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MULTIGENERATIONAL EFFECTS OF FLOWERING AND FRUITING PHENOLOGY IN *PLANTAGO LANCEOLATA*

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Abstract. Phenological patterns of flowering and fruiting can be influenced by the effects of reproductive time on seed production. We propose here that these patterns are also influenced by phenological effects on offspring quality. Furthermore, we hypothesize that there are cross-generational trade-offs between parental and offspring components of parental fitness influencing the evolution of reproductive phenology.

To test our hypothesis, we examined the multigenerational effects of flowering and fruiting phenology in *Plantago lanceolata*. Offspring of 30 families were transplanted into field plots to measure the effects of onsets of flowering and fruiting, duration of fruiting, percentage fungal infection, and damage by grasshoppers on total seed production, our measure of the within-generational component of parental fitness. To gather information about cross-generational contributions to parental fitness, we assessed the quality of offspring produced at different times in terms of seed mass and germination.

Families significantly differed in flowering and fruiting onsets. Larger plants began flowering earlier, and earlier flowering plants matured fruits earlier and produced fruits for a longer time. Significant family-mean correlations among these traits suggest that selection on any one trait will change all three traits. A negative family-mean correlation between fruiting onset and seed production suggests that we can expect an antagonistic trade-off in response to selection on these two traits. Early fruiting significantly reduced seed predation by grasshoppers and increased seed production. In contrast, late-maturing seeds were significantly heavier and germinated more rapidly than did early-maturing seeds produced by the same plants. The directions of the multigenerational effects support the hypothesis that there are cross-generational trade-offs between parental and offspring components of parental fitness. The experiments indicate that multigenerational fitness effects should be considered in future studies addressing the evolution of flowering and fruiting phenology.

Key words: *flowering; fruiting; multigenerational fitness; parental effects; path analysis; pathogen infection; phenology;* Plantago lanceolata; *seed predation.*

INTRODUCTION

The time of flowering and fruit ripening in plants can evolve in response to many selective pressures that influence seed production. Onset of flowering/fruiting must occur early enough during a growing season to allow for successful pollination, fertilization, and fruit maturation (e.g., see reviews by Rathcke and Lacey 1985, Primack 1987, Ollerton and Lack 1992). The length of this growing season can be determined by physical factors, such as temperature and water availability. Within this window, seed production can be influenced by the timing of pollinators, pathogens, seed predators, resource availability, and the synchronous flowering of individuals having the same ploidy level (e.g., Augspurger 1980, Bawa 1983, Schmitt 1983, Schemske 1984, Campbell and Motten 1985, Rathcke and Lacey 1985, Primack 1987, de Jong and Klinkhamer 1991, English-Loeb and Karban 1992, Diggle 1995, Bishop and Schemske 1998, Ollerton and Diaz 1999, O'Neil 1999, Husband and Schemske 2000, Picó and Retana 2000). For many species, these selection pressures may function in concert to modify reproductive phenology (e.g., Schemske et al. 1978, Bawa 1983, Evans et al. 1989, English-Loeb and Karban 1992, Brody 1997, Picó and Retana 2000, Pilson 2000).

In contrast to the many empirical studies focusing on seed production, few studies have examined how flowering and fruiting phenology may evolve in response to selection pressures acting on the progeny. This lack of attention to offspring fitness is also manifest in theoretical models of flowering time (e.g., Paltridge and Denholm 1974, Cohen 1976, Vincent and Pulliam 1980, Chiariello and Roughgarden 1984, de Jong et al. 1992). Individual seed mass can change over a reproductive season (e.g., Cavers and Steel 1984, Thompson and Pellmyr 1989, Galen and Stanton 1991, Winn 1991, Wolfe 1992, Diggle 1995, Ollerton and Diaz 1999, Simons and Johnston 2000), and flowering

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time can influence offspring germination, survival, and/or fitness (Lacey and Pace 1983, Case et al. 1996, Ollerton and Diaz 1999, Picó and Retana 2000, Wolf and Burns 2001, Galloway 2002). Thus, offspring produced at different times are not necessarily equally fit. These studies suggest that offspring quality, as well as quantity, could influence the evolution of flowering and fruiting phenology.

The influence of offspring quality on the evolution of reproductive timing is also suggested by recent studies of parental environmental effects. Greenhouse and growth-chamber studies show that the environment during fertilization and early embryonic development of an offspring while still attached to the maternal parent can influence offspring seed mass and germination (e.g., Riddell and Gries 1958, Koller 1962, Robertson et al. 1962, Sawhney and Naylor 1979, Gutterman 1980–1981, Siddique and Goodwin 1980, Wulff 1986, Case et al. 1996, Lacey 1996). It can also influence offspring fitness in the field (Lacey and Herr 2000). The studies cited here examined the specific effects of postzygotic temperature and/or photoperiod on offspring traits. Because temperature and photoperiod, on average, change predictably throughout a growing season, these studies suggest that by altering flowering/fruiting phenology, an individual plant has the potential to change its postzygotic temperature or photoperiod, and thereby also change the quality of its offspring. Differences in offspring quality could favor certain flowering/fruiting times over others.

The above studies suggest that if we wish to understand why plants flower and fruit when they do, we should assess the multigenerational fitness effects of flowering/fruiting phenology. Ideally, one should assess the effect of parental environment on parental fitness in the parental habitat, the within-generational fitness component for a parent, and offspring fitness in the offspring habitat, the cross-generational component of parental fitness (Donohue and Schmitt 1998). Two attempts to do this, in contexts unrelated to flowering/fruiting phenology, suggest that there may be trade-offs between these parental and offspring components (Donohue and Schmitt 1998, Donohue 1999).

To explore further the cross-generational interactions between fitness components in parental and offspring generations, which might arise because of parental environmental effects and which might influence the evolution of flowering and fruiting phenology, we examined the multigenerational consequences of flowering and fruiting phenology in *Plantago lanceolata*. Our study was motivated by Lacey and Herr's (2000) observation that postzygotic temperature can influence offspring fitness in field-grown *P. lanceolata*. Using two parental temperature regimes that resemble mean monthly temperatures for May and July, during which time *P. lanceolata* flowers in North Carolina, Lacey and Herr observed that high postzygotic temperature increased offspring fitness by almost 50%. Based on their results, Lacey and Herr hypothesized that from the perspective of offspring fitness, natural selection favors parents that flower later in the flowering season when it is warmer in the piedmont, North Carolina. If they are correct, then one must ask why *P. lanceolata* begins flowering so much earlier, when it is cooler. Some genotypes begin flowering in late April. Our hypothesis is that there are cross-generational trade-offs between parental and offspring components of parental fitness. Thus, although early-flowering parents may produce less fit offspring, they may produce sufficiently more offspring to offset the reduction in offspring fitness.

To test our hypothesis we conducted two types of experiments: one to measure the within-generational component of parental fitness, reproductive success, and one to measure the cross-generational component, i.e., offspring fitness, with *Plantago lanceolata*. In the first, we examined the phenological patterns of flowering and fruiting and their effects on seed production, our within-generational fitness measure. Also, we examined the phenological patterns of pathogen and seed predator attack. We hoped to answer the following questions: (1) What are the phenological patterns of flowering, fruiting, pathogen attack, and seed predation in a North Carolina population of *P. lanceolata*? (2) How do flowering and fruiting times affect within-generational fitness, as measured in terms of seed production? (3) Can fitness differences associated with flowering/fruiting phenology be explained by timing of pathogen attack and seed predation? We collected data on three phenological traits: flowering onset, fruiting onset, and fruiting duration. We assessed the impact of family and maternal plant size on these traits and determined their phenotypic and family-mean correlations. Then we used structural equation modeling (e.g., Mitchell 1992, Petraitis et al. 1996, Grace and Pugesek 1998, Scheiner et al. 2000) to examine the causal relationships among plant size, flowering/fruiting phenology, infection, predation, and seed production.

In the second set of experiments, in the laboratory we assessed the impact of fruiting phenology and family on two offspring traits: seed mass and germination. Lacey and Herr (2000) observed that high postzygotic temperature reduced seed mass but increased germination (Lacey 1996, Lacey and Herr 2000). Percentage germination strongly influenced overall offspring fitness. Therefore, we asked the following questions: (1) How does fruiting time affect these fitness components? (2) Are the effects in the directions suggested by the previous experiments? In other words, does delayed fruiting reduce seed mass and increase germination?

Methods

Biology of experimental species

Plantago lanceolata L. (Plantaginaceae), ribwort plantain, is a short-lived perennial herb that grows in

disturbed sites, abandoned crop fields, and lawns in temperate North America and in its native Eurasia. The species was introduced into North America ~100–200 years ago (Cavers et al. 1980). Individuals typically live from 2 to 5 years (e.g., Cavers et al. 1980, Antonovics and Primack 1982, Lacey and Herr 2000, Roach 2003). After germinating in the fall or spring, an individual grows vegetatively as a rosette. With the onset of flowering, a plant produces reproductive spikes at the ends of long stalks arising from leaf axils. The flowering season for *P. lanceolata* in the North Carolina (NC) piedmont extends from late April into August. Growth chamber and field experiments show that flowering phenology is both genetically and environmentally determined (e.g., Antonovics and Primack 1982, Wolff 1987, Wolff and van Delden 1987, van der Toorn and van Tienderen 1992). Mowing regime is known to influence the evolution of flowering time (van der Toorn and van Tienderen 1992).

Various pathogens and phytophagous insects can attack reproductive spikes during the flowering/fruiting season (e.g., de Nooij and Mook 1992). We focused on two pathogens that appeared in our study population. The disease symptoms matched those produced by two fungal pathogens that are known to infect *P. lanceolata*: *Diaporthe adunca* (Rob.) Niessl, syn. *Phomopsis subordinaria* (Desm.) Trav. and *Fusarium moniliforme* var. *subglutinans* (Booth 1971). *Diaporthe adunca* (hereafter referred to as *Diaporthe*) causes the stalk of a spike just below the flowers to turn brownish-black and wither about three days after fungal entry (de Nooij and van der Aa 1986). Spike development is arrested, which reduces seed production, and the infected spike may or may not turn downward. Uninfected spikes on the same rosette develop normally. *Diaporthe* is transmitted by weevils, which puncture spikes during feeding. These wounds provide doors for fungal entry (de Nooij and van der Aa 1986). Infection can also occur in high humidity in the absence of wounding (Linders et al. 1996). *Fusarium moniliforme* var. *subglutinans*, (hereafter referred to as *Fusarium*) produces a pink mycelium over a variable portion of a spike, and it also reduces seed production (Alexander 1982). Infection occurs during flowering, and one to many spikes of an individual plant may be infected. Data suggest that infection is not systemic (Alexander 1982). Both diseases produce a distinctive phenotype on the spikes. Thus, we assume that the above species were the sources of infection for our study plants.

Insect herbivores that feed on reproductive spikes include weevils (de Nooij and van der Aa 1986) and grasshoppers (E. P. Lacey and D. Roach, *personal observation*). We focused on grasshoppers because grasshopper damage could be easily detected. Grasshoppers chew the tips of maturing infructescences and/or create lateral indentations on an infructescence. A previous experiment suggested that grasshopper damage worsens during the latter part of the flowering/fruiting season (E. P. Lacey, *unpublished data*). Therefore, in this experiment, we sought to estimate the damage throughout the season and its impact on seed production.

Experimental designs

Seeds for our experiment were collected from 30 plants naturally growing along a transect in a field on the campus of Duke University in Durham, NC. Because this species is self-incompatible and predominantly wind pollinated, we treated seeds collected from a particular individual as constituting a maternal half-sib family. We germinated seeds in the Duke University Phytotron to minimize juvenile mortality and transplanted a total of 1200 six-week-old seedlings back into the field where the maternal parents grew. Seedlings from each family were planted in a randomized block design within two 10.3 × 4.4 m blocks. Within each block, plants were located every 15 cm with staggered rows 10 cm apart. Except for a 3-cm hole created for each seedling, the surrounding plant community was left undisturbed. The plants used in our experiment constituted a subset of a much larger set of plants established to study age-specific demography (Roach 2003).

Seedlings were planted in April 1997. In November, we counted the number of leaves on each individual and measured the length and width of the longest leaf. These data were used to estimate total leaf area, a good estimate of plant biomass (see Lacey 1996). Total leaf area was estimated as the product of leaf number, longest leaf length, and longest leaf width. Plants first flowered in May 1998, their second year. We collected our phenological data in summer 1998. Data from 2562 mature spikes produced by 444 plants were used in our analyses. Because some offspring died before flowering or did not flower, offspring sample sizes differed among families. There were from 4–10 offspring per block. For 92% of the family by block combinations, we collected data from six or more offspring per family per block. Data were analyzed by week, starting with week 1, which began Monday, 4 May 1998, and ending with week 14, which began 3 August 1998. Each week, we collected phenological data for each plant. We defined "onset of flowering" as the week that we first observed a developing spike on a plant. As soon as a spike began to turn brown and before it had dispersed seeds, it was collected. We define "onset of fruiting" as the week when the first mature spike was collected. Duration of fruiting, an estimate of the fruit maturation period, equaled the week of the last collection minus the week of the first collection. At collection time, evidence of fungal presence was recorded. If a spike showed no withering and darkening of its stalk and no mycelium, we assumed that the spike was healthy, i.e., not infected. In the laboratory, we inspected each spike for evidence of grasshopper damage and again looked for evidence of a mycelium using a dissecting microscope. Each spike was weighed to the nearest 0.001 mg.

To evaluate the quality of seeds produced at different times during the fruiting season, we compared the seed mass and the percentage of germination of seeds maturing during two weeks: week 8 (19–26 June) and week 11 (13–17 July). These two weeks represented early and mid-season fruiting. To control for maternal and offspring variation, seeds from these maturation times were compared for the same set of maternal families and offspring. Thus, the experimental design for seed mass was 12 half-sib maternal families × 3–6 offspring nested within family × 10 seeds per offspring × 2 seed maturation times, for a total of 900 seeds. Seeds were weighed individually to the nearest 0.001 mg. For the germination experiment, we used 30 seeds per offspring for a total of 2700 seeds.

To examine germination, we sowed seeds on filter paper in covered petri plates. Filter paper was moistened daily. Each plate was marked with a grid to keep track of individual seeds. Twenty-five seeds were assigned to each plate. The seeds from each offspring were randomly assigned across all plates, with the constraint that no more than one seed/offspring/maturation time could be assigned to any particular plate. Plates were kept in a growth chamber at 85% relative humidity and a 16-h light:8-h dark cycle. Temperatures resembled those of Durham, NC, in May: night; 15°C and day; 20°C (Teramura et al. 1981). Day of germination was recorded for each seed over 43 days, the last seven of which showed no new germination. Seeds had been stored dry in envelopes in the lab for one year prior to this test. These storage conditions maximize seed viability (Steinbauer and Grigsby 1975), and the storage time allowed for completion of most, if not all, after-ripening of seeds (Blom 1992).

Statistical analyses

Because the phenological data could not be normalized, we used nonparametric tests to examine flowering/fruiting onset and duration. We used the multivariate G test (log-likelihood ratio) (Bishop et al. 1975, Sokal and Rohlf 1995) to examine the effects of maternal family and block on onset of flowering, onset of fruiting, and fruiting duration (G test program, M. Rausher, *personal communication*). The G test can be used to test the independence of three or more variables when each variable corresponds to a dimension of a multidimensional contingency table. In our case, family and block were two dimensions of the table, and onset of flowering, onset of fruiting, or fruiting duration was the third dimension. To reduce the number of empty cells in the table, we lumped some weeks together for flowering/fruiting onset and duration (flowering onset levels used: week 2, weeks 3–4, weeks 5–8; fruiting onset levels: weeks 7–8, weeks 9–10, weeks 11–14; duration levels: <1 wk, 1–3 wk, 4–6 wk).

The Spearman rank correlation test (SAS 2000) was used to examine phenotypic and family-mean correlations among flowering onset, fruiting onset, and fruiting duration, and the phenotypic correlation between fruiting onset and skewness of the fruit maturation curve. For the last analysis, we computed the skewness of the fruit maturation (duration) curve for each fruiting onset. Only weeks 7–11 had large enough sample sizes of individual plants to be used in this analysis. Then we examined the correlation between skewness and fruiting onset. A correlation between these two traits could indicate that fruiting onset and the duration of fruit maturation undergo correlated evolution.

ANOVA (SAS 2000) was used to examine the effect of maternal family and block on fall leaf area. Before the analysis, we examined every family by block combination to check for normality. Thirteen outliers, which were greater than three interquartile ranges from the median leaf area value, were deleted to normalize the data for the analysis ($N = 431$). Family and its interaction were treated as random variables.

To estimate seed production for each spike and to compare the effects of infection and grasshopper damage on seed production, we counted all healthy seeds (i.e., all seeds that were brown and plump) on four subsets of spikes. We did not count aborted seeds, which are black and flat. The four subsets were: (1) undamaged spikes, which showed no signs of pathogen infection or predation ($N = 101$), (2) spikes showing evidence of grasshopper damage only ($N = 29$), (3) spikes showing evidence of infection only by *Fusarium* ($N = 28$), and (4) spikes showing evidence of infection only by *Diaporthe* ($N = 20$). In our total data set, only a handful of plants were both infected and eaten, and none showed signs of infection by both fungi. The spikes whose seeds were counted were chosen so that a range of families and spike masses would be represented. These data were used in linear regression analyses (SAS 2000) to determine how seed number changed with spike mass. We also tested quadratic and cubic regression models, but for all subsets the relationship between seed number and spike mass was strongly linear. Therefore, the linear regression equations were used to estimate seed number for spikes whose seeds were not counted. The t test was used to compare the slopes of regression lines for three of the four subsets of spikes. The requirement that the residuals be normally distributed was satisfied in those subsets. No statistical test was needed to evaluate the effect of the fourth subset.

To explore the interactions among flowering/fruiting phenology, predation, infection, and seed production, we used a structural equation modeling (SEM) procedure (PROC CALIS, SAS 2000) to perform a path analysis of the data (e.g., Mitchell 1992, Petraitis et al. 1996, Grace and Pugesek 1998, Scheiner et al. 2000). SEM allows one to perform path analyses of variables of interest. Given an a priori path model that defines the causal relationships between multiple dependent variables, one uses path analysis to measure the strength of the causal relationships among the variables

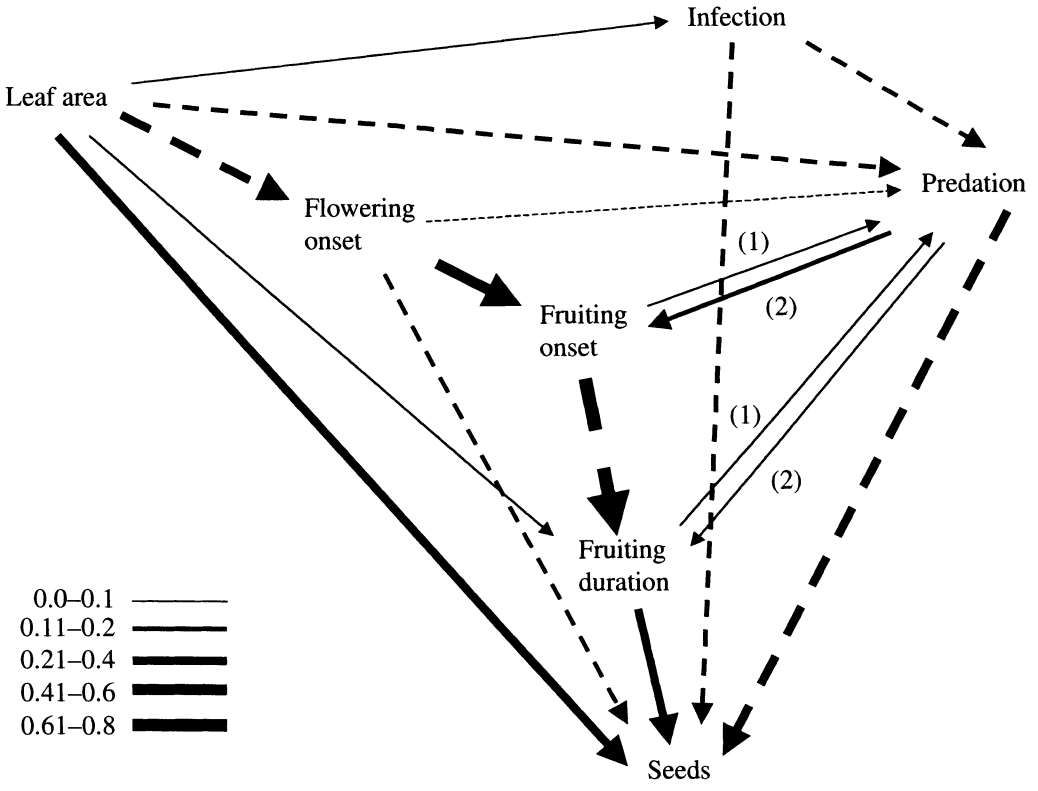

FIG. 1. Alternative path models tested for goodness-of-fit to our data for *Plantago lanceolata*. In Model 1, paths go from fruiting onset and duration to predation ("1" in parentheses). In Model 2, these paths are reversed ("2" in parentheses). Model 2 is used to measure the causal relationships among size, phenological traits, infection, seed predation, and seed production. Model 2 was used to calculate path coefficients. The strength of each coefficient is indicated by the width of the arrow. Solid lines indicate positive direct effects; dotted lines indicate negative effects.

(Wright 1934, Li 1975). One can also use SEM to test whether or not a path model chosen for the analysis fits the data. This allows the testing of alternative models to see which model provides the best fit. We tested two models, first with a goodness-of-fit chi-square (χ^2) test. A significant χ^2 value indicates that a model does not fit the data. We also report Bentler's and Bonett's Normed Fit Index (NFI), which is based on the model χ^2 relative to that of a model that assumes independence of all variables. NFI ranges between 0 and 1, with NFI > 0.90 indicating a good fit (Bentler 1989). Because our variables were not normally distributed, we used a weighted least squares procedure to test for goodness of fit of our models to the data. This procedure assumes no particular distribution for the variables in the models.

Our path models ($N = 444$) included leaf area, the three flowering/fruiting traits, percentage spikes infected by either fungus (Infection), percentage of spikes damaged by grasshoppers (Predation), and seed production (Fig. 1). Family was not included because it is a categorical variable and has no quantitative value that is biologically meaningful. Because of the temporal nature of the data, the causal relationships, i.e., direct paths, between most variables are straightforward. Leaf area directly influences flowering onset, flowering onset influences onset of fruiting, fruiting onset influences duration of fruiting, and fruiting duration, predation, and infection all directly influence seed production (Seeds). Because grasshopper damage showed seasonal change, we also included paths between the three phenological traits and predation. The two models differed in the directions of these paths. We did not include paths between the phenological traits and infection because probability of infection did not show seasonal change. Because leaf area is a good indicator of resource accumulation by a plant, we included paths from leaf area to infection, predation, fruiting duration, and seed production. Larger plants might be more susceptible to infection and predation because larger plants produce more targets (spikes) for infection and predation. Dudycha and Roach (*in press*) observed that the frequency of *Fusarium* increases with spike number. Also, larger plants have more resources available for lengthening the fruiting period and producing more seeds. We included a path from flowering onset directly to seed production because onset might influence seed production independently of fruiting if, for example, it affects pollinator activity. *Plantago lanceolata* is pollinated by both wind and insects. We also

included a path from infection to predation because we thought it possible that grasshoppers may discriminate between infected and uninfected spikes. Discrimination would explain the negligible number of spikes that were both damaged and infected.

Our two models differed in the direction of the paths between predation and fruiting onset and duration (Fig. 1). One could argue that time of onset of fruit maturation and length of the fruit maturation period influence the probability of predation. Because grasshopper abundance likely increases throughout the reproductive season, the later flowering and fruiting plants are likely to suffer more predation. Alternatively, one could argue that predation might alter a plant's rate of reproductive development, thereby affecting fruit ripening and duration. Therefore, we tested both. In Model 1 (Fruiting to Predation), paths go from both fruiting onset and duration to predation (paths marked with a "1" in Fig. 1). In Model 2 (Predation to Fruiting), the direction of the paths is reversed (marked with a "2").

Using our better path model, we estimated the path coefficients, which measure the strength of the causal relationships among variables. A path coefficient estimates the direct effect between pairs of traits, for example, the effect of infection directly on seeds. Path coefficients can also be used to estimate an indirect effect between two traits as mediated by one or more other variables, for example, the path from infection to seeds via predation. The indirect effect is the product of the path coefficient from infection to predation and the coefficient from predation to seeds.

To evaluate the phenological effects on seed mass, we used a three-way fixed-model ANOVA to test the effects of maturation time, family, and offspring nested within family on seed mass, that is, the mass of seeds produced by the offspring. All independent variables were treated as fixed. The variable offspring was treated as fixed because we deliberately chose only offspring whose fruiting duration extended over at least four weeks (weeks 8–11). Choosing only these offspring and ensuring that we had replicate offspring values within family also restricted the families used in the experiment. These restrictions limit the breadth of our conclusions to the particular families used in the experiment. However, the restrictions allow us to examine the effects of maturation time independently of maternal genetic effects, which would have been a confounding factor if we had ignored the origin of the seeds. No transformation of the seed mass data was required.

We used the χ^2 test to examine the effects of seed maturation time on total germination and percentage germination by day 2. This day was chosen because by day 2, ~40% of all seeds germinating throughout the test had germinated. By day 3, 70% of the seeds had germinated. Thus, using day 2 gave us the greatest ability to detect whether or not maturation time influences early germination. To examine the effect of seed mass on germination, we performed a linear regression (SAS 2000) of germination day on seed mass for each maturation time. The t test was used to compare the slopes of regression lines for each time. Germination day was logit-transformed to improve the normality of residuals.

Results

Flowering and fruiting patterns

Flowering onset occurred throughout May and June (from week 2 to 8) across all plants. However, because most plants began flowering in May, family means for onset ranged from 2.4 to 4.6 wk (Fig. 2). Fruits matured from mid-June to early August (week 7 to 14). Family means for onset of fruiting ranged from 8.5 to 10.6 wk. Spike maturation for the whole population climbed from 53 spikes maturing in week 7 to a peak of 1111 spikes in week 11, and then dropped to 19 in week 14. Estimated seed number per spike for all spikes collected, regardless of attack by pathogens or predators, declined from 99 seeds per spike in week 7 to 13 seeds in week 14 (Fig. 3a).

Flowering onset, fruiting onset, and fruiting duration were all significantly correlated with each other (Table 1). Earlier flowering plants began fruiting earlier and produced fruits for a longer time. Family-mean and phenotypic correlations were highly significant. Additionally, the family-mean correlation between fruiting onset and seed production was significant. The shape of the spike maturation curve for an individual plant, on average, changed as onset of fruiting was delayed. Most plants that began maturation in week 7 matured their spikes over 4–6 weeks. Few plants matured spikes more quickly. Most plants that began maturation in week 8 matured spikes over 2–4 weeks. Plants that began fruit maturation latest (in week 12, 13, or 14) all matured spikes within one week. Skewness of the fruit maturation curve was significantly correlated with fruiting onset ($N = 5$, Spearman rank correlation coefficient = 1.00, $P < 0$ .0001; Kendall's tau = 1.00, $P < 0.014$). Skewness changed from negative to positive as onset of fruiting was delayed.

Onset and duration of flowering differed among families. Family, but not block, significantly influenced onsets of flowering ($G = 101.73$, df = 58, $P < 0.001$) and fruiting ($G = 82.16$, df = 58, $P < 0.05$). The analysis of duration indicated that there was a significant block-by-family interaction ($G = 102.59$, df = 58, $P < 0.001$), which is explained by the fact that the families contributing most to these differences differed between blocks. In the two-way ANOVA of fall total leaf area, the block-by-family interaction was significant ($P < 0.043$). When each block was analyzed separately, families did not differ significantly in fall leaf area (Block 1, $F = 1.43$, df = 29, 197, $P < 0.084$; Block 2, $F = 1.02$, df = 29, 174, $P > 0.4$).

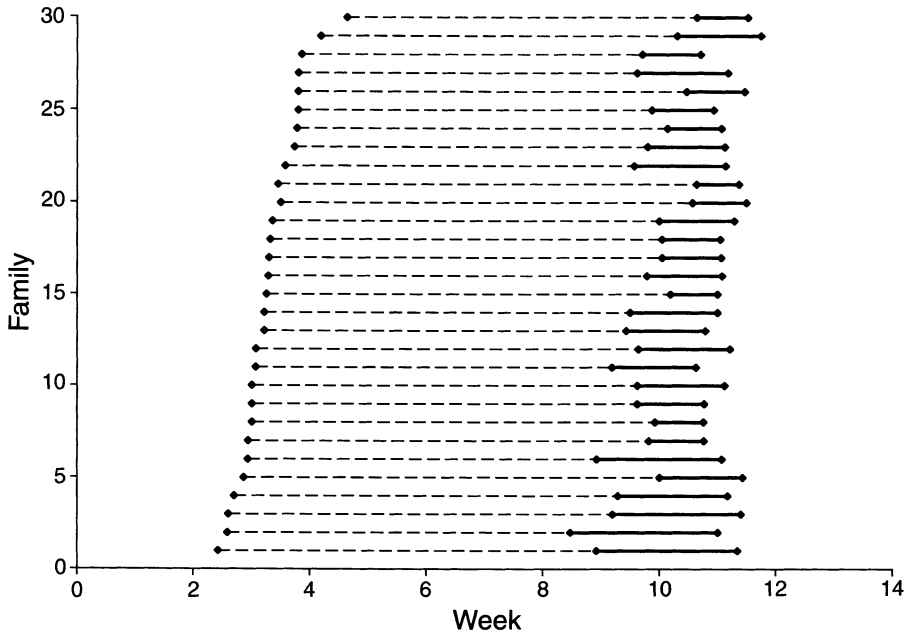

FIG. 2. Mean onsets of flowering and fruiting and mean end of fruiting for offspring of the 30 experimental families of *Plantago lanceolata*. The dotted line shows the time between initiation of flowering and onset of fruit maturation. The solid line shows the duration of fruiting.

Temporal patterns of grasshopper damage and fungal infection

Damage by grasshoppers increased throughout the summer and was always at a higher frequency than the incidence of fungal infection (Fig. 3b). Infection by *Diaporthe* never exceeded 4.2% of spikes collected in any one week. Infection by *Fusarium* never exceeded 6.9% for any week. In contrast, the percentage of spikes damaged by grasshoppers rose from 15% in week 7 to 84% in week 14.

Damage by fungi or grasshoppers reduced seed production per spike, as evidenced by the regressions of seed production on spike mass for the four subsets of spikes (Fig. 4). The t tests showed that grasshopper damage and *Fusarium* infection reduced seed production significantly and by a similar amount when compared to healthy spikes, even when one lowers the critical P value to account for multiple comparisons (healthy vs. eaten spikes, $t = 3.79$, $P < 0.001$; healthy vs. *Fusarium*-infected spikes, $t = 3.13$, $P < 0.002$; eaten vs. *Fusarium*-infected spikes, $t = 0.45$, $P > 0.6$). Infection by *Diaporthe* was most deleterious. *Diaporthe*-infected spikes produced no seeds.

Phenological effects on seed production

Both path models provided a good overall fit to the data. The χ^2 values of both were nonsignificant, indicating that the covariance structure specified by both models could not be rejected given the covariance structure of the data (Fruiting to Predation Model 1, $\chi^2 = 10.41$, df = 6, $P > 0.1$; Predation to Fruiting Model 2, $\chi^2 = 7.17$, df = 6, $P > 0.3$). The Bentler and Bonett NFI exceeded 0.90 for both models, indicating that both provided an excellent fit when compared to a null model that assumes independence among all variables (Model 1, NFI = 0.996; Model 2, NFI = 0.997). Because the P value associated with the χ^2 test for Model 2 was more than twice the P value for Model 1, we used Model 2 to explore further the interactions between the phenological variables, predation, and seed production.

Relationships between variables in the model varied greatly in strength, which can be seen by looking at the path diagram (Fig. 1) and the causal effects (Table 2). The absolute values for the direct effects ranged from 0.03 (flowering onset to predation) to 0.73 (fruiting onset to fruiting duration). The stronger relationships among variables (path coefficients >0.21) were contained in three paths. Two run from leaf area to seed production: (1) the direct path, and (2) the indirect path through flowering and fruiting (Fig. 1). The direct effect, however, explained most (71%) of the total causal effect of leaf area on seed production. Of the total causal effect of flowering onset on seed production, the indirect effect through fruiting was greater. It explained 56% of the total effect. The third path is the direct effect of predation on seed production. This direct effect explained almost all (96%) of the total effect of predation on seed production. Finally, considering the paths between predation and the three phenological traits, the coefficient for the path from predation to fruiting onset is at least double the coefficients for the other paths (Table 2). Moreover, this is the only phenological trait that has a significant family-mean correlation with seed production (Table 1).

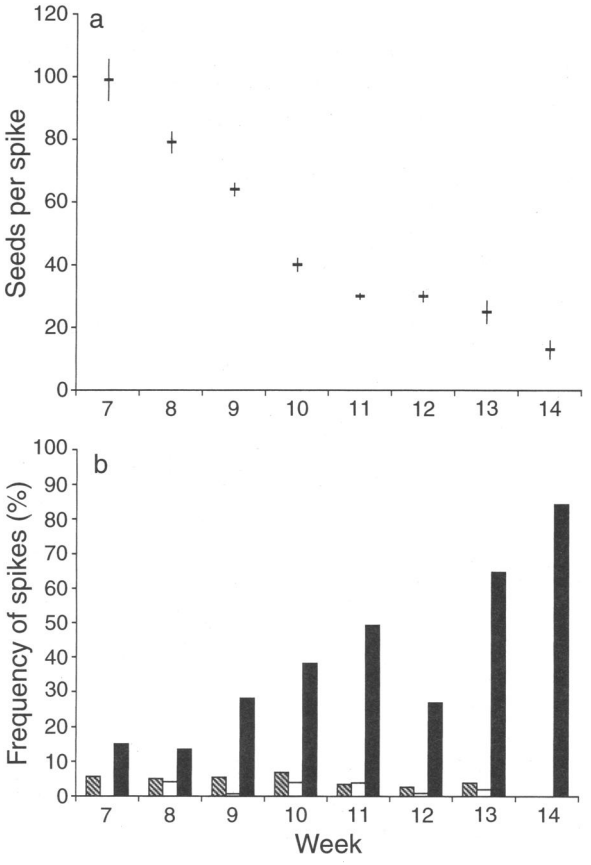

FIG. 3. (a) Weekly reproduction as mean seeds per spike (horizontal bars, with vertical bars representing ±1 SE) and (b) disease and grasshopper damage at the field site as frequency of mature spikes either infected by *Diaporthe adunca* (open bars), *Fusarium moniliforme* (striped bars), or grasshopper damaged (solid bars). The number of spikes infected by both fungi or that showed evidence of grasshopper damage and infection was negligible.

Effects on seed mass and germination

Late-maturing seeds were significantly heavier than early-maturing seeds when averaged over families (1.21 ± 0.01 mg for week 8, 1.26 ± 0.01 mg for week 11 [means ± 1 SE]; Table 3). Also, there was a significant family-by-time interaction, indicating that the effect of maturation time on seed mass had a genetic component.

Total percentage germination, i.e., over 43 days, was very high for both seed maturation times (88.03% and 89.47% for weeks 8 and 11, respectively), and early- and late-maturing seeds did not differ in final germination ($\chi^2 = 1.36$, df = 1, $P > 0.2$). However, percentage germination by day 2 did differ significantly. Later maturing seeds had a higher percentage germination (32.7% for week 8, 38.2% for week 11; $\chi^2 = 7.92$, df = 1, $P < 0.005$). Seed mass significantly influenced germination day for both maturation times (early maturation, day = 0.005[mass] + 0.07, $R^2 = 0.015$, $P < 0.014$; late maturation, day = 0.006[mass] + 0.07, $R^2 = 0.019$, $P < 0.005$). This relationship was similar for both maturation times, as evidenced by the lack of difference between the slopes of the regression lines for the maturation groups ($t = -1.03$, $P > 0.30$).

DISCUSSION

Even though we recognize that our data are limited to one season and one population, we feel that the patterns observed here provide useful information about general patterns concerning flowering/fruiting phenology. Our data show that flowering and fruiting times can produce multigenerational effects in *P. lanceolata*. Within the parental generation, plants that flowered and matured fruit earlier produced more seeds. When considering the seeds produced by an individual over its reproductive season, late-maturing seeds were heavier and germinated faster than did early-maturing seeds. When combined with the results of the experiment by Lacey and Herr (2000), these data provide evidence that reproductive phenology affects both offspring quality and quantity in *P. lanceolata*. The fact that several other studies (Lacey and Pace 1983, Case et al. 1996, Ollerton and Diaz 1999, Picó and Retana 2000, Wolf and Burns 2001, Galloway 2002) have also detected phenological effects on offspring phenotype suggests that such multigenerational effects may be widespread in plants. Effects on seed size and germination likely affect offspring fitness. Van

TABLE 1. Phenotypic and family-mean Spearmann rank correlation coefficients are shown for pairs of variables in *Plantago lanceolata*.

Pairs of variables	Coefficient	
	Phenotypic	Family-mean
LA–FL	−0.32***	−0.13 ns
LA–FR	−0.19***	−0.04 ns
LA–DUR	0.18***	0.04 ns
LA–PRED	−0.13 ns	−0.12 ns
LA–INF	0.04**	0.37*
LA–SEEDS	0.39***	0.25 ns
FL–FR	0.50***	0.64***
FL–DUR	−0.39***	−0.49**
FL–PRED	−0.02 ns	0.28 ns
FL–INF	−0.10***	0.07 ns
FL–SEEDS	−0.34***	−0.20 ns
FR–DUR	−0.73***	−0.82***
FR–PRED	0.16*	0.23 ns
FR–INF	−0.06**	−0.03 ns
FR–SEEDS	−0.44***	−0.36*
DUR–PRED	−0.04 ns	−0.003 ns
DUR–INF	0.02**	0.03 ns
DUR–SEEDS	0.48***	0.25 ns
PRED–INF	−0.18**	−0.16 ns
PRED–SEEDS	−0.27***	0.04 ns
INF–SEEDS	−0.04*	−0.06 ns

Notes: Abbreviations are: LA, leaf area; FL, flowering onset; FR, fruiting onset; DUR, fruiting duration; INF, infection; PRED, predation; SEEDS, seed production. Total offspring for all 30 families = 444.

* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; ns, $P > 0.05$.

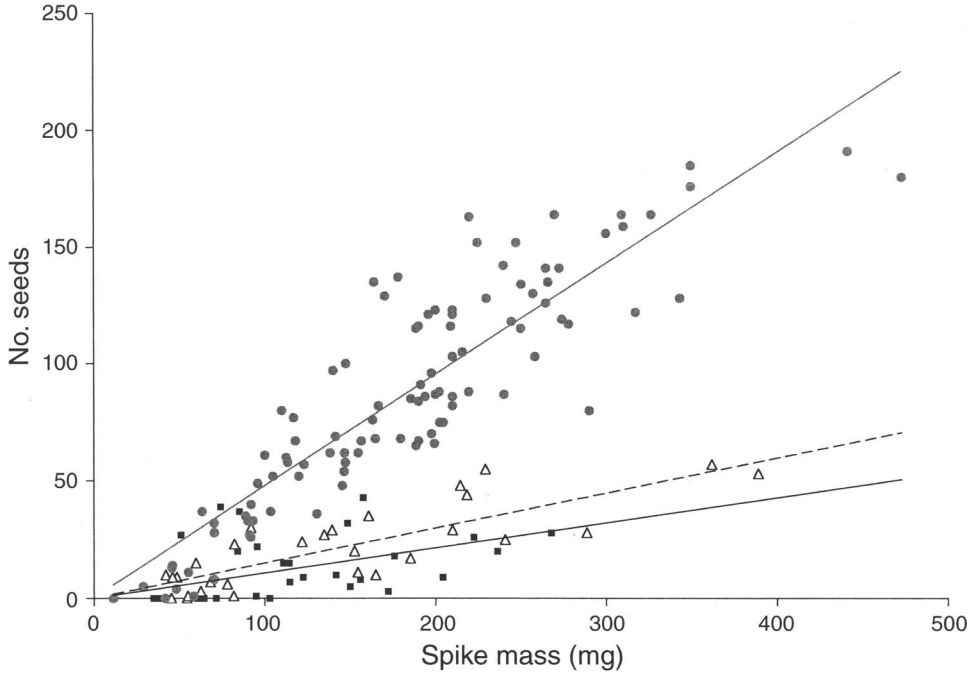

FIG. 4. Relationship between spike mass (mass) and seed number (seeds) with associated regression lines for three groups of spikes of *Plantago lanceolata*: (1) undamaged and uninfected (gray circles; regression equation: seeds = −1.310 + 0.4830[mass] [solid line], $R^2 = 0.79$, $P < 0.0001$), (2) *Fusarium*-infected (solid squares; regression equation: seeds = 4.225 + 0.0843[mass] [solid line], $R^2 = 0.14$, $P < 0.05$), and (3) grasshopper-damaged (open triangles; regression equation: seeds = 0.170 + 0.1486[mass] [dashed line], $R^2 = 0.67$, $P < 0.0001$). *Diaporthe*-infected spikes are not shown because they never produced seeds.

der Toorn and Pons (1988) observed that early-germinating plants have a competitive advantage in *P. lanceolata*. Seed mass and germination can influence lifetime fitness or its components in other species (e.g., Schaal 1980, Stanton 1984, Roach 1986, Winn 1988, Bière 1991, Galen and Stanton 1991, Donohue and Schmitt 1998, Simons and Johnston 2000).

Multigenerational effects are likely to influence the evolution of reproductive timing in *P. lanceolata*. Our data, as well as those of others (Primack and Antonovics 1981, Wolff 1987, Wolff and van Delden 1987, van Tienderen and van der Toorn 1991, Lacey 1996, Lacey and Herr 2000; D. Roach, *unpublished data*) show that genetic variation underlies the phenotypic variation in reproductive phenology in natural populations and that the genetic control is partially independent of plant size, which is also under partial genetic control. Therefore, selection pressures acting in the parental and progeny generations can potentially interact to influence the evolution of flowering and fruiting phenology. The amount of genetic variation underlying the phenotypic variation in phenological traits determines the degree of response to selection. The significant family-mean correlations between flowering and fruiting onsets and duration suggest that all three phenological traits are genetically correlated with each other, and therefore should coevolve. Fruiting onset was the only phenological trait that showed a significant family-mean correlation with seed production. This suggests that selection for earlier fruit maturation should increase seed production, our within-generational fitness component.

Multigenerational effects could act synergistically or antagonistically to determine the net direction of evolutionary change in reproductive phenologies (Lacey and Herr 2000). Our data support the hypothesis that the multigenerational effects are antagonistic, i.e., that there are cross-generational trade-offs between parental and offspring components of parental fitness in *P. lanceolata*. From the point of view of reproductive output (e.g., seed production), which is how fitness is typically measured in ecological and evolutionary studies, selection appears to favor plants that begin to flower and fruit early in the season. In contrast, from the point of view of offspring fitness, selection appears to favor parents that flower and fruit later. This latter point is supported by the Lacey and Herr (2000) study. If the relative contributions of parental and offspring components of parental fitness change little over years, then one would predict that flowering/fruiting time would contract around the period that maximizes the joint fitness effects. However, if yearly fluctuations in weather cause fluctuations in the relative balance between parental and offspring components, then a prolonged

TABLE 2. Total causal, direct, and indirect effects for pairs of variables in Path Model 2.

Variables (from–to)	Effect		
	Total	Direct	Indirect†
LA–FL	−0.33	−0.33	0
LA–FR	−0.19	0	−0.19
LA–DUR	0.17	0.04	0.13
LA–PRED	−0.14	−0.13	−0.0005
LA–INF	0.06	0.06	0
LA–SEEDS	0.42	0.30	0.12
FL–FR	0.50	0.50	−0.005
FL–DUR	−0.37	0	−0.37
FL–PRED	−0.03	−0.03	0
FL–SEEDS	−0.24	−0.10	−0.13
FR–DUR	−0.73	−0.73	0
FR–SEEDS	−0.27	0	−0.27
DUR–SEEDS	0.37	0.37	0
INF–PRED	−0.19	−0.19	0
INF–FR	−0.03	0	−0.03
INF–DUR	0.01	0	0.01
INF–SEEDS	−0.06	−0.11	0.05
PRED–FR	0.16	0.16	0
PRED–DUR	−0.03	0.08	−0.12
PRED–SEEDS	−0.24	−0.23	−0.01

Notes: Total causal effects = direct + indirect effects. See Table 1 for abbreviations.

† 0 = no effect.

flowering/fruiting season would be expected. Such fluctuations could help to explain why *P. lanceolata* has such a long flowering season. While our experiments do not allow us to quantify the relative contributions of parental and offspring components of parental fitness, nor quantify their annual fluctuations, they do indicate that both components should be considered in studies addressing the evolution of reproductive phenology and that, ultimately, such quantification is needed.

Seed predation intensity changes over the reproductive season in many species. For some, seed predation is highest early in the season (e.g., Evans et al. 1989, Bière and Honders 1996, Pilson 2000), for others, it is highest late (e.g., Schemske 1984, Bière and Honders 1996, Bishop and Schemske 1998), and for a few species, it is highest during the time of peak reproduction (e.g., Marquis 1988, Evans et al. 1989, English-Loeb and Karban 1992). In our study, seed predation increased throughout the season. Spike damage by grasshoppers exceeded 80% at season's end. As evidenced by the path analysis results, predation negatively affected seed production per plant. Grasshopper reproduction and survival generally increase as weather becomes warm, dry, and sunny (Dempster 1963), which typifies the changing weather conditions from April to August, the reproductive season for *P. lanceolata*, in piedmont, North Carolina.

The data suggest that seed predation by grasshoppers, unlike fungal infection, favors earlier flowering and fruiting in *P. lanceolata*. In contrast to predation, the percentage of spike infection by *Fusarium* has never been observed to exceed 10% in this population (Alexander 1982, Dudycha and Roach, *in press*). Because both path models fit our data well, the relationship between predation and fruiting is unclear. Earlier maturing fruits suffered less grasshopper damage, but whether or not damage influences maturation rate is unknown. These relationships need further study. Also, as *P. lanceolata* individuals become older/larger, their fruiting duration is extended later and later into the season (D. Roach, *unpublished data*). One can speculate that with increasing size, a plant is better able to tolerate seed loss from grasshoppers.

Our observation that late-maturing seeds were heavier than were early-maturing seeds did not match our predicted results based on Lacey's experiment (1996). In a growth chamber experiment, she observed that low, rather than high, temperature increased seed mass. This observation is consistent with results of other growth chamber experiments examining the effects of temperature in other species (e.g., Robertson et al. 1962, Sawhney and Naylor 1979, Gutterman 1980–1981, Siddique and Goodwin 1980, Wulff 1986). Our results suggest that a seasonal change in some variable other than temperature more than offsets the effect of high temperature on seed mass. Most studies documenting seasonal change in seed mass have reported a decline in mass over time, attributable to declining resources (e.g., Cavers and Steel 1984, Thompson and Pellmyr 1989, Galen and Stanton 1991, Winn 1991, Wolfe 1992, Diggle 1995, Simons and Johnston 2000). Clearly, declining resources do not explain our results. One possible explanation is that plants may alter their resource allocation within a spike throughout the reproductive season such that later spikes produce fewer but larger seeds. Alternatively, maturation time effects might be caused by differences in pollen donors. These paternal effects could be genetically or environmentally based. At this point, the cause of the observed increase in mass is unknown. In spite of this, our results are in the predicted direction, if the hypothesis that delayed flowering/fruiting enhances offspring fitness is true. Large seeds often germinate more quickly and produce larger seedlings than do small seeds (e.g., Black 1958, Harper 1977, Schaal 1980, Stanton 1984, Roach 1986, Winn 1988, Galen and Stanton 1991, Si-

TABLE 3. Fixed-effects model ANOVA results for the effects of maturation week, family, and offspring nested within family on seed mass.

Source	df	*F*	*P*
Maturation week	1	8.76	0.003
Family	11	1.35	0.2
Offspring (Family)	34	8.92	<0.0001
Week × Family	11	4.24	<0.0001
Error	859		

Note: *F* values were calculated using Type III ss.

mons and Johnston 2000). Larger seeds typically are more strongly favored in competitive, shaded, or stressful habitats, although there are some exceptions (reviewed in Donohue and Schmitt 1998).

Our germination results are also in the predicted direction, although they do not exactly match expectations based on the experiment by Lacey and Herr (2000). On average, temperature increases from April to August in piedmont, NC (Teramura 1978), and in our experiment, the daily temperature averaged over the two weeks prior to our seed collections in weeks 8 and 11 were 20.1 ± 1.2°C (mean ± 1 SE) and 27.2 ± 0.4°C, respectively (Roach 2003). Lacey and Herr observed that high postzygotic temperature increased total germination under field conditions in spring, but we found no difference in total germination between early- and late-maturing seeds. However, we did find that late-maturing seeds germinated more quickly. This difference is not likely explained by a difference in dormancy because seeds in both groups showed very high germination overall, and they had ample time to complete after-ripening prior to the germination test. Also, because early and late seeds were produced by the same maternal parents, the difference is not explained by maternal genetic effects. Our experimental design did not allow us to test for possible paternal effects associated with maturation time.

The biological significance of a one-day difference in germination is presently unclear. One could argue that it is likely to be important in highly competitive situations, where *P. lanceolata* can and does thrive, e.g., lawns. Rapidly germinating individuals have a competitive advantage over slowly germinating individuals in *P. lanceolata* (van der Toorn and Pons 1988) and in other species (e.g., Black 1958, Harper 1977, Schaal 1980, Roach 1986, Winn 1988, Bière 1991, Galen and Stanton 1991, Simons and Johnston 2000). Earlier germination may be more advantageous among fall-germinating seeds if it speeds establishment rate and, thereby, reduces winter mortality. Further studies will have to be conducted to assess the effect of reproductive timing on fall vs. spring germination. In *Campanula americana* (Galloway 2002) and in *Daucus carota* (Lacey and Pace 1983), earlier flowering plants produce seeds that are more likely to germinate in the fall because earlier flowering plants disperse their seeds earlier, in time for fall germination. This situation does not apply to NC piedmont populations of *P. lanceolata* because even later flowering plants disperse their seeds well before the fall germination season.

Finally, many studies have documented parental environmental effects in plants (e.g., see reviews: Roach and Wulff 1987, Gutterman 1992, Wulff 1995, Byers and Shaw 1998, Donohue and Schmitt 1998). Studies show that environmental factors such as parental nutrient level, shading, substrate material, drought stress, temperature, photoperiod, and density can affect offspring phenotype (e.g., Durrant 1962, Gutterman 1980–1981, Schaal 1984, Wulff 1986, Miao et al. 1991*a*, *b*, Schmitt et al. 1992, Platenkamp and Shaw 1993, Schmid and Dolt 1994, Lacey 1996, Sultan 1996, Mazer and Wolfe 1998), as well as offspring fitness or its components in nature (Schmitt et al. 1992, Galloway 1995, Donohue and Schmitt 1998, Lacey and Herr 2000). There is presently, however, little evidence that these effects are evolutionarily important (e.g., see Donohue and Schmitt 1998, Mazer and Wolfe 1998). One reason may be that most attention has been directed toward measuring the phenotypic effect of the parental environment, and little attention has been directed toward traits that produce a parental environmental effect. Traits that produce parental environmental effects should evolve in response to the direction and magnitude of the effects on offspring phenotype and to the selective pressures faced by offspring. Our data suggest that reproductive phenology evolves partially in response to the parental environmental effects that it induces.

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