heredity* 72: 446-458.

Ellner, S. and N. G. Hairston, Jr. 1994. Role of overlapping generations in maintaining genetic variation in a fluctuating environment. *Am. Nat.* 14: 403-417.

Ellstrand, N. C. 1992. Gene flow by pollen: implications for plant conservation genetics. *Oikos* 63: 77-86.

Ellstrand, N. C. and D. R. Elam, 1993. Population genetic consequences of small population size: implications for plant conservation. *Annu. Rev. Ecol. Syst.* 24: 217-42.

Ellstrand, N. C. and J. M. Lee. 1987. Cultivar identification of Cherimoya (*Annona cherimola* Mill.) using isozyme markers. *Evolution* 14: 238-255.

Ennos, R.A. 1989. Detection and measurement of selection: genetic and ecological approaches. Pages 200-214 in A. H. D. Brown, M.T. Clegg, A.L. Kahler, and B.S. Weir, eds. **Plant population genetics, breeding, and genetic resources**, Sinaur, Sunderland, MA.

Epling C., H. Lewis, and F. M. Ball. 1960. The breeding group and seed storage: a study in population dynamics. *Evolution* 14: 238-255.

Ferguson, N. J. 1999. Demographic and Genetic Variation in *Dodecahema leptoceras* (Gray) Rev. & Hardham. Ph.D. Dissertation. University of California, Riverside. 210 pages.

Ferguson, N. J., R. Whitkus, and N. C. Ellstrand. 1996. Investigation into the population biology of *Dodecahema leptoceras* (slender-horned spineflower). Prepared for Calif. Dept. of Fish and Game, Region 5, Long Beach, CA. 25 pages.

Hardham, C. B. 1989. Chromosome number of some annual species of *Chorizanthe* and related genera (Polygonaceae: Eriogonoideae). *Phytologia* 66(2): 89-94.

Hamrick J.L. and M.J.W. Godt, 1989. Allozyme diversity in plant species. Pages 43-63 in A.H.D. Brown, M.T. Clegg, A.L. Kahler and B.S. Weir, eds. **Plant population genetics, breeding and germplasm resources,** Sinaur Associates, Inc., Sunderland, MA.

Hedrick, P. W. 1995. Genetic polymorphism in a temporally varying environment: effects of delayed germination of diapause. *Heredity* 75: 164-170.

Hickman, J.C., ed. 1993. **The Jepson Manual**. University of California Press, Berkeley, CA.

Huenneke, L. F. 1991. Ecological implications of genetic variation in plant populations. Pages 31-44 in D. A. Falk and K. Holsinger, eds. **Genetics and conservation of rare plants,** Oxford Univ. Press, New York.

Jain S. K. 1994. Genetics and demography of rare plants and patchily distributed colonizing species. Pages 292-307 in V. Loeschcke, J. Tomiuk, and S.K. Jain, eds. **Conservation Genetics,** Birkhauser Verlag, Basel, Switzerland.

Kalisz, S. 1989. Fitness consequences of mating system, seed weight, and emergence date in a winter annual, Collinsia verna. *Evolution* 43: 1263-1272.

Levin, D. A. 1990. The seed bank as a source of genetic novelty in plants. *Am. Nat.* 135: 563-572.

Lewis, P. O., 1992. *GeneStat-PC 3.3.* Dept. of Statitics, Box 8203, North Carolina State Univ., Raleigh, NC.

Lewis, P. O. and D. Zaykin. 1999. *Genetic Data Analysis.* Dept. of Ecology and Evolutionary Biology, University of Connecticut, Storrs, CT.

McCue, K. A. and T. P. Holtsford, 1998. Seed Bank Influences on Genetic Diversity in the rare annual *Clarkia springvillensis* (Onagraceae). *Am. J. Bot.* 85(1):30-36.

Morden, C. W., J. F. Doebley, and K. F.Schertz, 1987. A manual of techniques for starch gel electrophoresis of Sorghum isozyme. Texas Agricultural Experiment Station Miscellaneous Publications No. 1635. College Station, TX.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. *Proc. Nat. Acad. Sci., USA* 70: 3321-3323.

Nei, M. 1986. Definition and estimation of fixation indices. *Evolution* 40: 643-645.

Rey-Vizgirdas, E., 1994. Status and conservation of the endangered slender-horned spineflower (*Dodecahema leptoceras* (Gray) Rev. & Hardham) and alluvial scrub habitat in Southern California. M. A. Thesis. Calif. State University, Fullerton. 110 pages.

Riddoch, B. J. 1993. The adaptive significance of electrophoretic mobility in phosphoglucose isomerase (PGI). *Biol. J. Linn. Soc.* 50: 1-17.

Sokal, R. R. and F. J. Rohlf. 1995. **Biometry**. W. H. Freeman, San Francisco, CA.

Templeton A .R. and D. A. Levin, 1979. Evolutionary consequences of seed pools. *Am. Nat.*, 114: 232-249.

Tonsor, S. J., S. Kalisz, J. Fisher, and T. P. Holtsford. 1993. A life-history based study of population genetic structure: seed bank to adults in *Plantago lanceolata*. *Evolution* 47: 833-843.

United States Fish and Wildlife Service (USFWS). 1987. Endangered status for *Eriastrum densifollium* ssp. *sanctorum* (Santa Ana River wooly-star) and *Centrostegia leptoceras* (slender-horned spineflower). Final rule. **Federal Register** 52: 36265-36270.

Wendel J. F. and N. F. Weeden, 1989. Visualization and interpretation of plant iozymes. Pages 5-45 in D.E. Soltis and P.S. Soltis, eds. **Isozymes in Plant Biology**, Dioscorides Press, Portland, OR.

Weir, B. S. and C. C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* 38:1358-1370.

Wright, S. 1938. Size of a population and breeding structure in relation to evolution. *Science* 87:430-431.

Table 1. Estimates of *D. leptoceras* population sizes at dates of collection. The estimates of populations numbering under 100 plants represent direct counts. All other estimates are based on the observation of each plant collected representing 1 in X number of plants surrounding it.

	Number of Plants:		
Site:	**3/98**	**8/98**	**4/99**
Dripping Springs:			
DS1	750	475	500
DS2	500	500	12
Bautista Creek:			
BA1	650	492	500
BA2	900	500	400
San Jacinto Wash:			
SJ	2300	>3,000	500
Santa Ana Wash at Orange St.:			
SA	70	66	30

Table 2. Genetic diversity statistics (unbiased for sample size and population number) for *D. leptoceras* based on 12 isozyme loci. Data are pooled over 5 populations in each year. Data from the 1995 study, modified for 12 loci and 5 populations are included for comparison. H_T: total gene diversity; H_S: within population gene diversity; D_{ST}: difference between H_T and H_S; G_{ST}: co-efficient of genetic differentiation.

Year/Stage		H_S	D_{ST}	H_T	G_{ST}
1995 seed	mean	**0.12**	**0.02**	**0.14**	**0.13**
	s.e.	0.05	'*****	0.06	'*****
1998 plant	mean	**0.11**	**0.02**	**0.13**	**0.16**
	s.e.	0.04	'*****	0.05	'*****
1998 seed	mean	**0.08**	**0.01**	**0.09**	**0.12**
	s.e.	0.04	'*****	0.05	'*****
1999 plant	mean	**0.14**	**0.04**	**0.18**	**0.22**
	s.e.	0.04	'*****	0.06	'*****

Table 3. Polymorphism indices for 5 populations of *D. leptoceras* based on 12 isozyme loci. N: mean number of individuals sampled; *A*: alleles per locus; A_p; alleles per polymorphic locus; *P* (%); percent polymorphic loci. Modified data from 1995 study are included for comparison.

Year/Stage		N	A	A_p	P (%)
1995 Seed	mean	**18.1**	**1.63**	**2.37**	**44**
	s.e.	2.9	0.09	0.10	4
1998 Plant	mean	**65.1**	**2.15**	**2.65**	**70**
	s.e.	7.6	0.09	0.04	6
1998 Seed	mean	**45.3**	**1.78**	**2.96**	**40**
	s.e.	3	0.08	0.18	4
1999 Plant	mean	**36.4**	**2.05**	**2.51**	**70**
	s.e.	5.3	0.11	0.05	7

Table 4. Gene diversity (means and standard errors) for 5 populations of *D. leptoceras* in 1998-99. Data cover two life-history stages, seeds and plants.

Population	Year: Stage:	1998 plants	1998 seeds	1999 plants
DS	**mean**	**0.12**	**0.08**	**0.10**
	s.e	0.06	0.05	0.04
BA1	**mean**	**0.10**	**0.09**	**0.16**
	s.e.	0.05	0.05	0.05
BA2	**mean**	**0.10**	**0.08**	**0.16**
	s.e	0.05	0.05	0.07
SJ	**mean**	**0.11**	**0.07**	**0.15**
	s.e	0.04	0.04	0.05
SA	**mean**	**0.14**	**0.08**	**0.15**
	s.e.	0.05	0.05	0.05

DS= Dripping Springs; BA = Bautista Creek; SJ = San Jacinto Wash; SA = Santa Ana Wash.

Table 5. Individual population indices of polymorphism for *D. leptoceras* based on 12 isozyme loci. N: census size estimated at time of collection; n: sample size (mean n over all loci in population/12 loci); *A*: alleles per locus; *Ap*: alleles per polymorphic locus; *P* (%): percent polymorphic loci.

	SITE	N	n	*A*	*Ap*	*P* (%)
1998 PLANTS	DS	750	66.7	2.33	2.78	75
	BA1	650	84.1	1.83	2.67	50
	BA2	900	41.8	2.17	2.56	75
	SJ	2300	77.6	2.33	2.60	83
	SA	70	55.3	2.08	2.63	67
1998 SEEDS	DS	475	43.6	1.67	3.00	33
	BA1	492	44.7	1.83	3.00	42
	BA2	500	41.2	1.92	3.75	33
	SJ	3000	59.9	2.08	2.86	58
	SA	66	42.8	1.58	2.75	33
1999 PLANTS	DS	500	46.2	2.25	2.67	75
	BA1	500	39.8	1.75	2.50	50
	BA2	400	21.7	1.83	2.43	58
	SJ	500	48.1	2.27	2.40	91
	SA	30	26	2.17	2.56	75

DS = Dripping Springs; BA = Bautista Creek; SJ = San Jacinto Wash; SA = Santa Ana Wash

Table 6. Expected (H_e) and observed (H_o) heterozygosity, and the inbreeding coefficient (f) for 5 populations of *D. leptoceras* over two years and two life-history stages.

Year/Stage	Population	H_e	H_o	f
1998 plant	DS	0.12	0.07	0.43
	BA1	0.10	0.05	0.47
	BA2	0.10	0.05	0.47
	SJ	0.11	0.06	0.45
	SA	0.13	0.09	0.36
	mean	0.11	0.06	0.43
1998 seed	DS	0.08	0.05	0.44
	BA1	0.09	0.06	0.45
	BA2	0.08	0.04	0.32
	SJ	0.07	0.04	0.41
	SA	0.08	0.04	0.47
	mean	0.08	0.05	0.42
1999 plant	DS	0.09	0.05	0.48
	BA1	0.16	0.11	0.35
	BA2	0.16	0.12	0.21
	SJ	0.15	0.13	0.13
	SA	0.15	0.11	0.27
	mean	0.14	0.10	0.28

DS = Dripping Springs; BA = Bautista Creek; SJ = San Jacinto Wash; SA = Santa Ana Wash

Table 7. Chi-square test values comparing allele frequencies of plants (1998-9) and seed rain (1998) in five populations of *D. leptoceras*. Only loci in which samples varied significantly are included. Details on population labels and category of change are in the text.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; sequential Bonferroni for tests of individual loci.

Population	Change	Locus	Comparison of generations and stages:		
			'98 Plants-'98 Seeds	'98 Seeds-'99 Plants	'98 Plants-'99 Plants
DS	1	Pgm-3		15.15***	21.38***
	1	Udp			6.51*
BA1	3	Pgi	10.43**		25.72***
	2	Pgm-1	8.12*	34.87***	26.51***
	1,2	Udp		70.03***	137.00***
BA2	1	Pgm-1		41.67***	44.21***
	1	Udp		10.82***	86.43***
SJ	2	Aco	179.73***	198.09***	
	1,2	Mdh-2		6.48*	
	1	Pgi		17.81***	15.25**
	4	Pgm-1	23.71***		14.73***
	1	Udp		94.30***	82.96***
SA	1,2	Mdh-1		8.18**	5.76*
	1,2	6Pgd		4.71*	6.25*
	1	Pgi			21.26***

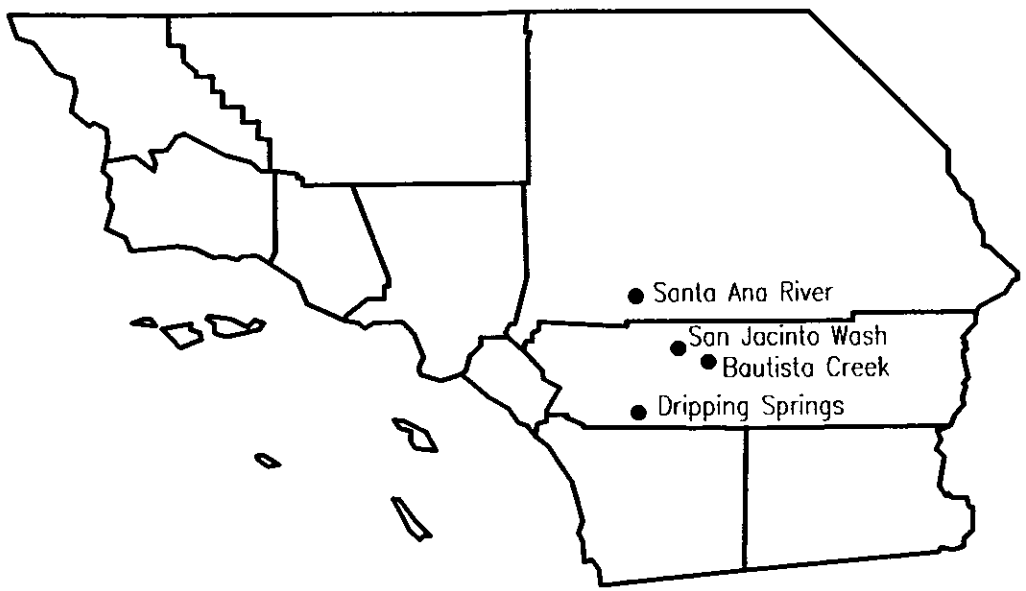

Figure 1. Locations of *D. leptoceras* populations surveyed by enzyme electrophoresis in San Bernardino, and Riverside counties of southern California.

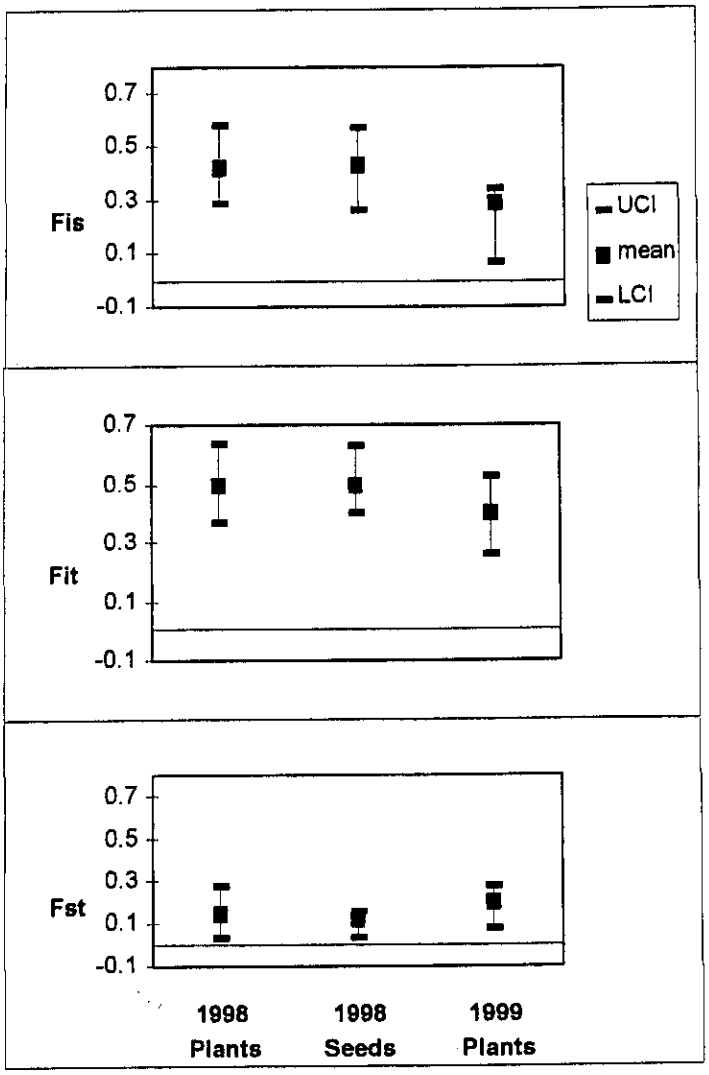

Figure 2. Wright's *F*-statistics estimated from 5 populations of *D. leptoceras* plants and seed rain. 95% confidence intervals were calculated from 1000 bootstrap iterations over 12 electrophoretic loci.

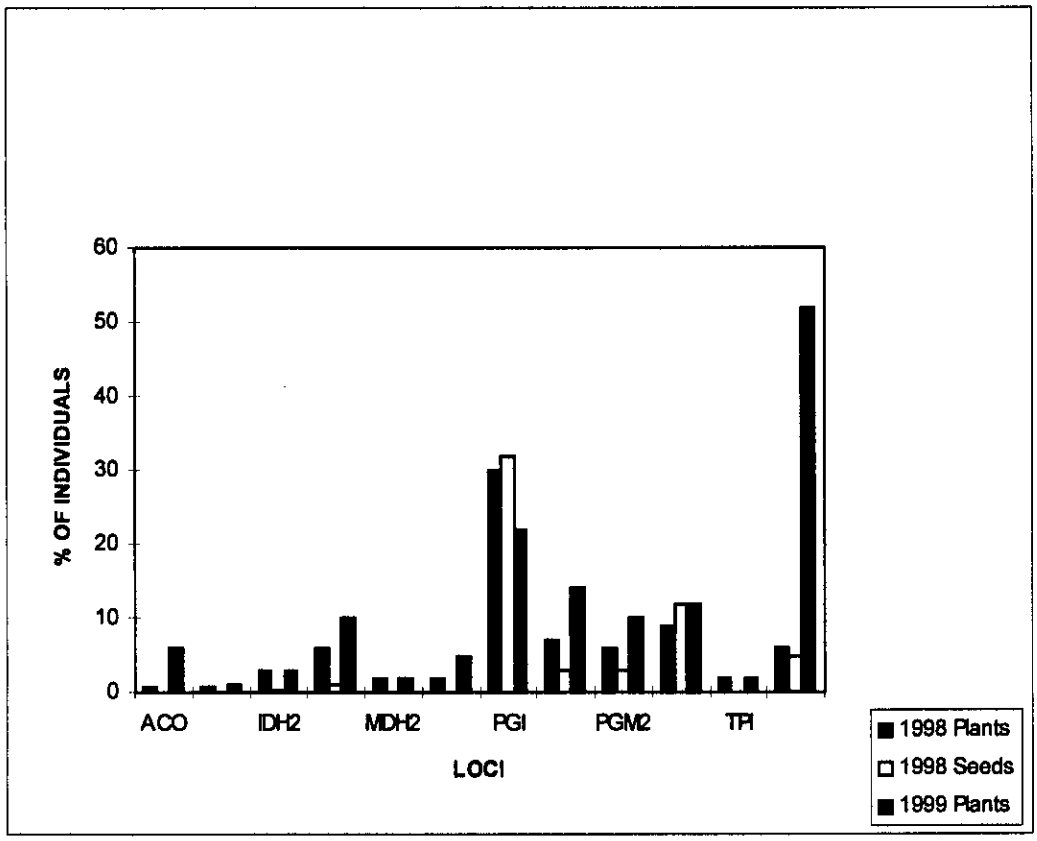

Figure 3. Histogram of the number of loci heterozygous for individual *D. leptoceras* seeds and germinated plants. Data are pooled over populations for life-history stages and years.

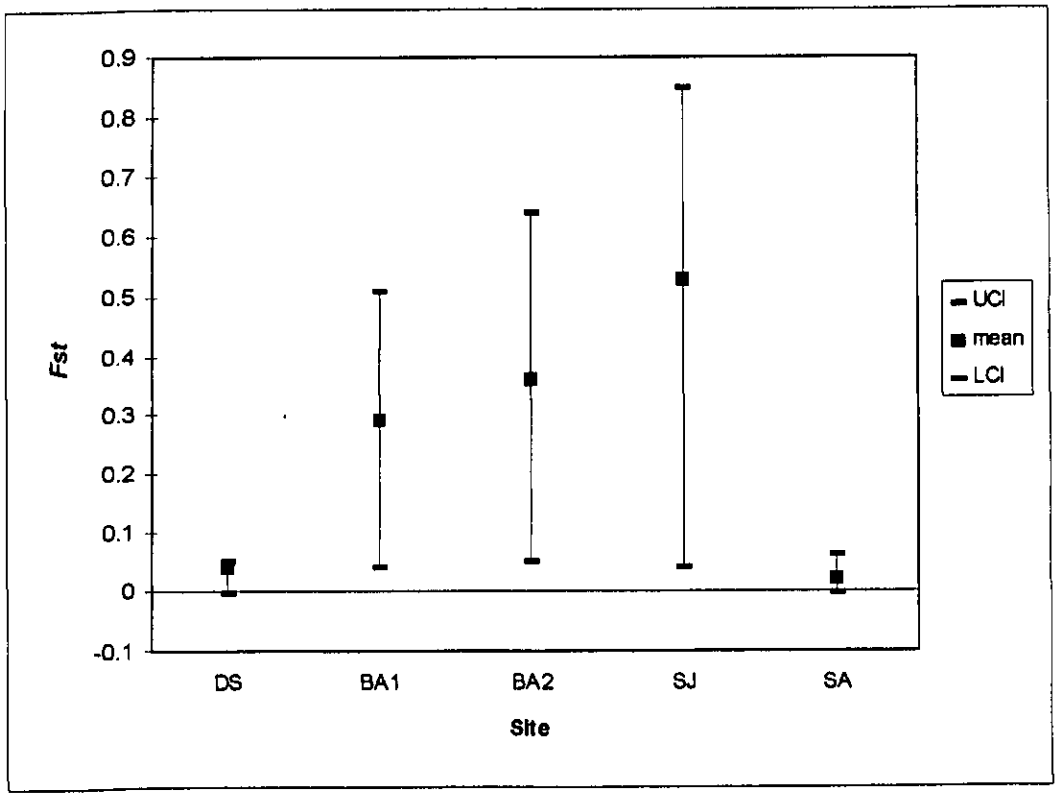

Figure 4. Wright's *F* statistics comparing population divergence between seed rain (1998) and germinating plants (1999) over 5 populations of *D. leptoceras*. 95% confidence intervals were calculated from 1000 bootstrap iterations over 12 electrophoretic loci.

Appendix A. Gene frequencies for 12 loci in 5 populations of *D. leptoceras* as determined by enzyme electrophoresis. Data are presented for plants (1998-9) and seed rain (1999). n: number of individuals scored per locus. DS: Dripping Springs; BA: Bautista Creek; SJ: San Jacinto Wash; SA: Santa Ana Wash.

	Population	DS1:			BA1:			BA2:			SJ:			SA:		
	Stage	plant	seed	plant	plant	seed	plant	plant	seed	plant	plant	seed	plant	plant	seed	plant
	Year	1998	1998	1999	1998	1998	1999	1998	1998	1999	1998	1998	1999	1998	1998	1999
PGM1	*n*	*39*	*53*	*47*	*91*	*51*	*41*	*45*	*44*	*21*	*49*	*68*	*49*	*53*	*36*	*26*
	1				0.06		0.29	0.02	0.02	0.48	0.07	0.02	0.02			
	2	0.99	1.00	1.00	0.94	0.99	0.71	0.96	0.91	0.48	0.80	0.98	0.97	0.92	0.99	1.00
	3	0.01				0.01		0.02	0.05	0.04	0.11		0.01	0.08	0.01	
	4								0.02		0.02					
PGM2	*n*	*39*	*53*	*47*	*91*	*51*	*41*	*47*	*44*	*21*	*50*	*57*	*49*	*55*	*36*	*26*
	1	0.03			0.07	0.08	0.11			0.02	0.01	0.01	0.02	0.09		0.02
	2	0.97	1.00	1.00	0.87	0.91	0.84	1.00	1.00	0.98	0.96	0.98	0.98	0.89	1.00	0.98
	3				0.06	0.01	0.05				0.03	0.01	0.00	0.02		
PGM3	*n*	*44*	*38*	*32*	*94*	*51*	*41*	*46*	*44*	*21*	*51*	*57*	*49*	*54*	*36*	*26*
	1	0.42	0.36	0.31	0.11	0.13	0.17	0.02			0.02	0.01	0.02	0.10	0.06	0.02
	2	0.38	0.46	0.69	0.88	0.83	0.81	0.93	0.91	1.00	0.89	0.96	0.98	0.85	0.94	0.90
	3	0.18														
	4	0.02	0.18		0.01	0.04	0.02	0.05	0.09		0.09	0.03		0.05		0.08
TPI	*n*	*101*	*28*	*50*	*96*	*22*	*34*	*48*	*21*	*22*	*84*	*54*		*61*	*28*	*26*
	1	0.02	0.04	0.01									nd	0.06		0.04
	2	0.97	0.96	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	nd	0.94	1.00	0.96
	3	0.01											nd			
UDP	*n*	*66*	*38*	*28*	*94*	*34*	*40*	*48*	*58*	*22*	*56*	*57*	*47*	*59*	*52*	*26*
	1			0.03	0.03		0.70		0.01	0.73			0.65	0.06	0.02	0.04
	2	1.00	1.00	0.94	0.97	0.98	0.30	1.00	0.96	0.27	0.96	0.98	0.35	0.81	0.84	0.79
	3			0.03		0.02			0.02		0.04	0.02		0.13	0.12	0.17
	4								0.01						0.02	

	Population	DS1:			BA1:			BA2:			SJ:			SA:		
	Stage	plant	seed	plant	plant	seed	plant	plant	seed	plant	plant	seed	plant	plant	seed	plant
	Year	1998	1998	1999	1998	1998	1999	1998	1998	1999	1998	1998	1999	1998	1998	1999
PGM1	*n*	*39*	*53*	*47*	*91*	*51*	*41*	*45*	*44*	*21*	*49*	*68*	*49*	*53*	*36*	*26*
	1				0.06		0.29	0.02	0.02	0.48	0.07	0.02	0.02			
	2	0.99	1.00	1.00	0.94	0.99	0.71	0.96	0.91	0.48	0.80	0.98	0.97	0.92	0.99	1.00
	3	0.01				0.01		0.02	0.05	0.04	0.11		0.01	0.08	0.01	
	4								0.02		0.02					
PGM2	*n*	*39*	*53*	*47*	*91*	*51*	*41*	*47*	*44*	*21*	*50*	*57*	*49*	*55*	*36*	*26*
	1	0.03			0.07	0.08	0.11			0.02	0.01	0.01	0.02	0.09		0.02
	2	0.97	1.00	1.00	0.87	0.91	0.84	1.00	1.00	0.98	0.96	0.98	0.98	0.89	1.00	0.98
	3				0.06	0.01	0.05				0.03	0.01	0.00	0.02		
PGM3	*n*	*44*	*38*	*32*	*94*	*51*	*41*	*46*	*44*	*21*	*51*	*57*	*49*	*54*	*36*	*26*
	1	0.42	0.36	0.31	0.11	0.13	0.17	0.02			0.02	0.01	0.02	0.10	0.06	0.02
	2	0.38	0.46	0.69	0.88	0.83	0.81	0.93	0.91	1.00	0.89	0.96	0.98	0.85	0.94	0.90
	3	0.18														
	4	0.02	0.18		0.01	0.04	0.02	0.05	0.09		0.09	0.03		0.05		0.08
TPI	*n*	*101*	*28*	*50*	*96*	*22*	*34*	*48*	*21*	*22*	*84*	*54*		*61*	*28*	*26*
	1	0.02	0.04	0.01									nd	0.06		0.04
	2	0.97	0.96	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	nd	0.94	1.00	0.96
	3	0.01											nd			
UDP	*n*	*66*	*38*	*28*	*94*	*34*	*40*	*48*	*58*	*22*	*56*	*57*	*47*	*59*	*52*	*26*
	1			0.03	0.03		0.70		0.01	0.73			0.65	0.06	0.02	0.04
	2	1.00	1.00	0.94	0.97	0.98	0.30	1.00	0.96	0.27	0.96	0.98	0.35	0.81	0.84	0.79
	3			0.03		0.02			0.02		0.04	0.02		0.13	0.12	0.17
	4								0.01						0.02	