

**DO SURFACE PLANT AND SOIL SEED BANK  
POPULATIONS DIFFER GENETICALLY? A  
MULTIPOPULATION STUDY OF THE DESERT MUSTARD  
*LESQUERELLA FENDLERI* (BRASSICACEAE)<sup>1</sup>**

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Seed banks are an important component of many plant populations, but few empirical studies have investigated the genetic relationship between soil seeds and surface plants. We compared the genetic structure of soil seeds and surface plants of the desert mustard *Lesquerella fendleri* within and among five ecologically diverse populations at the Sevilleta National Wildlife Refuge in Central New Mexico. At each site, 40 *Lesquerella* surface plants and 40 samples of soil seeds were mapped and genetically analyzed using starch gel electrophoresis. Overall allele frequencies of soil seeds and surface plants showed significant differences across the five populations and within three of the five individual populations. Surface plants had significantly greater amounts of single and multilocus heterozygosity, and mean surface plant heterozygosity was also greater at the total population level and in four of the five individual populations. Overall soil seed (but not surface plant) homozygosity was significantly greater than predicted by Hardy-Weinberg expectations at the total and individual population levels. Although  $F_{st}$  estimates revealed similarly small but significant genetic divergence within each life-history stage, estimates of coancestry showed that fine-scale (0.5–2 m) genetic correlations among the surface plant genotypes were roughly twice those of soil seed genotypes. An unweighted pair group method with arithmetic mean cluster analysis indicated that in the two geographically closest sites, the surface plants were slightly more genetically similar to each other than to their own respective seed banks. We also found weak and/or negative demographic associations between *Lesquerella* soil seed and surface plant densities within each of the five sites. We discuss the difficulties involved with sampling and genetically comparing these two life-history stages.

**Key words:** allele frequencies; Brassicaceae; coefficient of coancestry; ecological genetics; heterozygosity; *Lesquerella fendleri*; population genetic structure; seed banks; Wright's F statistics.

Soil seed banks are an important component of many plant populations in habitats ranging from tropical forests (Garwood, 1989) to deserts (Kemp, 1989). Despite the intensive study seed banks have received over the past 20 yr (see reviews in Roberts, 1981; Fenner, 1985; Leck, Parker, and Simpson, 1989; Thompson, 1992), all that is known about most seed banks is their size and species composition, as estimated at a single point in space and time. Although a few recent studies have begun to empirically investigate the demographic relationship between seed banks and the aboveground vegetation (Kalisz, 1991; Kalisz and McPeck, 1992; del Castillo, 1994; Cabin, 1995), our understanding of the potential for seed banks to affect the evolutionary and genetic dynamics of

surface plant populations comes largely from theoretical discussions and mathematical models (e.g., Templeton and Levin, 1979; Venable and Lawlor, 1980; Ritland, 1983; Brown and Venable, 1986; Klinkhamer et al., 1987; Venable and Brown, 1988; Venable, 1989).

These theoretical investigations have generated several intriguing hypotheses concerning how seed banks might affect the genetic structure of aboveground plant populations. For example, seed banks may function as a kind of genetic memory, accumulating and storing seed genotypes matured in different years and under potentially different selective regimes. Seed banks thus have the potential to dampen the response of plant populations to new selective pressures by introducing genotypes from the past, or alternatively, seed banks may serve as genetic reservoirs and ultimately increase the rate of evolution by maintaining large amounts of genetic variation for selection to act on. Finally, the ability of seeds in the soil to track and predict the environment (reviewed in Mayer and Poljakoff-Mayber, 1975; Baskin and Baskin, 1989) may also have resulted in the coadaptation of genes regulating dormancy and germination with genes controlling postgermination traits expressed later in the life cycle.

Although empirical investigation of the evolutionary effects of soil seeds are few, there is evidence that supports some of the above theoretical ideas. For example, some empirical studies (e.g., Epling, Lewis, and Ball, 1960; del Castillo, 1994) suggest that seed banks preserve local genetic diversity and buffer effective popu-

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lation sizes during periods when there are few or no aboveground plants. Work by McGraw and his associates within a tundra ecosystem in Alaska (Bennington, McGraw, and Vavrek, 1991; McGraw, Vavrek, and Bennington, 1991; Vavrek, McGraw, and Bennington, 1991; McGraw, 1993) has also demonstrated phenotypic and genetic divergence among populations derived from young soil seeds, old soil seeds, and the extant surface plants of several different species. Although the causal mechanisms behind these differences could not be determined, their results are consistent with the idea that seed banks may function as genetic memories of past regimes. Finally, Evans and Cabin (1995) documented the existence of conditions favoring the joint evolution of dormancy and postgermination traits in the desert mustard *Lesquerella fendleri* and argued that such associations may be adaptive.

While there are now a wealth of studies that investigate the distribution of genetic variation within and among the surface plant populations of many diverse species (reviewed in Hamrick, Mitton, and Linhart, 1979; Loveless and Hamrick, 1984; Hamrick, 1989; Mitton, 1989; Heywood, 1991), there are almost no empirical data on the distribution of genetic variation within seed bank populations. Although two empirical studies have directly analyzed the genetic structure of seed banks (Tonsor, Kalisz, and Fisher, 1993; Cabin, 1996), both of these studies examined only a single seed bank population. Intriguingly, both studies found significant genetic differences between the above- and belowground populations, suggesting that the distribution of genetic variation may differ across these life-history stages. However, no empirical studies have investigated the genetic relationship between seed banks and surface plants across larger spatial scales. These larger scale, multipopulation studies are necessary to test whether there are broad genetic differences between soil seeds and surface plants, rather than merely idiosyncratic differences between these life-history stages in particular populations. Such studies also allow us to calculate and compare the variability of local genetic statistics within and among the above- and below ground plant populations. Finally, this approach may ultimately improve our understanding of whether and to what extent soil seed banks affect the genetic structure of entire surface plant populations (e.g., do seed banks retard or enhance the genetic divergence of aboveground populations?).

Here we present the results of the first study to examine the genetic relationship of soil seeds and surface plants across several populations. Due to the complete absence of empirical data bearing on this question, this is necessarily an exploratory study that simply asks whether there are genetic differences between soil seeds and surface plants at scales larger than a single population. To investigate this question, we compare the genetic relationship of *Lesquerella fendleri* soil seeds and surface plants among five ecologically diverse populations across the Sevilleta National Wildlife Refuge in central New Mexico. We assess this genetic relationship using four kinds of analyses. First, we compare allele frequencies and heterozygosity of *Lesquerella* soil seeds and surface plants within and among the five populations. Second, we use Wright's (1951) F statistics to compare the par-

tioning of genetic variation within each life-history stage. Third, we estimate the coefficient of coancestry to compare the genetic structure of soil seeds and surface plants along a continuous spatial scale, and fourth, we examine the overall genetic relationship among the ten individual populations by constructing a phenogram using Nei's (1978) measure of genetic distance.

## MATERIALS AND METHODS

**Study system**—*Lesquerella fendleri* (Brassicaceae) is a short-lived, self-incompatible, perennial mustard native to the southwestern United States and northern Mexico (Rollins and Shaw, 1973). All field work was performed using *Lesquerella* populations at the National Science Foundation's Sevilleta Long-Term Ecological Research Site, ~100 km south of Albuquerque, New Mexico. At the Sevilleta, flowering of *Lesquerella* plants normally occurs in late April and early May, and often following suitable rains in the fall. Field plants may produce one to several hundred fruits, each containing 1–30 seeds, which mature between June and July. Seed dispersal experiments using naturally dehiscent fruits have shown that the vast majority of uneaten *Lesquerella* seeds remain within 1 m of the parent plant (R. Cabin and R. Mitchell, University of New Mexico, unpublished data). Artificial seed bank experiments (R. Cabin, University of New Mexico, unpublished data) and demographic comparisons of natural *Lesquerella* seed production and soil seed abundance (Evans and Cabin, 1995) at the Sevilleta indicate the majority of soil seeds can remain viable for at least 3 yr. Germination of *Lesquerella* seeds occurs primarily between the fall and early spring, depending on the amount and timing of rainfall (R. Cabin, University of New Mexico, personal observations). For more details of this species and the Sevilleta ecosystem, see Evans and Cabin (1995), Cabin (1996), and Cabin et al. (1997a, b).

**Sampling design**—To investigate the genetic relationship between *Lesquerella* surface plants and soil seeds, we selected five *Lesquerella* populations from ecologically diverse habitats across an 8 × 9 km section of the Sevilleta. Site A consists of open, clayey washes surrounded by grasses and herbs. Site F is dominated by creosote shrubs (*Larrea tridentata*) and largely open, barren patches in the interspaces between the shrubs. Site G lies in a transition zone between creosote shrublands and desert grassland habitats. Site M runs across a kangaroo rat (*Dipodomys* sp.) mound within a relatively sparse desert grassland habitat. Site H runs across the top of a largely barren hill with thin gravelly soil. We sampled all populations in April and May of 1994.

At each site, we established a line transect through the *Lesquerella* surface plant population. Based on field observations in the previous 3 yr, we estimated that the *Lesquerella* plants at each site were composed of roughly equivalent proportions of seedlings and older, established plants (probably between 1–4 yr old). To sample the aboveground population, we collected one to two leaf tissue samples per plant (depending on the amount of tissue available) from the first 40 *Lesquerella* plants that intersected the line transect at each site (40 plants/site × five sites = 200 total plants sampled). Each sample was placed into a chilled microtube containing an extraction buffer, then transported to the laboratory and homogenized. All tissue samples were stored at –70°C until allozyme electrophoresis was performed.

To sample the *Lesquerella* seed bank, we collected soil samples adjacent to each of the 40 surface plants sampled at each site, resulting in 200 total soil samples. Each soil sample covered a surface area of ~0.21 m<sup>2</sup>. Since previous desert seed bank studies have shown that 80–90% of all soil seeds are in the top 2 cm of soil (Childs and Goodall, 1973; Reichman, 1975) and that many desert seeds including *Lesquerella fendleri* (I. Ortiz, University of New Mexico, unpublished data) are unable to emerge from below this soil depth (Freas and Kemp, 1983; Kemp, 1989), soil was sampled to a depth of ~2 cm by carefully skimming the soil surface with a flat shovel. Previous pilot studies indicated

TABLE 1. Allele frequencies and heterozygosity for surface plants and soil seeds. *N* = the number of individuals scored at each locus. Het. = the observed proportion of individuals heterozygous at each locus. Values for the total population of surface plants and soil seeds (pooled across the five sites) and each individual population are shown separately for each locus.

| Locus        | Pop.         | Stage         | <i>N</i>      | Allele |       |       |       |       | Het.  |       |
|--------------|--------------|---------------|---------------|--------|-------|-------|-------|-------|-------|-------|
|              |              |               |               | 1      | 2     | 3     | 4     | 5     |       |       |
| <i>Idh</i>   | Total        | surface-plant | 197           | 0.015  | 0.962 | 0.003 | 0.020 |       | 0.066 |       |
|              |              | soil seed     | 193           | 0.018  | 0.940 | 0.010 | 0.031 |       | 0.119 |       |
|              | A            | surface-plant | 39            | 0.026  | 0.962 | 0.000 | 0.013 |       | 0.077 |       |
|              |              | soil seed     | 38            | 0.013  | 0.974 | 0.000 | 0.013 |       | 0.053 |       |
|              | F            | surface-plant | 39            | 0.013  | 0.974 | 0.013 | 0.000 |       | 0.051 |       |
|              |              | soil seed     | 38            | 0.000  | 0.961 | 0.013 | 0.026 |       | 0.079 |       |
|              | G            | surface-plant | 39            | 0.038  | 0.910 | 0.000 | 0.051 |       | 0.128 |       |
|              |              | soil seed     | 38            | 0.039  | 0.947 | 0.000 | 0.013 |       | 0.105 |       |
|              | H            | surface-plant | 40            | 0.000  | 0.962 | 0.000 | 0.038 |       | 0.075 |       |
|              |              | soil seed     | 39            | 0.000  | 0.885 | 0.026 | 0.090 |       | 0.231 |       |
|              | M            | surface-plant | 40            | 0.000  | 1.000 | 0.000 | 0.000 |       | 0.000 |       |
|              |              | soil seed     | 40            | 0.038  | 0.938 | 0.013 | 0.013 |       | 0.125 |       |
|              | <i>Pgd-1</i> | Total         | surface-plant | 172    | 0.003 | 0.453 | 0.544 |       |       | 0.453 |
|              |              |               | soil seed     | 181    | 0.006 | 0.381 | 0.613 |       |       | 0.337 |
| A            |              | surface-plant | 36            | 0.000  | 0.319 | 0.681 |       |       | 0.361 |       |
|              |              | soil seed     | 36            | 0.014  | 0.167 | 0.819 |       |       | 0.222 |       |
| F            |              | surface-plant | 26            | 0.019  | 0.423 | 0.558 |       |       | 0.385 |       |
|              |              | soil seed     | 34            | 0.000  | 0.426 | 0.574 |       |       | 0.441 |       |
| G            |              | surface-plant | 37            | 0.000  | 0.595 | 0.405 |       |       | 0.486 |       |
|              |              | soil seed     | 35            | 0.000  | 0.443 | 0.557 |       |       | 0.257 |       |
| H            |              | surface-plant | 36            | 0.000  | 0.431 | 0.569 |       |       | 0.417 |       |
|              |              | soil seed     | 37            | 0.014  | 0.514 | 0.473 |       |       | 0.514 |       |
| M            |              | surface-plant | 37            | 0.000  | 0.486 | 0.514 |       |       | 0.595 |       |
|              |              | soil seed     | 39            | 0.000  | 0.359 | 0.641 |       |       | 0.256 |       |
| <i>Pgd-2</i> |              | Total         | surface-plant | 160    | 0.016 | 0.256 | 0.728 |       |       | 0.419 |
|              |              |               | soil seed     | 175    | 0.029 | 0.217 | 0.754 |       |       | 0.331 |
|              | A            | surface-plant | 38            | 0.000  | 0.224 | 0.776 |       |       | 0.342 |       |
|              |              | soil seed     | 37            | 0.014  | 0.203 | 0.784 |       |       | 0.324 |       |
|              | F            | surface-plant | 23            | 0.065  | 0.174 | 0.761 |       |       | 0.478 |       |
|              |              | soil seed     | 32            | 0.000  | 0.203 | 0.797 |       |       | 0.333 |       |
|              | G            | surface-plant | 37            | 0.027  | 0.331 | 0.662 |       |       | 0.459 |       |
|              |              | soil seed     | 32            | 0.031  | 0.250 | 0.719 |       |       | 0.313 |       |
|              | H            | surface-plant | 30            | 0.000  | 0.233 | 0.767 |       |       | 0.467 |       |
|              |              | soil seed     | 37            | 0.014  | 0.230 | 0.757 |       |       | 0.378 |       |
|              | M            | surface-plant | 32            | 0.000  | 0.313 | 0.688 |       |       | 0.375 |       |
|              |              | soil seed     | 37            | 0.081  | 0.203 | 0.716 |       |       | 0.297 |       |
|              | <i>Pgm-1</i> | Total         | surface-plant | 200    | 0.188 | 0.813 |       |       |       | 0.295 |
|              |              |               | soil seed     | 193    | 0.127 | 0.873 |       |       |       | 0.202 |
| A            |              | surface-plant | 40            | 0.188  | 0.813 |       |       |       | 0.275 |       |
|              |              | soil seed     | 38            | 0.171  | 0.829 |       |       |       | 0.289 |       |
| F            |              | surface-plant | 40            | 0.225  | 0.775 |       |       |       | 0.450 |       |
|              |              | soil seed     | 38            | 0.158  | 0.842 |       |       |       | 0.263 |       |
| G            |              | surface-plant | 40            | 0.225  | 0.775 |       |       |       | 0.300 |       |
|              |              | soil seed     | 38            | 0.145  | 0.855 |       |       |       | 0.237 |       |
| H            |              | surface-plant | 40            | 0.087  | 0.913 |       |       |       | 0.025 |       |
|              |              | soil seed     | 39            | 0.064  | 0.936 |       |       |       | 0.026 |       |
| M            |              | surface-plant | 40            | 0.213  | 0.788 |       |       |       | 0.425 |       |
|              |              | soil seed     | 40            | 0.100  | 0.900 |       |       |       | 0.200 |       |
| <i>Pgm-2</i> |              | Total         | surface-plant | 200    | 0.755 | 0.245 |       |       |       | 0.370 |
|              |              |               | soil seed     | 192    | 0.758 | 0.242 |       |       |       | 0.339 |
|              | A            | surface-plant | 40            | 0.913  | 0.087 |       |       |       | 0.125 |       |
|              |              | soil seed     | 38            | 0.868  | 0.132 |       |       |       | 0.158 |       |
|              | F            | surface-plant | 40            | 0.675  | 0.325 |       |       |       | 0.400 |       |
|              |              | soil seed     | 38            | 0.645  | 0.355 |       |       |       | 0.395 |       |
|              | G            | surface-plant | 40            | 0.700  | 0.300 |       |       |       | 0.450 |       |
|              |              | soil seed     | 38            | 0.842  | 0.158 |       |       |       | 0.211 |       |
|              | H            | surface-plant | 40            | 0.812  | 0.188 |       |       |       | 0.375 |       |
|              |              | soil seed     | 39            | 0.769  | 0.231 |       |       |       | 0.359 |       |
|              | M            | surface-plant | 40            | 0.675  | 0.325 |       |       |       | 0.500 |       |
|              |              | soil seed     | 39            | 0.667  | 0.333 |       |       |       | 0.564 |       |
|              | <i>Pgi</i>   | Total         | surface-plant | 179    | 0.017 | 0.056 | 0.335 | 0.486 | 0.106 | 0.715 |
|              |              |               | soil seed     | 171    | 0.015 | 0.050 | 0.389 | 0.506 | 0.041 | 0.596 |
| A            |              | surface-plant | 35            | 0.057  | 0.100 | 0.443 | 0.386 | 0.014 | 0.714 |       |
|              |              | soil seed     | 36            | 0.069  | 0.028 | 0.528 | 0.361 | 0.014 | 0.500 |       |
| F            |              | surface-plant | 39            | 0.000  | 0.000 | 0.423 | 0.385 | 0.192 | 0.846 |       |
|              |              | soil seed     | 34            | 0.000  | 0.029 | 0.500 | 0.441 | 0.029 | 0.765 |       |
| G            |              | surface-plant | 37            | 0.027  | 0.162 | 0.243 | 0.473 | 0.095 | 0.730 |       |
|              |              | soil seed     | 34            | 0.000  | 0.118 | 0.279 | 0.544 | 0.059 | 0.588 |       |

TABLE 1. Continued.

| Locus      | Pop.  | Stage         | N   | Allele |       |       |       |       | Het.  |
|------------|-------|---------------|-----|--------|-------|-------|-------|-------|-------|
|            |       |               |     | 1      | 2     | 3     | 4     | 5     |       |
| <i>Lap</i> | H     | surface-plant | 33  | 0.000  | 0.000 | 0.273 | 0.652 | 0.076 | 0.485 |
|            |       | soil seed     | 34  | 0.000  | 0.029 | 0.309 | 0.618 | 0.044 | 0.588 |
|            | M     | surface-plant | 35  | 0.000  | 0.014 | 0.286 | 0.557 | 0.143 | 0.771 |
|            |       | soil seed     | 33  | 0.000  | 0.045 | 0.318 | 0.576 | 0.061 | 0.514 |
|            | Total | surface-plant | 189 | 0.048  | 0.225 | 0.450 | 0.262 | 0.016 | 0.550 |
|            |       | soil seed     | 181 | 0.041  | 0.240 | 0.478 | 0.224 | 0.017 | 0.380 |
|            | A     | surface-plant | 36  | 0.042  | 0.153 | 0.625 | 0.181 | 0.000 | 0.472 |
|            |       | soil seed     | 36  | 0.056  | 0.153 | 0.597 | 0.194 | 0.000 | 0.389 |
|            | F     | surface-plant | 38  | 0.079  | 0.224 | 0.434 | 0.263 | 0.000 | 0.684 |
|            |       | soil seed     | 37  | 0.054  | 0.230 | 0.486 | 0.203 | 0.027 | 0.243 |
|            | G     | surface-plant | 40  | 0.038  | 0.237 | 0.375 | 0.300 | 0.050 | 0.475 |
|            |       | soil seed     | 35  | 0.057  | 0.200 | 0.543 | 0.200 | 0.000 | 0.371 |
|            | H     | surface-plant | 36  | 0.028  | 0.236 | 0.333 | 0.403 | 0.000 | 0.472 |
|            |       | soil seed     | 38  | 0.000  | 0.197 | 0.368 | 0.382 | 0.053 | 0.474 |
|            | M     | surface-plant | 39  | 0.051  | 0.269 | 0.487 | 0.167 | 0.026 | 0.641 |
|            |       | soil seed     | 35  | 0.043  | 0.429 | 0.400 | 0.129 | 0.000 | 0.400 |

that this soil sampling procedure would yield at least one *Lesquerella* soil seed in most samples.

To analyze the genetic structure of the *Lesquerella* seed bank at each of the five populations, we first passed each soil sample through 1.98 and 0.59 mm mesh sieves to remove soil particles much larger and smaller than the *Lesquerella* soil seeds. Because the soils within and among the sites differed considerably in texture and organic content, different amounts of soil from each sample remained after this sieving process. We therefore measured the remaining volume of soil in each sample after sieving and subsampled 250 mL of this sieved soil for *Lesquerella* seed bank quantification and tissue collection (mean  $\pm$  1 SD of remaining soil = 480  $\pm$  243 mL). Each 250-mL soil sample was spread in a thin layer on top of sand-filled plastic flats, sprayed with 100 ppm gibberellic acid to break dormancy (Sharir and Gelmond, 1971; Evans, Mitchell, and Cabin, 1996), and placed in a greenhouse receiving ambient light and 21° day and 10°C night temperatures. Previous studies (Cabin, 1996) testing the accuracy of this procedure have shown that the number of *Lesquerella* soil seeds per sample recovered via greenhouse germination is strongly and significantly correlated with the number of viable soil seeds per sample found via hand-extraction under a dissecting microscope ( $r = 0.83$ ,  $P < 0.0001$ ).

We censused each of the 200 germinated soil samples in the greenhouse for emerging *Lesquerella* seedlings for 6 wk, and randomly collected and processed up to two *Lesquerella* seedlings per soil sample throughout this time as described above for the *Lesquerella* surface plant sampling at the Sevilleta. For the samples that contained fewer than two *Lesquerella* seedlings (8.5% of the original 200 soil samples), we germinated the remaining sieved soil and censused these samples for an additional 6 wk. After this second round of germination, we were able to collect at least one *Lesquerella* seedling from 195 of the original 200 soil samples. To estimate the total number of viable soil seeds within each sample, we multiplied the total amount of sieved soil from each sample by the number of *Lesquerella* seedlings found per millilitre of germinated sieved soil.

**Genetic data collection and analysis**—We characterized *Lesquerella* genotypes using starch gel electrophoresis. We assayed five enzymes and seven polymorphic loci using methods described in Cabin (1996). We electrophoresed all 200 samples from the *Lesquerella* surface plant population, and one seedling from all germinated soil samples containing *Lesquerella* plants. Since both greenhouse-germinated and field-collected plants were randomly sampled without regard to their size and tissue quality, some of these samples (~15%) resulted in missing scores at one or more electrophoretic loci. In these cases we ran an additional sample (if available) from the same spatial location and life-history

stage and used only the data from whichever sample produced the most complete electrophoretic genotype. Thus for all genetic analyses, only one soil seed genotype and one surface plant genotype were analyzed at each of the 40 positions along each of the five transects.

To compare allele frequencies and observed heterozygosity of *Lesquerella* soil seeds and surface plants, we used log-likelihood  $g$  tests. Rare alleles were combined when necessary to meet the assumptions of the test (Fienberg, 1980).  $P$  values for  $G$  tests with small expected cell values were calculated by comparing the observed  $G$  value with  $G$  values generated from 1000 iterations of a randomization program (see related discussion in Potvin and Roff [1993] and Cabin [1996]). The overall null hypothesis of no genetic differences between *Lesquerella* soil seeds and surface plants was tested by pooling the data over all loci and populations (the  $G$  values in the lower left-hand corner of Table 2). To explore the details of these overall tests, we also present separate analyses for the seven loci within each of the five populations, and use a sequential Bonferroni adjustment (Rice, 1989) to correct for multiple tests of the same data set. Sample sizes, allele frequencies, and mean observed heterozygosity for each locus are shown by life-history stage for each of the five populations in Table 1.

To compare the partitioning of genetic variation within each life-history stage among the five populations, we used Wright's  $F$  statistics.  $F_{st}$  estimates the amount of genetic differentiation among the five individual populations.  $F_{it}$  estimates the amount of homozygosity within individuals relative to that which would occur if gametes from all individual populations were randomly united under the assumptions of the Hardy-Weinberg equilibrium.  $F_{is}$  estimates the amount of homozygosity within individuals relative to that which would occur if each individual population conformed to the assumptions of the Hardy-Weinberg equilibrium.  $F$  statistics were calculated following the equations of Weir and Cockerham (1984) using the program Genetic Data Analysis, written by Paul Lewis (University of New Mexico). Single-locus standard deviations were obtained by jackknifing over the five individual populations within each life-history stage, while multilocus 95% confidence intervals were generated by 2000 bootstrap iterations over the seven polymorphic loci.

To compare the genetic relationship of soil seeds and surface plants across a continuous spatial scale, we estimated the coefficient of coancestry separately for each life-history stage. Unlike spatial autocorrelation measures like Moran's  $I$ , genetic statistics such as the coefficient of coancestry have a firm foundation in population genetics theory and may be easily combined over alleles at each locus and over all loci for a more powerful test of overall spatial genetic structure (Heywood, 1991; Loiselle et al., 1995). Assuming the electrophoretic loci represent neutral, unlinked markers, this estimate equals the probability that an

allele drawn from two different individuals are identical by descent (Hartl and Clark, 1989). Values at each distance interval were calculated for all alleles at each locus and weighted across all loci using a method analogous to that described for F statistics in Weir and Cockerham (1984). See Loiselle et al. (1995) for a more detailed description of this analysis.

Finally, to visualize the overall genetic relationship between *Lesquerella* surface plants and soil seeds among the five populations, we constructed a phenogram based on unweighted pair group method with arithmetic mean clustering of the ten individual populations using Nei's (1978) unbiased genetic distance. The data were analyzed using BIOSYS-I (Swofford and Selander, 1981), and the UPGMA clustering was performed using NTSYS-pc (Rohlf, 1993).

## RESULTS

***Lesquerella* seed bank and surface plant demography**—Due to the variation in surface plant density, the transect lengths for the five *Lesquerella* populations examined in this study ranged from 20 to 45 m, and the mean interplant distance along the line varied from 0.4 to 1.2 m (Fig. 1). Although there was no consistent relationship between the mean interplant distance of the surface plants and the mean and coefficient of variation of the number of soil seeds per sample, there was a general trend for surface plant and soil seed densities to be inversely related. For example, the site with the lowest density of surface plants, and thus the greatest mean interplant distance (population F), also had the greatest mean and maximum number of soil seeds per sample.

**Genetic comparisons of *Lesquerella* seed bank and surface plant populations**—**Allele frequency comparisons**—When data from all five populations were pooled, all alleles at each locus were found in both the soil seed and surface plant populations (Table 1). However, within each of the five sites, there was at least one case in which an allele found at one locus in one life-history stage was absent from the other life-history stage. For example, allele 4 at the *Idh* locus was present within the soil seed samples but absent in the surface plant samples of populations F and M. Overall, the surface plant population was the stage with this missing allele in twice as many cases as the soil seed population (14 vs. 7 cases for surface plants and soil seeds, respectively; see Table 1).

There were significant differences in the overall allele frequencies (pooled over all loci) between the soil seed and surface plant life-history stages (Table 2). There were also significant overall differences between these life-history stages in three of the five individual populations, and within some individual loci at both the total and individual population levels. For the two loci that differed significantly at the total population level (*Pgm-1* and *Pgi*), the relative frequency of some alleles in the soil seed and surface plant populations was consistent across all five individual populations. For example, the frequencies of *Pgi* allele 5 and *Pgm-1* allele 1 in the surface plant population were always greater than or equal to the frequency of these alleles in the corresponding soil seed population (Table 1).

**Observed heterozygosity**—In general, the *Lesquerella* surface plants were more heterozygous than the soil seeds beneath them. When data were pooled across all loci,

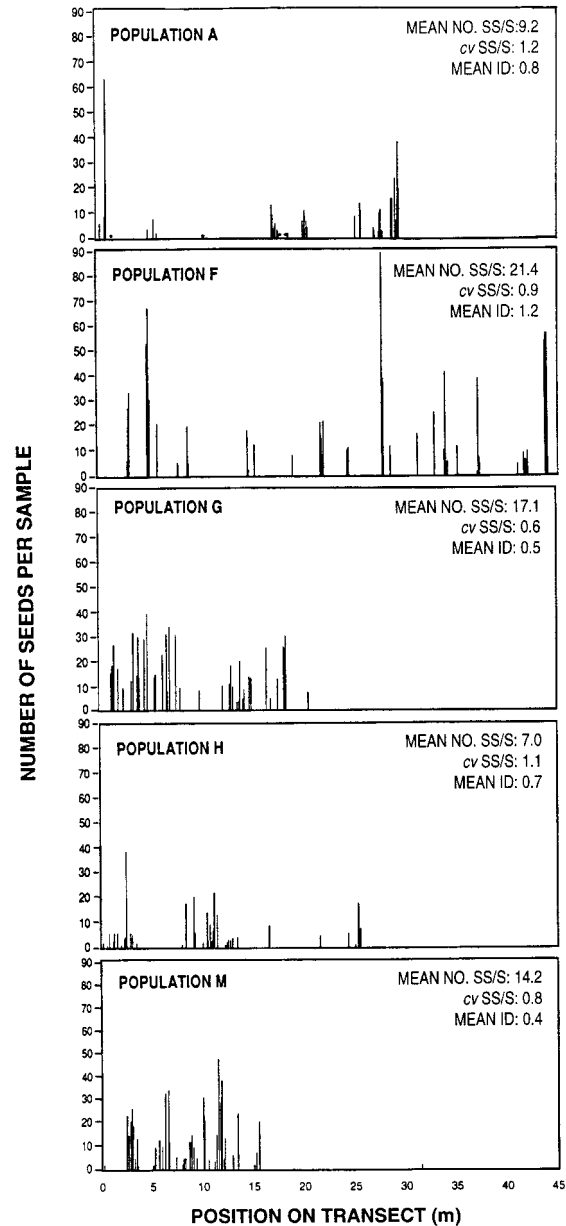


Fig. 1. *Lesquerella* soil seed densities in the five populations. The x axis shows the spatial position of each soil sample along the line transect established at each site. Each soil sample was collected adjacent to a *Lesquerella* surface plant that intersected the transect. Thus the greater the surface plant density, the shorter the distance needed to sample 40 plants. The number of *Lesquerella* soil seeds within each sample was calculated by counting *Lesquerella* seedlings that germinated from known volumes of these samples in the greenhouse. Asterisks indicate soil samples that contained no viable *Lesquerella* soil seeds. Also shown are the mean and coefficient of variation for *Lesquerella* soil seeds per soil sample (SS/S) and the mean interplant distance (ID) of the *Lesquerella* surface plants along each transect.

there were highly significant differences in heterozygosity between soil seeds and surface plants at the total population level, and within two of the five individual populations. There were also significant differences in heterozygosity within some individual loci at both the total and individual population levels (bottom line of Table 2). Mean heterozygosity (averaged over all loci) was also

TABLE 2. G tests comparing allele frequencies and observed heterozygosity of *Lesquerella* surface plants and soil seeds. The total population represents comparisons of all surface plants and soil seeds (pooled across the five sites). The individual locus comparisons show the details of where the overall differences originated. We performed a separate sequential Bonferroni adjustment over these seven individual (but not the total) comparisons for the allele frequency and heterozygosity G tests within each of the five populations. Randomization procedures were used to generate significance values for G tests containing any expected cell values less than five. For the allele frequency tests, rare alleles were pooled so that no expected cell values were less than one; the degrees of freedom are one less than the number of alleles at each locus. Sample sizes for all tests are as indicated in Table 1. Allele Freq. = allele frequency comparisons, Het. = heterozygosity comparisons.

| Locus        | Total population |                 | Individual populations |      |                 |                  |               |              |              |              |               |                 |
|--------------|------------------|-----------------|------------------------|------|-----------------|------------------|---------------|--------------|--------------|--------------|---------------|-----------------|
|              | Allele freq.     | Het.            | A                      |      | F               |                  | G             |              | H            |              | M             |                 |
|              |                  |                 | Allele freq.           | Het. | Allele freq.    | Het.             | Allele freq.  | Het.         | Allele freq. | Het.         | Allele freq.  | Het.            |
| <i>Idh</i>   | 2.33             | 3.33            | 0.18                   | 0.19 | 0.24            | 0.24             | 0.81          | 0.10         | 3.55         | <b>3.86*</b> | <b>7.09*</b>  | <b>7.27**†</b>  |
| <i>Pgd-1</i> | 3.51             | <b>5.02*</b>    | 3.74                   | 1.69 | 0.03            | 0.19             | 3.33          | <b>4.10*</b> | 0.05         | 0.70         | 2.55          | <b>9.09**†</b>  |
| <i>Pgd-2</i> | 2.52             | 2.73            | 0.01                   | 0.03 | 5.40            | 1.02             | 0.51          | 1.57         | 0.03         | 0.54         | 0.15          | 0.47            |
| <i>Pgm-1</i> | <b>5.46*</b>     | <b>4.56*</b>    | 0.07                   | 0.02 | 1.14            | 2.99             | 1.67          | 0.41         | 0.32         | 0.01         | <b>3.91*</b>  | 4.79            |
| <i>Pgm-2</i> | 0.01             | 0.42            | 0.79                   | 0.18 | 0.16            | 0.01             | <b>4.51*</b>  | <b>5.13*</b> | 0.46         | 0.02         | 0.01          | 0.33            |
| <i>Pgi</i>   | <b>12.51*</b>    | 5.47            | 3.76                   | 3.45 | <b>13.36**†</b> | 0.79             | 4.24          | 1.60         | 3.49         | 0.73         | 3.62          | <b>5.13*</b>    |
| <i>Lap</i>   | 1.78             | <b>10.78**</b>  | 0.23                   | 0.52 | 1.14            | <b>15.19***†</b> | 9.39          | 0.83         | 8.66         | 0.01         | 6.33          | <b>4.34*</b>    |
| Total        | <b>27.76*</b>    | <b>32.31***</b> | 8.78                   | 6.08 | <b>21.47*</b>   | <b>20.43**</b>   | <b>24.46*</b> | 13.74        | 16.56        | 5.87         | <b>23.66*</b> | <b>31.42***</b> |

Note: Significant G test values indicated by boldface Unadjusted probability: \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ . Sequential Bonferroni for tests of individual loci: †  $P < 0.05$  (adjusted).

greater in surface plants at the total population level, and in four of the five individual populations (Fig. 2). At the *Idh* locus, heterozygosity was greater in soil seeds than surface plants in each case where this difference was significant or marginally significant, while at all other loci, the converse was true (Tables 1, 2). Finally, surface plants exhibited significantly greater levels of multilocus heterozygosity ( $G = 16.47$ ,  $df = 6$ ,  $P = 0.011$ ), with proportionally fewer individuals in each low heterozygosity class (<4/7 loci heterozygous), and proportionally more individuals in each high heterozygosity class (>3/7 loci heterozygous; see Fig. 3).

*F statistics*—Single-locus estimates of Wright’s F statistics within each life-history stage varied from one ( $F_{st}$ ) to two ( $F_{it}$  and  $F_{is}$ ) orders of magnitude among the seven electrophoretic loci (Table 3). To obtain an overall measure of the distribution of genetic variation of soil seeds and surface plants, we averaged F statistics for individual loci as recommended by Nei (1987) following the equations of Weir and Cockerham (1984). These multilocus estimates of  $F_{st}$  showed that both the soil seed and surface plant populations contained similarly small but statistically significant genetic divergence (Fig. 4). Both  $F_{it}$  and  $F_{is}$  were significantly greater than zero in the soil seed but

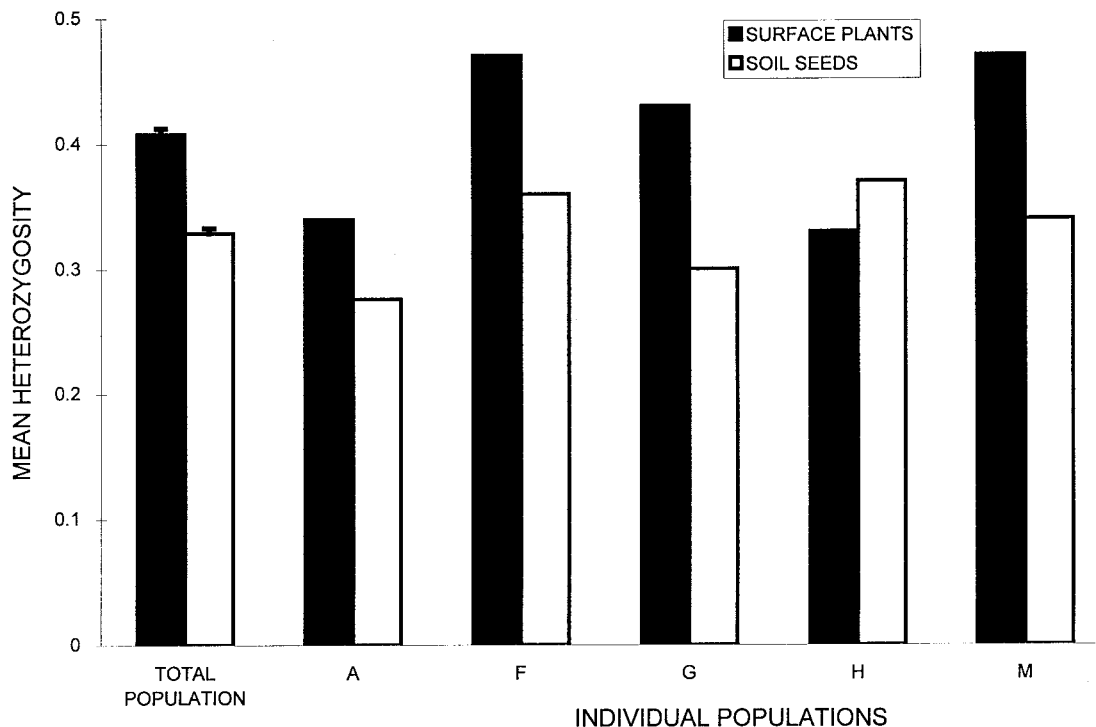


Fig. 2. Histogram comparing the mean heterozygosity of *Lesquerella* soil seeds and surface plants. The standard errors for the total population values were calculated from the five individual mean heterozygosity values within each life-history stage.

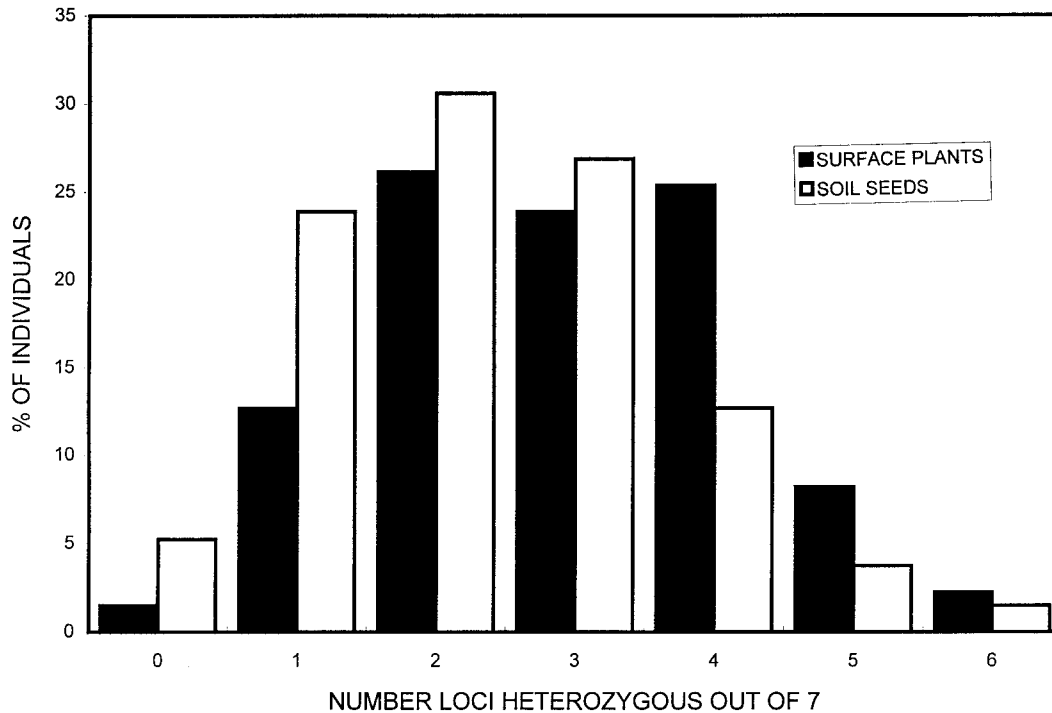


Fig. 3. Histogram of the number of loci heterozygous out of seven for individual soil seeds and surface plants. Only individuals with data at all seven electrophoretic loci were used in this analysis ( $N = 134$  for the surface plants and 140 for soil seeds). While the actual G test was performed on counts of individuals within each category, percentages are shown for illustration.

not the surface plant populations, although the large variance in these estimates resulted in overlapping confidence intervals between the two life-history stages.

*Spatial genetic structure*—Correlograms of the coefficient of coancestry revealed that the genetic relationships within each life-history stage were significantly greater than zero among individuals occurring within 2 m of each other and statistically indistinguishable from zero at all distance intervals  $>2$  m apart (Fig. 5). At the two intervals between 0.5 and 2 m, the correlation of surface plant genotypes was roughly twice that of the soil seed genotypes, indicating that surface plants were slightly more genetically similar at these relatively fine spatial scales. Finally, a UPGMA clustering analysis indicated that for three of the five population pairs, the surface plant populations showed the greatest similarity to their corresponding soil seed populations (Fig. 6). However, this was not the case for the two geographically closest sites (upper diagonal in Fig. 6), in which the surface

plants were more genetically similar to each other than to their own respective seed banks.

## DISCUSSION

We found significant genetic differences between the five ecologically diverse *Lesquerella* soil seed and surface plant populations examined in this study. This is the first study to document the existence of such differences across multiple populations and may suggest that the genetic structure of plant populations changes as cohorts pass from dormant seeds in the soil to established above-ground plants. However, the data collected in this study do not allow us to directly distinguish among several plausible and nonmutually exclusive hypotheses that could explain this genetic divergence. We thus hope that our necessarily speculative discussion will stimulate other researchers to perform similar empirical studies in other systems so that the generality of and causal mechanisms

TABLE 3. Single-locus F statistics calculated for *Lesquerella* surface plants and soil seeds. Also shown is  $\pm 1$  SD for each locus, calculated by jackknifing over the five populations within each life-history stage.

| Locus        | Surface plants     |                    |                    | Soil seeds         |                    |                    |
|--------------|--------------------|--------------------|--------------------|--------------------|--------------------|--------------------|
|              | $F_{st}$           | $F_{it}$           | $F_{is}$           | $F_{st}$           | $F_{it}$           | $F_{is}$           |
| <i>Idh</i>   | 0.012 $\pm$ 0.013  | 0.113 $\pm$ 0.123  | 0.103 $\pm$ 0.115  | 0.012 $\pm$ 0.016  | -0.038 $\pm$ 0.008 | -0.051 $\pm$ 0.024 |
| <i>Pgd-1</i> | 0.027 $\pm$ 0.032  | 0.099 $\pm$ 0.084  | 0.074 $\pm$ 0.080  | 0.054 $\pm$ 0.058  | 0.306 $\pm$ 0.096  | 0.268 $\pm$ 0.105  |
| <i>Pgd-2</i> | -0.002 $\pm$ 0.004 | -0.033 $\pm$ 0.073 | -0.035 $\pm$ 0.073 | -0.009 $\pm$ 0.002 | 0.136 $\pm$ 0.080  | 0.144 $\pm$ 0.079  |
| <i>Pgm-1</i> | 0.009 $\pm$ 0.020  | 0.036 $\pm$ 0.159  | 0.027 $\pm$ 0.147  | 0.004 $\pm$ 0.010  | 0.092 $\pm$ 0.101  | 0.088 $\pm$ 0.093  |
| <i>Pgm-2</i> | 0.047 $\pm$ 0.039  | 0.012 $\pm$ 0.086  | -0.037 $\pm$ 0.064 | 0.041 $\pm$ 0.019  | 0.088 $\pm$ 0.114  | 0.048 $\pm$ 0.110  |
| <i>Pgi</i>   | 0.039 $\pm$ 0.011  | -0.112 $\pm$ 0.069 | -0.157 $\pm$ 0.067 | 0.034 $\pm$ 0.017  | -0.004 $\pm$ 0.098 | -0.039 $\pm$ 0.092 |
| <i>Lap</i>   | 0.020 $\pm$ 0.019  | 0.192 $\pm$ 0.075  | 0.175 $\pm$ 0.072  | 0.027 $\pm$ 0.024  | 0.438 $\pm$ 0.051  | 0.422 $\pm$ 0.063  |

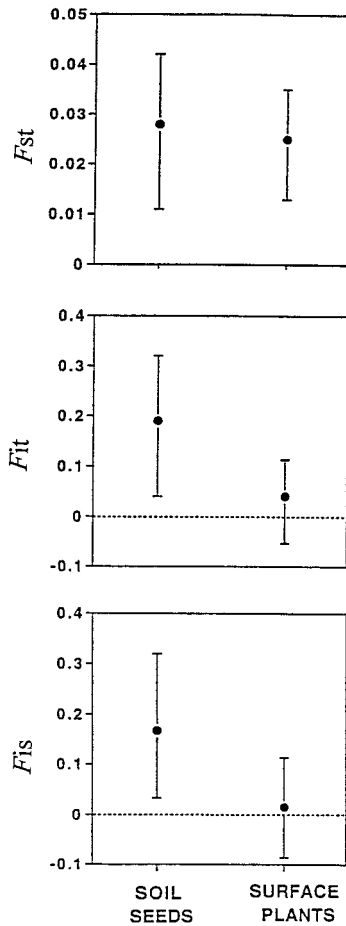


Fig. 4. Wright's F statistics estimated from the five populations of *Lesquerella* soil seeds and surface plants. Circles indicate the mean, and the vertical bars show the 95% confidence intervals calculated from 2000 bootstrap iterations over the seven electrophoretic loci.

behind these intriguing results may be better understood and incorporated into current evolutionary theory.

**Allele frequencies**—Despite the considerable ecological diversity of the five *Lesquerella* populations across the Sevilleta, we found significant genetic differences in the overall allele frequencies between the soil seed and surface plant life-history stages. These frequencies were also significantly different in some, but not all, of the individual populations, and at two of the five individual loci (*Pgm-1* and *Pgi*). In these instances, it is not clear whether these individual population and locus allele frequency differences were caused by natural selection, genetic drift, or a combination of both. Interestingly, however, the relative frequency of some alleles at both *Pgm-1* and *Pgi* tended to be higher in one life-history stage across all five populations. Since by definition drift should produce random allele frequency divergence of soil seeds and surface plants, these results suggest the action of selection, although it is not clear where in the *Lesquerella* life cycle (e.g., soil seed persistence, germination, establishment, and/or surface-plant survival) selection may have occurred.

In this study, soil seed and surface plant allele fre-

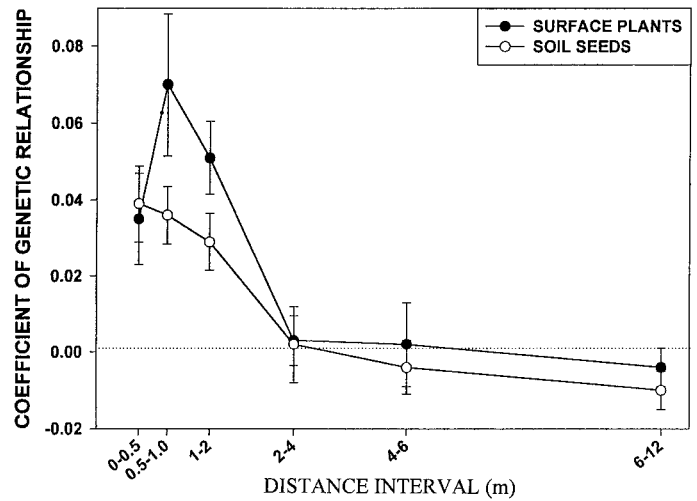
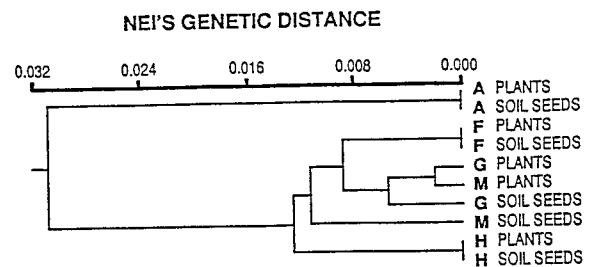


Fig. 5. Correlograms of the coefficient of coancestry for *Lesquerella* soil seeds and surface plants. At each distance interval from 168 to 1048 pairs were analyzed for soil seeds and 174 to 1122 pairs were analyzed for surface plants. Values shown are means  $\pm$  1 SE, calculated from the individual coefficient of relationship values within each life-history stage from the five separate populations.



| P | SURFACE PLANTS |       |       |       |       | SOIL SEEDS |       |       |       |       |
|---|----------------|-------|-------|-------|-------|------------|-------|-------|-------|-------|
|   | A              | F     | G     | H     | M     | A          | F     | G     | H     | M     |
| L | A              |       |       |       |       |            | 9.3   | 5.4   | 8.1   | 5.8   |
| A | F              | 0.018 |       |       |       |            |       | 6.1   | 10.2  | 4.5   |
| N | G              | 0.038 | 0.011 |       |       |            |       |       | 4.3   | 1.6   |
| T | H              | 0.029 | 0.015 | 0.013 |       |            |       |       |       | 6.0   |
| S | M              | 0.026 | 0.002 | 0.002 | 0.012 |            |       |       |       |       |
| S | A              | 0.000 | 0.024 | 0.065 | 0.041 | 0.042      |       |       |       |       |
| E | F              | 0.015 | 0.000 | 0.016 | 0.018 | 0.004      | 0.020 |       |       |       |
| E | G              | 0.004 | 0.010 | 0.008 | 0.006 | 0.003      | 0.022 | 0.010 |       |       |
| D | H              | 0.033 | 0.017 | 0.007 | 0.000 | 0.012      | 0.051 | 0.014 | 0.007 |       |
| S | M              | 0.031 | 0.011 | 0.022 | 0.013 | 0.006      | 0.035 | 0.007 | 0.011 | 0.017 |

Fig. 6. UPGMA phenogram based on Nei's unbiased genetic distances among the ten individual populations of *Lesquerella* soil seeds and surface plants. Pairwise genetic distances among the ten populations are given below the diagonal, and the geographic distances between the five sites are shown in kilometres in the upper right of the table.

quencies differed most strongly at the *Pgi* locus. Many other population genetic studies have also demonstrated nonrandom associations between electrophoretic variation at this locus and various ecological and environmental variables (reviewed in Riddoch, 1993). Previous studies that have compared soil seed and surface plant genetics (Tonsor, Kalisz, and Fisher, 1993; Cabin, 1996) have found the greatest differentiation at this locus. In addition, Zangerl and Bazzaz (1984a, b) demonstrated differential germination of *Pgi* genotypes of *Amaranthus retroflexus* in response to oxygen availability and soil moisture. These studies therefore suggest that the *Pgi* locus may be under selection or closely linked to other loci under selection. However, since electrophoretic marker loci are generally chosen more for their genetic resolution than for their ecological significance (Ennos, 1989), and allele frequencies at marker loci may be differentially affected by random sources of variation (Slatkin and Arter, 1991), the significance of these kinds of single-locus differences remains controversial and in need of experimental verification.

**Observed heterozygosity**—Single-locus, multilocus, and mean heterozygosity were generally higher among surface plants than among soil seeds. These results are consistent with those of Tonsor, Kalisz, and Fisher (1993) for *Plantago lanceolata*, but contradict the earlier *Lesquerella* work of Cabin (1996). One possible explanation for these apparent differences is that each of these studies examined different stages of the aboveground population lifecycle. Tonsor, Kalisz, and Fisher (1993) compared soil seeds to both seedling and to adult aboveground populations and found that although heterozygosity increased in each successive stage, the only significant differences were between the seed bank and adult populations. The authors argued that selection favoring the survival of relatively heterozygous seedlings (via inbreeding depression or overdominance) was the most likely mechanism generating the increase in heterozygosity from *Plantago* seedlings to adults. Although Cabin (1996) found that heterozygosity was significantly greater in the *Lesquerella* seed bank, he only compared soil seeds to the *Lesquerella* seedling population. In contrast, the present study compares the seed bank to a random sample of the aboveground population (including both seedlings and established adults). A variety of selective mechanisms favoring survival of relatively heterozygous *Lesquerella* seedlings (e.g., frequency-dependent selection favoring rare heterozygous genotypes, selection against inbred progeny, and/or overdominance at the marker loci) could explain the different results of these two *Lesquerella* studies. Indeed, several studies have shown that heterozygosity increases over successive life-history stages in many plant populations (reviewed in Ennos, 1989; Mitton, 1989). However, there is also considerable evidence that heterozygosity increases with seed age, perhaps by similar mechanisms (see discussion and review in Hamrick, 1989; Cabin, 1996); therefore, the relative heterozygosity of seed bank and aboveground populations may be a function of the relative age of these two life-history stages.

**F statistics**—Multilocus  $F_{st}$  estimates of genetic differentiation among the five *Lesquerella* populations were virtually identical for the seed bank and aboveground populations. Although  $F_{st}$  values for each stage were significantly different from zero, the amount of population differentiation was an order of magnitude less than the mean reported values for other outcrossing animal-pollinated plants (see table 4 in Hamrick and Godt, 1989). This result may be partially attributable to the persistent and demographically important *Lesquerella* seed bank at the Sevilleta (Evans and Cabin, 1995), as theoretical models (Levin, 1978; Templeton and Levin, 1979) and some empirical studies (Epling, Lewis, and Ball, 1960; del Castillo, 1994) indicate seed banks may reduce population divergence by increasing effective population sizes and decreasing the rate of allele frequency change and loss of alleles due to genetic drift. Indeed, pairwise comparisons of the five *Lesquerella* seed bank and surface plant populations found that the seed bank frequently contained alleles missing in the corresponding aboveground population, while the converse was less common. However, while seed banks may also be a source of novel genetic variation in other species (Levin, 1990), we found no seed bank alleles that were not also present in at least one aboveground population. Overall, these results suggest that while the *Lesquerella* seed bank may serve as a genetic reservoir on a local, population-level scale, it does not appear to store genetic variation on a larger, metapopulation scale.

The relatively higher observed heterozygosity of *Lesquerella* surface plants was also consistent with the results of the F statistic analyses. Both  $F_{it}$  and  $F_{is}$  multilocus estimates showed that homozygosity relative to Hardy-Weinberg expectations declined from the seed bank to the surface plant stage at both the total and individual population levels, although the large confidence intervals around these estimates resulted in no statistically significant differences. These large variances were most likely the result of having only seven loci available for bootstrapping (P. Lewis, University of New Mexico, personal communication). Nevertheless, it is interesting that both  $F_{it}$  and  $F_{is}$  values in the seed bank were significantly greater than zero, while these F statistics for the surface plants were statistically indistinguishable from Hardy-Weinberg expectations. This result may indicate that population subdivision differentially affects below- and aboveground plant populations. It is also noteworthy that while the  $F_{it}$  and  $F_{is}$  estimates for the *Lesquerella* seed bank in this study are very close to those found by Tonsor, Kalisz, and Fisher (1993) for the *Plantago* seed bank, the *Lesquerella* surface plant values for these statistics lie between those found for the *Plantago* seedling and adult populations.

Higher than expected soil seed homozygosity is also consistent with the Wahlund effect; that is, when pooled allele frequencies obtained from distinct populations are used to calculate an expected homozygote frequency based on Hardy-Weinberg expectations, the observed frequency will always be higher than expected (Hartl and Clark, 1989). Although this effect is usually considered for spatially subdivided populations, populations that become genetically subdivided over time could also generate a “temporal” Wahlund effect (see related discussion

in Tonsor, Kalisz, and Fisher, 1993). Thus if the *Lesquerella* seed bank sampled in this study comprised several seed cohorts with different original allele frequencies, our soil samples would have effectively combined seeds from several distinct genetic populations. While we know that many *Lesquerella* soil seeds in this system have remained viable for at least 3 yr (Evans and Cabin, 1995), we do not know whether and to what extent the allele frequencies of new seeds varies from year to year, and thus this hypothesis cannot be tested without long-term data.

**Spatial genetic structure**—Correlograms of the coefficient of coancestry revealed different patterns of genetic relationship in the below- and aboveground *Lesquerella* populations. Although both stages exhibited significantly positive genetic correlations among individuals occurring 0.5–2.0 m apart, these correlations were roughly twice as great among the surface plants within these distance intervals. These surface plant correlations were also greater than the correlations among surface plants at the smallest distance interval (0.0–0.5 m apart), whereas the soil seed genetic correlations steadily declined with geographic distance. Unfortunately, our data do not allow us to determine whether these different spatial patterns reflect a biological (e.g., spatially varying selection) or methodological (e.g., differential sampling of above- and belowground genotypes; see discussion below) phenomenon.

Cabin (1996) also found that the genetic correlations among fine-scale patches of *Lesquerella* seedlings were greater than the correlations among the underlying soil seeds. In that study he suggested two hypotheses to explain this result: (1) temporal variation in localized biparental inbreeding could generate spatial genetic differences between relatively new and old soil seeds. Seeds that germinate at any particular time may be differentially comprised of more recently matured seeds, while the underlying seed bank may contain a more genetically diverse group of seeds matured over many years, and/or (2) at small spatial scales, germination and establishment may be under stronger selection than is persistence in the soil seed bank. Although the fine-scale soil seed genetic correlations in the present study are similar to those found by Cabin (1996) for the *Lesquerella* seed bank, the surface plant correlations in the present study are greater than those found in Cabin's previous study of *Lesquerella* seedlings. If small patches of *Lesquerella* surface plants become more genetically similar as the plants mature (e.g., due to fine-scale spatially variable selection), this could explain the different results of these two studies. However, neither *Lesquerella* study, nor to our knowledge any other study, has directly tested either of the above causal hypotheses.

On a larger spatial scale, a UPGMA genetic clustering analysis suggested that *Lesquerella* surface plants were generally more similar to their own underlying soil seed banks than they were to nearby populations of aboveground plants. Intriguingly, however, this was not the case for the two geographically closest sites, in which the genetic distance between the two adjacent surface plant populations was slightly less than the genetic distance between each of these populations and their respective soil seed banks. These results could indicate that over

large spatial scales, surface plants are genetically most similar to the soil seeds directly below, while at finer spatial scales, evolutionary forces may differentially affect the below- and aboveground populations and cause them to more closely match members of their own life-history stage. However, many more empirical studies investigating the genetic relationship of seed banks and surface plant populations are needed to test these ideas.

**Methodological issues**—This study is the first to demonstrate that the genetic structure of soil seed banks may differ from the genetic structure of surface plants across multiple populations. However, we feel that it is important to point out that whereas all of the surface plants along each transect were sampled and genetically analyzed, only a small fraction of the viable *Lesquerella* soil seeds within the soil samples was actually electrophoresed (see Fig. 1). This fact raises an important sampling issue: although the distribution of surface plant populations is relatively easy to quantify and sample, the underlying soil seed distribution cannot be assessed a priori. This is particularly problematic because seed banks in many ecosystems are notoriously patchy in space and time and often may not match aboveground plant distributions (see reviews in Leck, Parker, and Simpson, 1989). Indeed, in the present study, we found weak and/or negative associations between *Lesquerella* soil seed and surface plant densities at each of the five sites.

Given the patchy and elusive nature of soil seed populations, what is the best way to sample seed banks? We chose to spatially match each soil sample to a sampled *Lesquerella* surface plant, and to collect enough soil (based on preliminary studies) so that at least one *Lesquerella* seed would likely be available from each soil sample. There are two main advantages of this approach. First, it minimizes the potentially confounding effect of spatial genetic structure in the soil seed and/or surface plant populations. That is, if genotypes within one or both life-history stages are nonrandomly distributed over space, sampling soil seeds and surface plants at different spatial locations could generate spurious genetic differences (see discussion in Evans and Cabin [1995] and Cabin [1996]). Second, our sampling procedure results in roughly equal genetic sample sizes for both life-history stages within a site. The main disadvantages are (1) the sampling regime results in a spatially nonrandom sample of the underlying seed bank distribution, such that dense patches are underrepresented and sparse patches are overrepresented (whereas the converse may be true in the surface plant population), and (2) collecting, processing, and germinating the ~1500 kg of soil generated by this sampling design is extremely labor and space intensive.

Unfortunately, in most study systems, it does not seem possible to design a sampling procedure that simultaneously addresses all of the above considerations. For example, in our study, randomly sampling the *Lesquerella* seed bank at each site would require collecting continuous soil samples across the entire length of each surface plant transect, then germinating each sample and randomly selecting *Lesquerella* seedlings emerging within these samples. However, this technique would most likely result in comparisons of soil seed and surface plant genotypes from different spatial locations and over dif-

ferent spatial scales, and thus introduce potentially serious biases into the genetic analyses. In addition, collecting, processing, and germinating the enormous volume of soil that would result from this sampling regime would not be feasible for most investigators.

One alternative approach is to experimentally establish seed banks with a known genetic structure and spatial distribution. One of us (RJC) has initiated such experiments to test some of the above hypothesized causal mechanisms behind the genetic differentiation of below- and aboveground plant populations. However, such artificial seed banks may not realistically simulate real seed banks, which represent the product of many complex ecological and evolutionary interactions accumulating over potentially many years and diverse biotic and abiotic environments. We therefore feel that research that combines experimental and descriptive techniques may yield the most meaningful insights into the demographic and genetic dynamics of plant populations.

**Implications**—There are now a wealth of studies demonstrating nonrandom associations of allozyme markers and various ecological and environmental variables (see reviews in Soltis and Soltis, 1989; Linhart and Grant, 1996). However, most of these studies focus on adults and only examine the spatial distribution of genetic variation within these mature populations. In contrast, relatively few ecological genetic studies have investigated how genetic variation may change within populations over successive life-history stages, and thus the evolutionary importance of temporal genetic change remains unclear. This study suggests that the genetic structure of plant populations may change during the critical transition from dormant soil seeds to established aboveground plants. This kind of temporal genetic change may be an important mechanism for generating and maintaining population genetic structure in plants (Hamrick, 1989) and other diverse organisms with similar dormant stages (e.g., encysting in dinoflagellates, Binder and Anderson [1990]; egg dormancy in copepods, DeStasio [1989]).

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