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A LIFE-HISTORY BASED STUDY OF POPULATION GENETIC STRUCTURE: SEED BANK TO ADULTS IN *PLANTAGO LANCEOLATA*

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Abstract.—We explored the extent to which the soil seed bank differed genetically and spatially in comparison to two actively growing stages in a natural population of *Plantago lanceolata*. All seed-bank seeds, seedlings, and adults of *P. lanceolata* within eight subunits in a larger population were mapped, subjected to starch gel electrophoresis, and allozyme analysis in 1988. Gel electrophoresis was also used to estimate the mating system in two years, 1986 and 1988. The spatial distributions of seeds, seedlings, and adults were highly coincident. Allele frequencies of the dormant seeds differed significantly from those of the adults for four of the five polymorphic loci. In addition, a comparison of the genotype frequencies of the three life-history stages indicated that the seed bank had an excess of homozygotes. Homozygosity, relative to Hardy-Weinberg expectations, decreased during the life cycle (for seed bank, seedlings, and adults respectively: $F_{it} = 0.19, 0.09, 0.01$; $F_{is} = 0.14, 0.04, -0.12$). Spatial genetic differentiation increased sixfold during the life cycle: (for seed bank, seedling and adults: $F_{st} = 0.02, 0.05, 0.12$). The apparent selfing rate was 0.01 in 1986 and 0.09 in 1988. These selfing rates are not large enough to account for the elevated homozygosity of the seed bank. Inbreeding depression, overdominance for fitness, and a “temporal Wahlund’s effect” are discussed as possible mechanisms that could generate high homozygosity in the seed bank, relative to later life-history stages. In *Plantago lanceolata*, the influence of the mating system and the “genetic memory” of the seed bank are obscured by the time plants reach the reproductive stage.

Key words.—Demographic genetics, mating system, Plantaginaceae, *Plantago lanceolata*, population genetic structure, seed bank, Wright’s F -statistics.

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Persistent soil seed banks are a common feature of plant populations found over a wide range of life-history types, habitats, and climates (for a review, see Leck, et al. 1989). However, little is known about the basic ecological genetics of soil seed banks or other long-term dormant stages. Many questions about the relationship of soil seed banks to the evolutionary and population genetic dynamics remain unanswered. In our view, the most important questions are the following. Can seed banks act to disperse genes through time, and as a consequence (1) maintain genes in a population through periods in which they are selected against, and (2) damp out the effects of short-term variation in selection (e.g., Clegg et al. 1978b)? Can seed banks prevent the homogenization caused by random genetic drift in small populations by pooling the reproductive output of many generations and averaging out the effects of each generations’s allele frequency

sampling error? Do seed banks slow evolution by creating a lag time between the season in which selection takes place and the season in which the response to selection is observed (Templeton and Levin 1979)? Do seed banks change the long-term selection acting on a population by diluting the effects of selection in years that are poor in seed production compared to the effects of selection acting in years that seed production is good (Templeton and Levin 1979)? Alternatively, does the soil environment impose a selective regime on the phenotype that is substantially different and opposed to the selective regime experienced by aboveground plants? The answer to each of these questions can have substantial implications for the expected process and pattern of evolution in plant populations. Unfortunately, the population genetics of soil seed banks remains virtually unquantified.

This study is a first effort at exploring the interaction of the seed bank and below ground ecological genetic processes with growing plants and aboveground ecological genetic processes in *Plantago lanceolata*. We expect that the genetic

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similarity of the seed bank and adult plants will be a complex function of the mating system, seed dispersal distance, temporal and spatial patterning of selection, variance in survival and fecundity, factors affecting seed longevity in the seed bank, and the proportion of seedlings germinating from the persistent seed bank versus those germinating from the previous season's reproductive output. Obviously, substantial effort is required to completely understand genetic exchange between the seed bank and the reproductive plants. In this paper, we set a more modest goal and ask two main questions.

First, do the spatial distributions of seeds in the seed bank, seedlings, and adults coincide? If there is long-term persistence of seeds in the seed bank relative to the adults, and the locations of sites suitable for germination and growth vary from year to year, we might expect that the spatial distribution of seeds would be less patchy than that of the adults. Under these conditions, a more or less continuous distribution of seeds would develop because seeds could persist in areas that were not consistently favorable for survival of other stages. We could conclude from a continuous distribution that the seed bank is the longest lived life-history stage in this population. Alternatively, if the seed bank and the above-ground plants are coincident in their spatial distributions, then the seed bank may store temporal but not spatial variation. We address this question by comparing the distributions of mapped seeds in the seed bank, seedlings, and adults in a natural population.

Second, we are interested in determining if the population genetic structure differs among the seed bank and other life-history stages. We asked, to what extent do the allele and genotype frequencies of the seed bank, seedlings and adults vary? We addressed these questions using allozymes as genetic markers to quantify allele and genotype frequencies for dormant seed, seedlings, and reproductive plants. We also examined the seed crop by maternal plant to determine the mating system. We apply *F*-statistics (Wright 1951) and a mating system estimator (Ritland and Jain 1981; Ritland 1990) to allozyme data gathered from a mapped population.

MATERIALS AND METHODS

The Study Plant

Plantago lanceolata (Plantaginaceae) is a weedy perennial herb common in human-disturbed en-

vironments. In the study population, the majority of the seedlings emerge in the spring with less than 1% of the individuals emerging in summer and fall (Tonsor unpubl. data). Flowering spans early June through mid-August with its peak in July (Tonsor 1987). *Plantago lanceolata* is wind pollinated and has a gametophytic self-incompatibility system (Ross 1973). Fruit maturation and subsequent seed dispersal occur throughout the summer and fall. Average pollen-dispersal distance is approximately 1.5 m (Tonsor 1985; Bos et al. 1986) while average seed-dispersal distance is 0.08 m (Bos et al. 1986). Cavers et al. (1980) provide further details of the biology of this species. Although it is known that *P. lanceolata* forms a persistent soil seed bank, the longevity of seeds in the seed bank or average time spent as a dormant seed are unknown.

Study Site and Field Methods

The research was conducted in Turkey Meadow of the Kellogg Biological Station, Kalamazoo County, Michigan. Turkey Meadow, a herbaceous perennial community comprised of native and introduced grasses and forbs, occupies an 8-ha area, which has not been cultivated since 1960. *Plantago lanceolata* was undoubtedly present when this field was under cultivation, because *P. lanceolata* is present in all the adjacent cultivated fields. For a more detailed description of the vegetation of Turkey Meadow, see Burbank et al. (1992). The study area is a 480-m² plot. Since the fall of 1985, all aboveground plants in a 6-by-7-m area have been marked with permanent numbered tags. Since the fall of 1986, and every fall and spring thereafter, all reproductive plants within the study site have been marked with permanent numbered tags. In 1987, 13 1-m radius circles (subunits) were marked in the study area. Within these subunits, all aboveground plants and newly emerging seedlings are marked at germination and followed throughout their lives. In the spring of 1988, eight of these subunits were chosen for the present study (fig. 1A). Each subunit was gridded into squares 0.33 m on a side (fig. 1B), and the grid was used to define the locations of the seeds, seedlings, and adults.

The physical population structure of seedlings, seed bank, and adults within the subunits and their genotypes were determined as follows:

Seedlings.—In June, the locations of all seedlings that emerged within the subunits were mapped. A total of 161 seedlings emerged within

the eight subunits. These seedlings were collected, transported to the greenhouse, and planted in 4-cm pots filled with potting mix. The seedlings were grown in the greenhouse until they developed two sets of true leaves. Tissue samples of the most recently fully developed leaf were taken from each seedling to be used to assay their isozyme genotypes using starch gel electrophoresis.

Seed Bank.—After germination had ceased, the seeds that remained in the soil and were still viable represented the portion of the soil seed bank that could carry over to the following season. To sample the seed bank, 7.5-cm diameter soil cores were taken from 20 squares in the grid within each subunit (fig. 1B) without regard to the density of the aboveground plants. Preliminary work had demonstrated that the seeds could be found only in the top 5 cm of soil, thus, cores were taken to a depth of 7 to 8 cm. The cores were sieved from the soil using a U.S. Standard sieve no. 18 and all seeds of *P. lanceolata* were hand-picked from among the sand grains. The 207 seeds we recovered were planted into individual pots filled with potting mix and placed in growth chambers under simulated field conditions. Approximately 50% of the seeds germinated yielding a total sample size of 102 germinated individuals from the seed bank. Once the plants reached the two leaf stage, we collected tissue samples from the most recently fully expanded leaf for electrophoretic analysis. Ungerminated seeds were tested for viability using tetrazolium blue vital stain. Of the ungerminated seeds, 31% or about 15% of all seeds collected—were ungerminated and still viable.

Adults.—The location of each adult plant within the subunits was mapped to a section in the gridded circle (fig. 1B). Tissue samples were collected from the 214 plants within the subunits using the most recently fully expanded leaf from each.

Mating System Estimate Seeds.—In the fall of 1986, seeds were collected from a 42-m² plot in the center of the population. In 1988 seeds were collected from throughout the population. Eighty maternal parents were chosen at random from those collections for each year. For 1986, 16 seeds were placed on moist filter paper in a growth chamber for germination. Germination rate was about 50% for 1986. For 1988, 10 seeds were first cold treated, then germinated in the same way, resulting in approximately 90% germination. Six to eight seeds were electrophoresed from each of the maternal plants.

Electrophoretic Methods

Leaf tissue was homogenized at 4°C in the crushing buffer of Mitton et al. (1979). The electrode buffer was 0.3M boric acid and 0.1M NaOH, pH 8.6. The gels contained 0.015M Tris and 0.003M citric acid, pH 7.8. Gels were run for 4½ hours at 35 mA and 4°C. Gels were sliced horizontally and stained for four enzymes. For seed bank seeds, seedlings and adults, five polymorphic loci were scored: *Pgi2* (5.3.1.9), *Pgm1* (EC-2.7.5.1), *Pgm2* (EC-2.7.5.1), *Lap2*, and *Tpi2* (EC-5.3.1.1). In the mating system study, the 1986 offspring were scored for *Pgm1*, *Pgm2*, *Pgi2*, and *Tpi2*. Only *Pgi2* and *Pgm2* were scored for 1988 offspring.

Statistical Analyses

Spatial Association.—We tested the extent to which the distributions of the three life-history stages coincided on a small scale using the grid in each subunit. Each of the sections was categorized for the presence or absence of seeds, seedlings, and adults. (1) For comparisons between seedlings and adults, we scored for the presence or absence of seedlings and adults in each section of the grid. One subunit had no seedlings, and it was excluded from the comparison for seeds versus seedlings and adults versus seedlings. (2) To compare the distributions of seeds and the other two stages, we scored the presence or absence of seedlings or adults in the 20 sections containing soil cores. This allowed us to test for independence in the number of seeds, seedlings, and adults on the scale of the grid cells. We tested for spatial association among the stages using χ^2 tests.

Genetic Variation.—Allele frequencies were calculated for each of the five polymorphic loci. We tested for single-locus differences in allele frequencies by performing *G*-tests, which tested for differences in the distributions of allele frequencies between the seed bank and adult stage. Like the χ^2 statistic, *G*-tests are sensitive to cells that contain less than 5% of the total sample. Rare alleles (<5% of the total) were combined to meet the assumptions of the test (Sokal and Rohlf 1981).

Wright's *F*-statistics were calculated from the electrophoretic data. F_{st} estimates the extent to which the subunits are genetically differentiated. F_{it} estimates the magnitude of the homozygosity in the population, relative to the expectation given random breeding in the population as a whole. F_{is} measures the magnitude of the homozygosity relative to the expectation assuming random

TABLE 1. The number of individuals by life-history stage within the spatial subunits of the study population.

Stage	Spatial subunit							
	1	2	3	4	5	6	7	8
Seed bank	19	14	9	83	14	17	40	11
Seedling	0	25	7	65	23	7	26	8
Adult	19	18	17	63	21	16	42	18

mating within subunits. Single-locus F -statistics and F -statistics jackknifed over the five polymorphic loci were calculated following the equations of Weir and Cockerham (1984).

Mating System.—Inbreeding coefficients were estimated as $f = 1 - h_o/h_e$, where h_o and h_e are the frequencies of heterozygotes observed and expected under random mating, respectively. Expected heterozygosity was adjusted to account for the number of individuals in the sample (n), using $h_e = (n/n - 1)(h_e - h_o/2n)$ (Nei and Chesser 1983). Genotypes of maternal plants were inferred from the plants' progeny and then used for estimating f of the maternal plants (Brown and Allard 1970). One seedling genotype was drawn from each progeny array to create the data set for estimating progeny f . For 1988, where data for four loci were available, the multilocus f estimate was jackknifed over loci. For 1986, the two-locus f we present is a weighted (by gene frequency) average of the two single-locus f estimates (Reynolds et al. 1983; Weir and Cockerham 1984). Mating structure was estimated was by assuming the mixed mating model, that is that

all matings were either random outcrosses or self-fertilizations (Clegg 1980). True self-fertilization is precluded in *P. lanceolata* and there is no feasible means to assign apparent philopatric matings into the myriad possible categories (i.e., full-sib, half-sib, first-cousin, etc.). We therefore estimate mating structure as selfing equivalents, or apparent rate of self-fertilization. Panmixia produces an apparent outcrossing rate $t = 1.0$. Inbreeding results in $t < 1.0$, and negative assortative mating will result in $t > 1.0$ (Ritland and Jain 1981; Ritland 1990).

RESULTS

Spatial Distribution of Stages

The spatial distributions of the three life-history stages in this population were highly coincident when tested in two-way χ^2 contingency tests. The seed bank was not uniformly distributed throughout the study population. Rather, seeds in the seed bank were significantly associated with both seedlings and adults ($P < 0.0001$). Seedlings were significantly associated with adults ($P < 0.0001$) as well. The numbers of individuals collected for each life-history stage and each spatial subunit are listed in table 1. There appear to be localized areas of seed dispersal, seedling emergence and establishment in this population on the scale of tens of centimeters. Because we did not find seeds in the absence of seedlings or adults, these data suggest that this population of *Plantago lanceolata* does not have a seed bank of great longevity, or that the microsites suitable for growth and reproduction have

TABLE 2. Allele frequencies by stage for the five electrophoretic loci. Note the differences in allele frequencies between seed bank and adult stages.

Locus	Stage	Allele 1	Allele 2	Allele 3	Allele 4	Allele 5
<i>Tpi2</i>	Seed	0.01	0.99	0.0		
	Seedling	0.04	0.95	0.01		
	Adult	0.08	0.91	0.01		
<i>Pgi2</i>	Seed	0.36	0.53	0.05	0.04	0.01
	Seedling	0.35	0.53	0.04	0.08	0.01
	Adult	0.42	0.49	0.08	0.01	0.0
<i>Pgm1</i>	Seed	0.35	0.38	0.20	0.07	
	Seedling	0.33	0.40	0.27	0.01	
	Adult	0.34	0.39	0.26	0.0	
<i>Pgm2</i>	Seed	0.08	0.78	0.15	0.0	
	Seedling	0.0	0.82	0.18	0.0	
	Adult	0.002	0.87	0.10	0.02	
<i>Lap2</i>	Seed	0.27	0.53	0.19	0.01	
	Seedling	0.28	0.49	0.22	0.0	
	Adult	0.41	0.41	0.18	0.002	

TABLE 3. Single-locus G -tests for differences between soil seed bank and adult allele frequencies. Rare alleles' frequencies ($< 5\%$ see table 2) have been combined to meet the assumptions of the G -test.

Locus	G -value	Probability (P)
<i>Tpi2</i>	17.30	<0.001
<i>Pgi2</i>	62.14	<0.001
<i>Pgm1</i>	0.54	<0.80
<i>Pgm2</i>	36.36	<0.001
<i>Lap2</i>	14.12	<0.005

remained constant over several seasons, which is roughly equal to the longevity of the seed bank.

Comparison of Genetic Variation by Stage

For some loci, the seed bank did contain alleles different from those found in the adult stage and in general there were differences in allele frequencies between the seed bank and adult stages (tables 2, 3). Allele frequencies for the five polymorphic loci are reported by stage in table 2. There is no apparent pattern to this variation. For example, allele 3 of *Tpi2* is not present in the seed bank, but it is found in both the seedling and adult stages. Allele 5 of *Pgi2* and allele 4 of *Pgm1* are not present in the adults, but are found in both the seed bank and seedling stages. This stage-specific presence or absence for the rare alleles can be entirely attributed to chance. In addition, the frequencies of specific alleles changed between the seed bank and adult stages. For example, allele 1 of *Lap2* increased from 27% in the seed bank to 41% in the adults, while allele 2 decreased from 54% to 41% from the seed bank to the adults, respectively (table 2). The G -test to compare allele frequencies between the seed bank and adults indicated that the allele frequencies were significantly different for all loci except *Pgm1* (table 3).

The total genetic variance, as measured by Weir and Cockerham's (1984) method, was roughly equivalent among the three cohorts (Weir and

Cockerham's 'a' = 0.46, 0.49, and 0.50 for seed bank, seedlings, and adults, respectively).

F -statistics

Confidence intervals jackknifed over subunits show substantial overlap among the five single locus estimates for F_{st} and general overlap for F_{it} and F_{is} (table 4). Therefore, we treated the five single-locus estimates as independent estimates from the same underlying distribution and used them to calculate multilocus estimates of the F -statistics jackknifed across loci (Weir and Cockerham 1984) (fig. 2A).

Allele frequency divergence from the seed bank to the adult stages is evidenced by the increasing value of F_{st} . F_{st} more than doubles from the seed bank to the adult cohort. At the same time, homozygosity decreases substantially from the seed bank to the adult stage as evidenced by the values of both F_{it} and F_{is} (fig. 2B,C). F_{it} declines from 0.19 in the seed bank to a value indistinguishable from zero in the adults. F_{is} declines from 0.15 in the seed bank to -0.12 in the adults. This value is significantly less than zero, indicating fewer homozygotes in the adults than would be expected.

Mating System

Mating structure and inbreeding coefficient estimates differed significantly between years (table 5). There is significant but slight inbreeding in 1986 with $t = 0.99$ (95% confidence interval based on Student's t : 0.992, 0.995). More inbreeding was evident in 1988, with $t = 0.91$ (95% c.i. as above: 0.850, 0.968). The inbreeding coefficient for maternal plants is indistinguishable from zero in 1986 (95% c.i. from Student's t for f : maternal plants: -0.022 , 0.002). The 1986 offspring inbreeding coefficient is negative (95% c.i. as above: -0.165 , -0.025). In 1988, neither inbreeding coefficient differs significantly from zero, although the confidence intervals are larger (95% c.i. as above, for mothers: -0.087 , 0.043; for offspring: -0.154 , 0.092).

TABLE 4. Single locus F -statistics calculated for the seed bank, seedlings, and adult stages of *Plantago lanceolata*.

	Seed bank			Seedlings			Adults		
	F_{st}	F_{it}	F_{is}	F_{st}	F_{it}	F_{is}	F_{st}	F_{it}	F_{is}
<i>Tpi2</i>	0.02	-0.01	-0.04	0.03	0.18	0.14	0.18	0.09	-0.11
<i>Pgi2</i>	0.04	0.19	0.16	0.03	0.10	0.08	0.09	0.13	0.05
<i>Pgm1</i>	0.04	0.28	0.25	0.07	0.01	-0.08	0.09	0.14	0.06
<i>Pgm2</i>	0.12	0.07	-0.05	0.03	0.01	-0.01	0.12	-0.11	-0.26
<i>Lap2</i>	0.04	0.16	0.13	0.04	0.19	0.15	0.13	-0.19	-0.36

DISCUSSION

Spatial Structure

In this population of *Plantago lanceolata*, the seed bank, seedlings, and adults exhibit coincident, patchy distributions in the field. This pattern is at least partially explained by very localized seed dispersal exhibited in this species (Bos et al. 1986; Tonsor unpubl. data). Seeds often remain within the fruit after the fruit is on the ground. Even when the seeds are individually dispersed they generally fall within 10 cm of the seed parent. This localized seed dispersal, coupled with the fact that the seeds exude mucilage upon wetting, means that the seeds are likely, on the average, to be deposited into the seed bank in the vicinity of their parents.

The spatial contagion of seeds and adults suggests that the microsites occupied by adults are extremely constant over long periods of time, or that seeds do not persist in the seed bank more than a few generations, or both. This population of *P. lanceolata* was mapped yearly from 1985 through 1992. There had been very little shift in the adult distribution during that time (Tonsor unpubl. data). In the Netherlands, van Groenendaal (1985) found 20% to 30% of seed survived their first year in the seed bank, and sites that differed substantially in their effects on other aspects of life history did not differ significantly in seed bank survivorship. If this is a reasonable estimate of seed bank survivorship in Michigan as well, then we can conclude, knowing that seed dispersal is 10 to 20 cm or less (Bos et al. 1986) and that the seed bank is confined to the immediate vicinity of adult plants, that the vast majority of local seed bank seeds were produced locally within the last five years.

Genetic Variation

Populationwide allele frequencies differ significantly between the seed bank and adult cohorts at four of the five loci surveyed (table 3). However, total genetic variance does not differ significantly from the seed bank to the adult cohort. Total genetic variance was slightly but insignificantly higher in the adult stage. This is counter to the expectation that the seed bank acts as a storehouse of greater amounts of genetic variation (Templeton and Levin 1979).

F_{ST} indicated genetic differentiation among the subunits in the adult cohort when compared to the seedling or seed bank cohorts (fig. 2A). The amount of population subdivision seen in the adults is typical for predominantly outcrossing

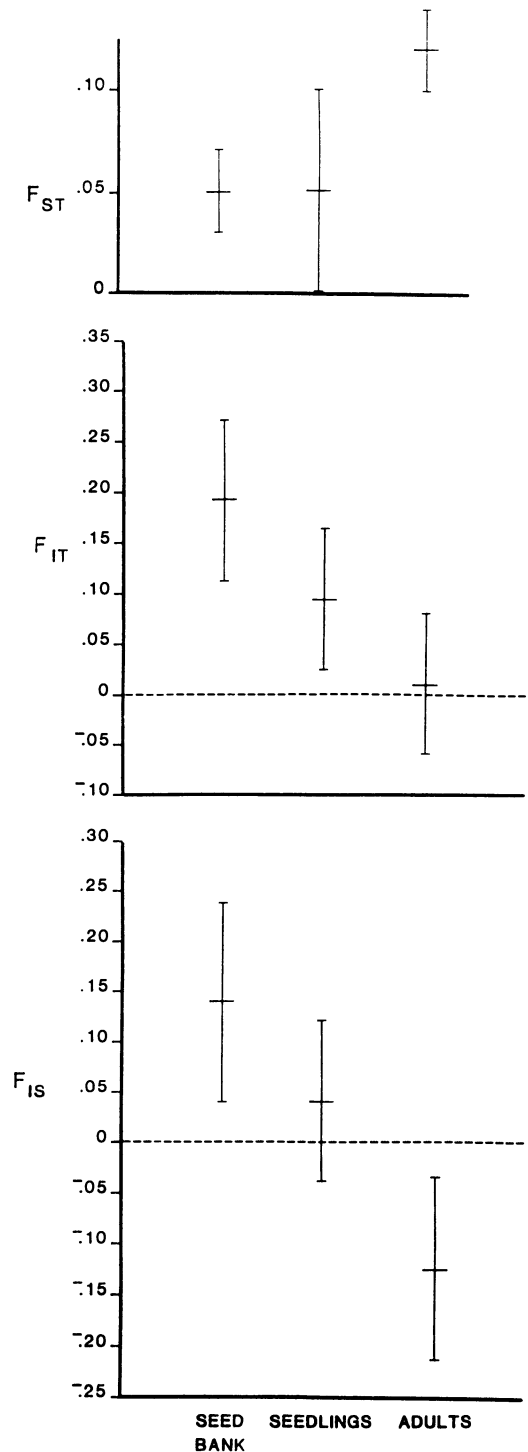


FIG. 2. Jackknifed F -statistics calculated using five polymorphic loci for seed bank, seedling, and adult stages of *Plantago lanceolata* in a study population in southwestern Michigan. Horizontal bars indicate the mean and the vertical bars indicate 95% confidence intervals. A, F_{ST} ; B, F_{IT} ; C, F_{IS} . See the text for details.

TABLE 5. Single-locus and multilocus mating system and fixation index estimates for two years. Bootstrapped standard errors are in parentheses for outcrossing rates, jackknifed standard errors for f , the fixation index.

Year	Locus	Outcrossing rate	Maternal f	Offspring f
1986	<i>Pti2</i>	1.115 (0.049)	-0.198	-0.220
	<i>Pgm2</i>	0.980 (0.041)	-0.039	0.074
	<i>Pgm1</i>	0.997 (0.046)	-0.059	-0.037
	<i>Tpi2</i>	0.968 (0.051)	-0.019	-0.076
	4-locus	0.993 (0.001)	-0.108 (0.056)	-0.095 (0.036)
1988	<i>Pgi2</i>	0.900 (0.042)	-0.035	0.032
	<i>Pgm2</i>	1.019 (0.064)	0.053	-0.146
	2-locus	0.914 (0.033)	-0.022 (0.031)	-0.031 (0.063)

plant species (e.g., $G_{st} = 0.118$; table 2 in Loveless and Hamrick 1984). Although the confidence interval on F_{st} is large for the seedling estimate, its estimate is virtually identical to the estimate of F_{st} for the seed bank. The seed bank therefore appears to function as a very local reservoir of genetic variation.

The increased subunit differentiation detected in the adult cohort appears to be the result of postemergence events. Local selection on the scale of the subunits could explain this pattern, although there may not be direct selection on the allozyme loci we assayed. Phenotypic selection among the limited number of families within each of the subunits could produce correlated selection on the allozyme loci, as a result of family-level gametic phase disequilibrium. Clegg et al. (1972) discuss a more extreme version of gametic phase disequilibrium-generated correlated selection in *Hordeum vulgare*, a highly selfing species.

Homozygosity, relative to random-mating expectations, is highest in the seed bank, declines in the seedlings, and declines further in the adults (fig. 2B,C). Two kinds of causal questions arise from examination of this pattern. (1) How do the (more) heterozygous adults give rise to (more) homozygous seeds in the seed bank? (2) How can the more homozygous seed bank give rise to more heterozygous adults? These are not simply two ways of asking the same question because the causes of change in genotype frequencies can be different for the seeds and the adults.

There are three potential explanations for the elevated homozygosity in the seed bank. First, if biparental inbreeding (Uyenoyama 1988) occurred in some years, it could elevate the proportion of homozygous offspring produced. This is unlikely because the inbreeding coefficients in the offspring were near zero or negative, based

on the mating system data (table 5). Unless some years have much higher inbreeding levels than those measured in this study, the level of inbreeding cannot by itself explain the elevated homozygosity level in the seed bank.

Second, the seed bank may exhibit a temporal (rather than spatial) Wahlund's effect. Wahlund (1928) described the effects on observed genotype frequencies when pooling multiple, spatially differentiated subunits, where random mating occurs within the subunits (see Crow and Kimura 1970, pp. 54–55). When data are pooled over such subunits and the pooled allele frequencies are used to calculate an expected frequency of homozygotes based on random mating, the observed frequency will always be higher than the expected frequency. This results from the parabolic relationship between the random-mating expectation for homozygote frequency versus allele frequency. The pooled allele frequency can be thought of as a weighted mean frequency of all seasons' inputs to the seed bank. It is a fundamental property of the mean that an individual year's deviation toward extreme frequencies must be counterbalanced by deviations toward equal frequencies of all alleles in other years. However, the associated homozygote frequencies cannot balance, because the homozygote frequencies are a function of the square of the allele frequencies. To detect a temporal Wahlund's effect would require that the allele frequencies of seeds produced each season vary. There is no significant between-season variation in the pollen-pool allele frequencies, based on estimates from the mating system study, and their bootstrapped confidence intervals (unpubl. data). Whether deviations in other years could be a sufficient cause for the excess seed bank homozygosity we measured depends on the number of seasons represented in the seed bank as well as

the among season variance in offspring allele frequencies. This hypothesis cannot be tested without long-term data.

A third possible explanation of the decrease in homozygosity through the life cycle involves inbreeding depression or heterozygote advantage (Charlesworth and Charlesworth 1987; Charlesworth et al. 1990). If the more homozygous individuals are more inbred, they may suffer from lower postemergence survivorship as a result of inbreeding depression. It is also possible that the more inbred seeds are less likely than outcrossed seeds to perceive germination cues, and they consequently have a longer residency time in the soil. Kalisz (1989) found that a higher percentage of selfed seeds remained in the soil without germinating than did seed produced by outcrossing in *Collinsia verna*. Inbreeding depression could be a mechanism that explains the change in the level of homozygosity between the seed bank and seedling stages for *P. lanceolata*.

Of the potential causal explanations for the increased homozygosity in the seed bank, only the third can also explain the decrease in homozygosity seen between the seedlings and adults. Inbreeding depression, exhibited as decreased survivorship of more inbred juveniles/adults, or overdominance, exhibited as higher survival of heterozygotes, could account for the decrease in homozygosity (F_{is} and F_{it}) with life-history stage. In the same experiment with *C. verna* described above, Kalisz (1989) also found that selfed seeds had lower mass. Seeds of *Plantago lanceolata*, which are more homozygous as determined by electrophoretic analysis, also have lower seed mass (Tonsor 1987). Lower seed mass has been associated with lower seedling survivorship in other species (Schaal 1984; Stanton 1984; Waller 1985; Winn 1988) and with lower seed bank survivorship in *Plantago lanceolata* (van Groenendaal 1985). These factors could also influence the change in the level of homozygosity between the seedling and adult stages seen in this study. Schaal and Levin (1976) also found an increase in heterozygosity with age/stage of aboveground plants in the perennial herb, *Liatris cylindracea*. However, the causal mechanism generating the shift in genotype frequencies could not be identified.

Mating System Estimates

Inbreeding, apparently through related-neighbor matings, occurs in some years in this population. Van Dijk et al. (1988) found that five of seven *P. lanceolata* populations in the Nether-

lands showed significant philopatry ($t = 0.84-0.90$). Therefore, it seems to be a general phenomenon that in spite of the self-incompatibility system, mating structure in *P. lanceolata* can involve detectable inbreeding, and that inbreeding varies among populations and seasons.

In this study, the difference in mating system between years is largely due to effects of the *Pgi* locus. Difference in germination rate among years may have contributed to the variation in estimates of mating system if germination rate of plants homozygous at *Pgi* were less likely to germinate than *Pgi* heterozygotes (for a potential causal explanation, see Zangerl and Bazaaz 1984).

Inbreeding coefficient estimates for maternal plants and their progeny also varied among years. In both years, however, maternal and progeny f were significantly less than $F_{eq} = (1 - t)/(1 + t)$, the expectation of f for a given mating structure t (Brown 1979). Selection favoring heterozygotes may be responsible for the inbreeding coefficients of maternal plants being lower than expected, given the mating structure estimates (Brown 1979).

The Role of the Seed Bank in Evolutionary Dynamics

Many studies of population genetic structure focus on the adults and infer causal mechanism based on differentiation of subpopulations of adults. This study indicates that for populations that have dormant stages, it is important to examine population structure at several times in the life history. If genetic structure varies between the seed bank and the adults, estimates gained from postestablishment sampling of such population-level traits as inbreeding, expected response to selection, or effective population size, may be incorrect. For example, the spatial genetic structure of adult *P. lanceolata* fits a model based on low seed dispersal and avoidance of inbreeding through outcrossing and relatively long-distance pollen movement (Crandall and Tonsor unpubl. MS). But the real cause is considerably more complicated and can be discovered only through studies that incorporate both genetics and demography (Clegg et al. 1978a,b; Ritland 1990). Understanding the changes in genetic structure from the seed bank to later life-history stages will be central to identifying causal mechanisms.

Soil seed banks are ubiquitous in the wild. Because many species that form persistent seed banks undergo population bottlenecks or expe-

rience habitat disturbance or other environmental stresses that can lead to a loss of genetic variation, seed banks may conserve or create genetic diversity (Levin 1990). Understanding seed-bank age structure in general, and genotype-specific differences in survival and emergence probabilities in particular, are of central importance for predicting evolutionary outcomes. The seed bank's unique potential for long-term storage of genetic variation makes knowledge of the genetic processes acting in and through soil seed banks essential for a general understanding of gene pool dynamics in plant populations, or other organisms that possess dormant stages as part of the life cycle (e.g., DeStasio 1989; Hairston and DeStasio 1988; Hairston and Dillon 1990). However, in this population, the effects of gene and genotype frequencies, as well as the effects of a slightly inbreeding mating system, appear to be substantially obscured by the time plants attain the adult stage. It is not clear that the seed bank plays a significant role in the microevolutionary dynamics of this population.

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LITERATURE CITED

- Bos, M., H. Harmens, and K. Vrieling. 1986. Gene flow in *Plantago* I. Gene flow and neighborhood size in *P. lanceolata*. *Heredity* 56:43-54.
- Brown, A.H.D. 1979. Enzyme polymorphism in plant populations. *Theoretical Population Biology* 15:1-42.
- Burbank, D. H., K. S. Pregitzer, and K. L. Gross. 1992. Vegetation of the W. K. Kellogg Biological Station. Michigan State University Agricultural Experiment Station Research Report #510.
- Cavers, P. B., I. J. Basset, and C. W. Compton. 1980. The biology of Canadian weeds. 47. *Plantago lanceolata* L. *Canadian Journal of Plant Science* 60: 1269-1282.
- Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* 18:237-268.
- Charlesworth, D., M. Morgan, and B. Charlesworth. 1990. Inbreeding depression, genetic load, and the evolution of outcrossing rates in a multi-locus system with no linkage. *Evolution* 44:1469-1489.
- Clegg, M. T. 1980. Measuring plant mating systems. *Bioscience* 30:814-818.
- Clegg, M. T., R. W. Allard, and A. L. Kahler. 1972. Is the gene the unit of selection? Evidence from two experimental plant populations. *Proceedings of the National Academy of Sciences, USA* 69:2474-2478.
- Clegg, M.T.A., A. L. Kahler, and R. W. Allard. 1978a. Estimation of life-cycle components of selection in an experimental plant population. *Genetics* 89:765-792.
- . 1978b. Genetic demography of plant populations. Pp. 173-188 in P. F. Brussard, ed. *Genetics and ecology: The interface*. Springer, Berlin.
- Crow, J. F., and M. Kimura. 1970. *An introduction to population genetic theory*. Harper and Row, New York.
- DeStasio, B. T. 1989. The seed bank of a freshwater crustacean: Copepodology for the plant ecologist. *Ecology* 70:1377-1389.
- Hairston, N. G., Jr., and B. T. DeStasio. 1988. Rate of evolution slowed by a dormant propagule pool. *Nature* 336:239-242.
- Hairston, N. G., Jr., and T. A. Dillon. 1990. Fluctuating selection and response in a population of freshwater copepods. *Evolution* 44:1796-1805.
- Loveless, M. D., and J. L. Hamrick. 1984. Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics* 15:65-95.
- Kalisz, S. 1989. Fitness consequences of mating system, seed weight, and emergence date in a winter annual, *Collinsia verna*. *Evolution* 43:1263-1272.
- Leck, M. A., V. T. Parker, and R. L. Simpson. 1989. *Ecology of soil seed banks*. Academic Press, San Diego.
- Levin, D. A. 1990. The seed bank as a source of genetic novelty in plants. *American Naturalist* 135: 563-572.
- Mitton, J. B., Y. B. Linhart, K. B. Sturgeon, and J. L. Hamrick. 1979. Allozyme polymorphism detected in mature needle tissue of ponderosa pine. *Journal of Heredity* 70:86-89.
- Nei, M., and R. K. Chesser. 1983. Estimation of fixation indices and gene diversities. *Annals of Human Genetics* 47:253-259.
- Reynolds, J., B. Weir, and C. C. Cockerham. 1983. Estimation of the coancestry coefficient: basis for a short-term genetic distance. *Genetics* 105:767-779.
- Ritland, K. 1990. A series of FORTRAN programs for estimating plant mating systems. *Journal of Heredity* 81:235-237.
- Ritland, K., and S. K. Jain. 1981. A model for estimation of outcrossing rate and gene frequencies using *n* independent loci. *Heredity* 47:135-152.
- . 1990. Gene identity and the genetic demography of plant populations. Pp. 181-199 in A.D.H. Brown, M. T. Clegg, A. L. Kahler and B. S. Weir, eds. *Plant population genetics, breeding, and genetic resources*. Sinauer, Sunderland, Mass.
- Ross, M. D. 1973. Inheritance of self-incompatibility in *Plantago lanceolata*. *Heredity* 30:169-176.
- Schaal, B. A. 1984. Life history variation, natural

- selection, and maternal effects in plant populations. Pp. 188–206 in R. Dirzo and J. Sarukhan, eds. *Perspectives on plant population ecology*. Sinauer, Sunderland, Mass.
- Schaal, B. A., and D. A. Levin. 1976. The demographic genetics of *Liatris cylindracea*. *American Naturalist* 109:511–528.
- Sokal, R. R., and F. J. Rohlf. 1981. *Biometry*, 2d ed. W. H. Freeman, San Francisco.
- Stanton, M. L. 1984. Seed variation in wild radish: Effects of seed size on components of seedling and adult fitness. *Ecology* 65:1105–1112.
- Templeton, A. R., and D. A. Levin. 1979. Evolutionary consequences of seedbanks. *American Naturalist* 114:232–249.
- Tonsor, S. J. 1985. Leptokurtic pollen flow, non-leptokurtic gene flow in a wind-pollinated herb, *Plantago lanceolata*. *Oecologia* 67:442–447.
- . 1987. The effect of flowering time on the number and quality of seeds in *Plantago lanceolata*. *Bulletin of the Ecological Society of America* 68:431.
- Uyenoyama, M. K. 1988. On the evolution of genetic incompatibility systems: incompatibility as a mechanism for the regulation of outcrossing distance. Pp. 212–232 in R. E. Michod and B. R. Levin, eds. *The evolution of sex*. Sinauer, Sunderland, Mass.
- van Dijk, H., K. Wolff, and A. De Vries. 1988. Genetic variability in *Plantago* species in relation to their ecology. 3. Genetic structure of populations of *P. major*, *P. lanceolata* and *P. coronopus*. *Theoretical and Applied Genetics* 75:518–528.
- van Groenendael, J. 1985. Selection for different life histories in *Plantago lanceolata*. Ph.D. dissertation. Catholic University of Nijmegen, The Netherlands.
- Wahlund, S. 1928. Zusammensetzung von Populationen und Korrelationserscheinungen vom Standpunkt der Vererbungslehre aus betrachtet. *Hereditas* 11:65–106.
- Waller, D. M. 1985. The genesis of size hierarchies in seedling populations of *Impatiens capensis* Meerb. *New Phytology* 100:243–260.
- Weir, B. S., and C. C. Cockerham. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* 38:1358–1370.
- Winn, A. A. 1988. The effects of seed size and microsite on seedling emergence in *Prunella vulgaris* in four habitats. *Journal of Ecology* 73:831–840.
- Wright, S. 1951. The genetical structure of populations. *Ann. Eugen.* 15:323–354.
- Zangerl, A. R., and F. A. Bazzaz. 1984. Niche partitioning between two phosphoglucose isomerase genotypes in *Amaranthus retroflexus*. *Ecology* 65:218–222.

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