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EVOLUTION IN STRESSFUL ENVIRONMENTS. I. PHENOTYPIC VARIABILITY, PHENOTYPIC SELECTION, AND RESPONSE TO SELECTION IN FIVE DISTINCT ENVIRONMENTAL STRESSES

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Abstract.—Considerable debate has accompanied efforts to integrate the selective impacts of environmental stresses into models of life-history evolution. This study was designed to determine if different environmental stresses have consistent phenotypic effects on life-history characters and whether selection under different stresses leads to consistent evolutionary responses. We created lineages of a wild mustard (*Sinapis arvensis*) that were selected for three generations under five stress regimes (high boron, high salt, low light, low water, or low nutrients) or under near-optimal conditions (control). Full-sibling families from the six selection histories were divided among the same six experimental treatments. In that test generation, lifetime plant fecundity and six phenotypic traits were measured for each plant. Throughout this greenhouse study, plants were grown individually and stresses were applied from the early seedling stage through senescence. Although all stresses consistently reduced lifetime fecundity and most size- and growth-related traits, different stresses had contrasting effects on flowering time. On average, stress delayed flowering compared to favorable conditions, although plants experiencing low nutrient stress flowered earliest and those experiencing low light flowered latest. Contrary to expectations of Grime's triangle model of life-history evolution, this ruderal species does not respond phenotypically to poor environments by flowering earlier. Most stresses enhanced the evolutionary potential of the study population. Compared with near-optimal conditions, stresses tended to increase the opportunity for selection as well as phenotypic variance, although both of these quantities were reduced in some stresses. Rather than favoring traits characteristic of stress tolerance, such as slow growth and delayed reproduction, phenotypic selection favored stress- avoidance traits: earlier flowering in all five stress regimes and faster seedling height growth in three stresses. Phenotypic correlations reinforced direct selection on these traits under stress, leading to predicted phenotypic change under stress, but no significant selection in the control environment. As a result of these factors, selection under stress resulted in an evolutionary shift toward earlier flowering. Environmental stresses may drive populations of ruderal plant species like *S. arvensis* toward a stress-avoidance strategy, rather than toward stress tolerance. Further studies will be needed to determine when selection in stressful environments leads to these alternative life-history strategies.

Key words.—Adaptation, C-S-R model, environmental stress, flowering time, life-history evolution, phenotypic selection, selection experiment, *Sinapis arvensis,* stress avoidance, stress tolerance.

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One of the central aims of evolutionary ecology is to determine how natural selection jointly molds the life histories and limits the distributions of organisms (Mayr 1963; Antonovics 1976). Following the lead of Darwin (1859), many biologists have focused on competition, predation, disturbance, and disease as the principal architects of life-history evolution (e.g., Lack 1966; Williams 1966; MacArthur and Wilson 1967; reviewed in Roff 1992; Stearns 1992). Others tend to view phenology and allocation traits as adaptations to abiotic environmental condition, and often attribute species characteristics and distributions to their tolerance for physical factors (Grubb 1977; Kohlmann et al. 1988; Root 1988; Prentice et al. 1991; Davis and Zabinski 1992; Woodward and Kelly 1997). As these two approaches have converged, increasing attention has been paid to the role that abiotic environmental stresses may play in shaping life histories and defining the limits of adaptation (Grime 1977, 1979; Parsons 1990; Bradshaw 1991; Hoffman and Parsons 1991; Geber and Dawson 1993; Arendt 1997).

Abiotic stresses may exert selection either for stress tolerance or for stress avoidance (Grime 1979; Berven and Gill 1983; Greenslade 1983; Southwood 1988; Taylor et al. 1990; Hoffman and Blows 1993). Evolution of stress tolerance is predicted by Grime's triangle (or C-S-R) life-history model (Grime 1977, 1979, 1988a), which is applied principally to plants and other non-motile organisms (Boorman 1982; Chapin and Shaver 1985; Grime 1988b; Kautsky 1988; Grime et al. 1990; but also see Winemiller and Rose 1992; Arendt 1997). In this view, stresses are defined as abiotic environmental conditions that substantially reduce the fitness of organisms and, specifically, the productivity of existing tissues. According to the triangle model, the interacting selection pressures imposed by frequency of disturbance and environmental stresses lead to the evolution of three distinct life-history strategies: ruderal, competitive, and stress tolerant. Stress tolerators are seen as poor competitors characterized by slow maximal growth rate, delayed reproduction, and reduced phenotypic plasticity. Consistent with these predictions, some quantitative models have suggested that slow growth and other resource-conserving traits will evolve in unproductive habitats (Iwasa and Cohen 1989; Arendt and Wilson 1997). Stress avoidance represents an alternate strategy to stress tolerance. In contrast to selection favoring slow growth and reduced tissue turnover, selection for stress avoidance is likely to favor rapid development and early reproduction (Levitt 1980; Ritland and Jain 1984; Rice and Mack 1991b; Aronson et al. 1992). Comparative studies of perennial plants find that species in poor habitats exhibit traits thought to confer stress tolerance (Grime and Hunt 1975; Chapin 1980; Chapin and Shaver 1985; Campbell and Grime

1992), whereas comparisons of ecotypes within annual species show divergence in traits associated with stress avoidance (Rice and Mack 1991b; Aronson et al. 1992). These contrasting findings may reflect life-history variation among the study species and/or differences in the types of environmental stresses those populations experience. It will be necessary to measure patterns of selection in response to different kinds of stresses and in species with different life-history characteristics to predict when stressful conditions will select for avoidance versus tolerance traits.

To make general predictions about the consequences of environmental stress for life- history evolution, we need to determine whether the selection pressures imposed by different stresses are consistent enough to lead to a common suite of adaptations (Chapin and Shaver 1985; Symeonidis et al. 1985; Sibly and Calow 1989; but see Hoffman and Parsons 1991). We examined this question using artificial selection in a weedy mustard species. Our factorial experiment used lineages that had experienced three generations of artificial selection under one of six different environments: five specific stresses and near-optimal control conditions. In a single test generation, we examined how lineages selected under different stresses responded to all six environments. Full-sib families from each lineage were divided among treatments, producing a common genetic background in each environment. This factorial experiment allowed us to examine the extent to which phenotypic selection was consistent across a range of environmental stresse, and whether this pattern of selection resulted in consistent, evolved life-history changes after three generations of selection. We address the following questions: (1) How does stress affect the phenotypic expression and fitness of individuals? Do different stresses result in similar changes in growth and phenology? (2) Does stress increase or decrease variability in relative fitness and other phenotypic traits, relative to benign conditions? (3) What growth and phenology traits are favored in stressful environments? Are similar traits favored in all stresses? (4) Do the selected lineages evolve in response to specific stresses? If so, are these responses consistent across different stresses?

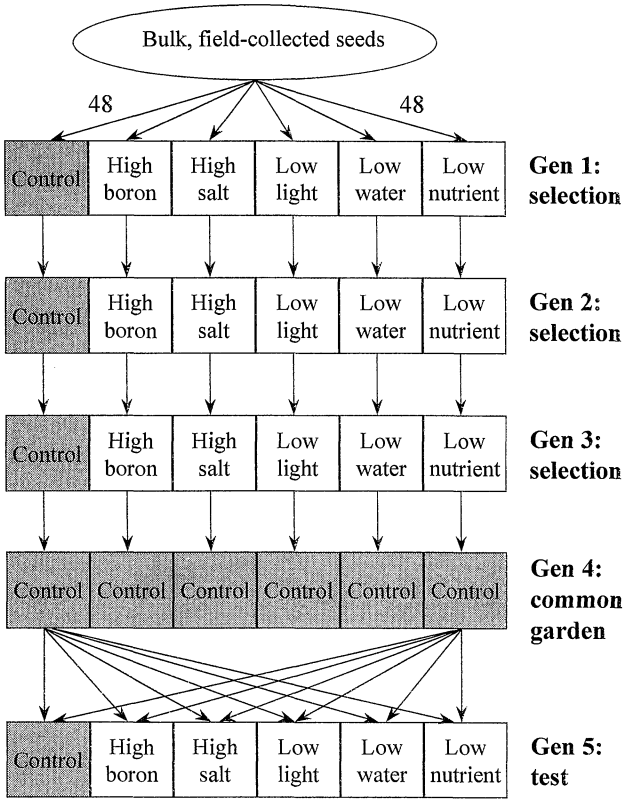

FIG. 1. Overview of the selection protocol and experimental design. In the greenhouse, individually grown plants were exposed to three generations of selection, followed by a single generation of near-optimal conditions. Each lineage (shown as a single box) was selected under one of six treatments, five stresses (unshaded) and a near-optimal control environment (shaded). Forty-eight randomly chosen individuals were used to found each lineage in each generation. Maternal environmental effects were minimized in the fourth generation by growing all lineages in a common (control) environment. Crosses were performed within lineages to produce full-sibling families. In the fifth (test) generation, eight randomly selected, full-sibling families from each selection history were divided among six environmental regimes, producing a 6×6 factorial design. Replicates were rotated between random locations twice each generation. One of eight replicates is shown here.

METHODS

Study Species and Source Population

Sinapis arvensis L. (= *Brassica kaber, Brassica arvensis*; ''wild charlock,'' ''wild mustard'') is native to Eurasia and is a common weed within disturbed sites in North America (Mulligan and Bailey 1975; Rollins 1981). In California's Mediterranean climate, *S. arvensis* is a winter annual that blooms in early to midspring, is visited by a variety of generalist pollinators, and sets seed by late June. The species is self-incompatible and populations can contain substantial genetic variability (Ford and Kay 1985; Lefol et al. 1996; Moodie et al. 1997). The seeds used in this study were collected from a very large, dense population of *S. arvensis* occupying a fallow field 1.5 km north of the University of California's Davis campus (Yolo County, CA). On three dates in June 1993, we walked along random transects through the population, collecting one to two fruits from many hundreds of plants each time. The three collections of over 100,000 seeds were combined. Seeds were removed from fruits and stored with desiccant at 4°C to retain maximum viability until used in experiments.

Artificial Selection in Six Environments

The design of the experiment is summarized in Figure 1. In January 1994, a subsample of the 1993 seed collection was divided into 48 lots with 48 seeds in each lot. Each lot was assigned at random to one of six experimental selection environments (described below). Eight replicate lineages experienced artificial selection in each environment. To minimize variation in seedling emergence time and seed dormancy, we induced seeds to germinate on filter paper using 1000 ppm gibberellic acid (GA). Two days before planting, seed lots were exposed to the GA treatment in random order. Lots were then kept in foil-covered petri dishes, and seeds with protruding hypocotyls were planted two days later into 200 cm^3 Conetainers (D-40 cells, Steuwe and Sons, Corvallis,

OR), filled with a 1:3 mixture of sterilized Yolo clay-loam and horticultural sand. To minimize competition throughout the growth cycle, a single germinated seed was placed into each container, and containers were spaced approximately 10 cm apart on greenhouse benches. Different lots were placed into randomly assigned locations within two adjacent greenhouses and watered gently from overhead twice each day. These procedures resulted in more than 90% seed germination and more than 95% seedling emergence and prevented plants from escaping stress by remaining dormant. The few seedlings that did not emerge within four to five days after planting were replaced with extras planted from the same seed lot. In all, each generation of selection involved 2304 plants (6 selection environments $\times$ 8 replicate lineages per environment $\times$ 48 plants per lineage).

The use of a relatively poor soil-sand mixture, in concert with subirrigation, allowed us to control the levels of nutrients, toxins, and water to which plants were exposed. One week after planting, by which time most seedlings had produced their first true leaf, we imposed six different environmental treatments on the seedlings. Five of the treatments exposed growing plants to conditions that had been shown in trial studies to substantially reduce size and fecundity, but without incurring severe mortality. Each of these putative environmental stresses differed from the control treatment by a single environmental factor, as described below. In the control treatment, plants were exposed to maximum sunlight on the greenhouse bench and subirrigated with a balanced fertilizer solution (Growmore® 4-18-38; Nacco Agricultural Chemicals, Gardena, CA; N:P:K = 2:1:2, supplemented with Hoagland's solution) for two-day intervals. Between watering intervals, trays were drained of the fertilizer solution and allowed to dry for 12 h before refilling. These conditions supported luxuriant growth and fruit production. In the high boron treatment, all conditions were the same as for controls, except that boric acid was added to the fertilizer solution to achieve a boron concentration of 9 mg L^{-1} (this was increased to 18 mg L^{-1} in generations 2 and 3). Boron toxicity occurs in arid agricultural regions, including many in California's Sacramento Valley, that rely heavily on irrigation from aquifers (Kelley 1928; Page and Chang 1990; Manyowa and Miller 1991). In the high-salt treatment, sodium chloride was added to the fertilizer solution to achieve a salt concentration of 6.43 g L^{-1}. Increases in salinity are also common in agricultural areas using irrigation (Shainberg and Shalhevet 1984). In the low-light treatment, plants were watered and fertilized as in the control treatment, but were grown under a 60% neutral shade canopy. In the low-nutrient treatment, plants received only deionized water after the expansion of their first true leaf. In the low-water treatment, plants received the same fertilizer solution as the control treatment, but were kept dry between two-day subirrigation intervals until all plants were severely wilted. The number of days without water ranged from three to five days. All stress regimes were applied to plants throughout their life cycle, until fruits were mature and natural senescence was nearly complete.

Artificial selection protocol.—We allowed the experimental treatments to generate natural variability both in pollen and seed production. Once plants began to flower, we performed random cross-pollinations with a brush or feather duster two days per week until flowering ceased. All pollinations were performed within a lineage. To prevent inadvertent pollen transfer between lineages, a separate brush was used for each, barriers were erected between lineages, and researchers wiped down with alcohol between pollinations. These procedures minimized genetic contamination between selection treatments. Self-incompatibility in *S. arvensis* ensures that all seeds were outcrossed, thus minimizing the loss of allelic diversity through inbreeding. After each generation we randomly sampled a subset of the seeds to produce the next generation of 48 individuals. Thus, our artificial selection protocol is a surrogate for natural selection, in which variation in both male and female fecundity can contribute to genetic change across generations in response to stress. We followed this selection protocol for three generations in all six environments.

During the three generations of selection, replicate lineages from the six environments were blocked together in the greenhouse for a total of eight blocks. Each block consisted of the same six lineages throughout the study, one representing each growth environment (hereafter referred to as ''selection history''). Block location in the greenhouse was randomly assigned twice each generation, so blocks represented a unique temporal sequence of greenhouse locations. Thus, two factors could contribute to variation among replicate lines within a given stress: random genetic drift and position effects from random locations of blocks in the greenhouse.

Achieving a common maternal environment.—To minimize the impact of maternal environmental effects (Roach and Wulff 1987; Galloway 1995; Weiner et al. 1997) on phenotypic and evolved responses to stress treatments, all plants were grown under near-optimal control conditions in the fourth generation. Within this common environment, we randomly assigned pairs of individuals within each lineage to be sire and dam and performed cross-pollinations to produce eight full-sib families from each replicate line (n = 384 full-sib families from unique sires for all lines).

The Test Generation

To examine phenotypic and evolved response to stress, we exposed plants from all selection histories to all stress and control environments in a 6 $\times$ 6 factorial experiment in the fifth generation. Germinated seeds from each full-sib family were sown on 30 August 1995 and then divided among the same six environmental treatments that had been used during the three generations of selection. Each replicate lineage within each selection history was represented by eight individuals, one from each of eight full-sib families, combining for a total of 2304 individuals. By stratifying families across the full range of experimental treatments, we ensured that plants exposed to each current environment came from very similar genetic backgrounds.

In the factorial experiment, we measured the following growth and phenology traits on all individuals: plant height (in cm) in weeks 2 and 3, length of the longest leaf (in cm) in weeks 2 and 3, the number of true leaves in week 3, Julian date of the first flower, and total weight (in g) of all seeds produced by each individual plant. In all analyses, total seed mass (fecundity) was used as our estimate of individual (fe-

male) fitness. We calculated two additional traits, relative change in plant height (height relative growth rate) and leaf length (leaf relative growth rate), as the difference between the natural log of the first and second measurements.

Statistical Analysis

ANOVA design and interpretation.—In this study we are interested in how the various stress treatments decrease fitness and concomitantly alter the phenotype. Phenotypic responses to stress are reflected by how all individuals respond to their current environment, whereas evolved responses to stress are reflected by how responses to the different environments are influenced by selection history. We conducted separate analyses of fecundity and phenotype, using MANOVA to protect our F-tests in the phenotypic analyses where we considered all six traits that we measured.

The mixed-model ANOVA includes four main effects: current environment, selection history, replicate, and family nested within each selection history and replicate combination, that is, within each unique lineage. Current environment and selection history are treated as fixed factors, whereas replicate and family are treated as random factors. Main effects of current environment are interpreted as purely phenotypic responses to stress. Evolved responses to stress resulting from artificial selection would be indicated by a main effect of selection history, if the response was consistent across test environments, or by interaction between selection history and other factors. To assess whether we could generalize about the nature of phenotypic and evolved responses to different stresses, we included two preplanned contrasts in the ANOVA model, in which either all stress environments or all stress selection histories were pooled in a contrast to the control current environment or the control selection history. The first of these contrasts tests whether there is a generalized phenotypic response to current stress environment; that is, is there a consistent change in fecundity or phenotype in all stress environments, relative to the control environment? The second contrast asks whether there is a generalized, evolved response to stress across all stress selection histories.

When the effects of stress on fecundity or phenotype varied among replicates (as indicated by a significant three-way interaction between current environment, selection history, and replicate), we conducted separate analyses by replicate to examine the nature of the observed response. To control our Type I error in these analyses, we adjusted significance to account for the eight separate tests, using a sequential Bonferroni adjustment for each source of variation (Rice 1989).

The construction of appropriate F-tests for random factors in a mixed-model framework affects the biological interpretation of those effects (Fry 1992; Newman et al. 1997). Two approaches for the construction of F-tests differ in their choice of the denominator. Hocking's model (used in SAS) tests random factors over their interactions, whereas the Scheffé approach is to test random factors over the mean square error. Fry (1992) points out that when families are reared in two environments, the family effect tested by the Hocking model reflects the genetic correlation between environments, whereas the Scheffé model reflects the cross-environment heritability. Extending the Hocking model to more than two environments requires an assumption that covariances among pairs of environments are equal. Because our experiment included six environments, the assumption of equality of covariances is likely to be violated for either of the random factors, replicate or family. Furthermore, a single representative of each family in a current environment precludes an estimate of the family by current environment interaction. We therefore take a conservative approach, using the Scheffé model for constructing appropriate F-tests. All fixed effects including interactions were tested over the appropriate random interaction, whereas all random effects were either tested over their nested random factor or over the mean square error. The existence of a family-by-current environment interaction, which we cannot estimate, inflates the significance of the replicate effect and deflates the significance of the family effect. Therefore, our test for cross-environment family effects, and thus cross-environment heritability (Fry 1992), is very conservative.

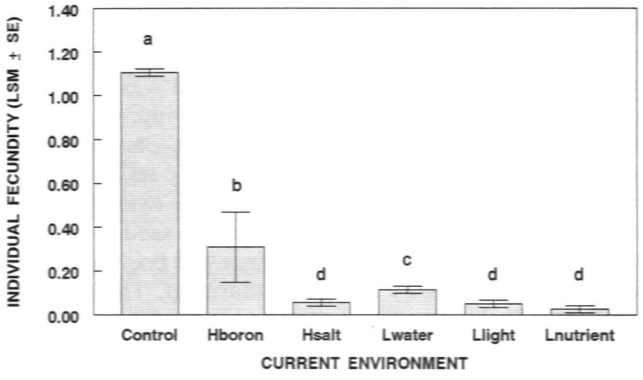

FIG. 2. Reduction of plant fecundity under five experimental stress regimes, compared to the near-optimal control environment. Fecundity is expressed as the total mass of seeds (in mg) produced by an individual plant. Least-square means for the main effect of current environment derived from the ANOVA model in Table 1 are shown, although fecundity was back-transformed for ease of interpretation here. Treatments not sharing the same label are significantly different at the $P < 0.05$ level, as demonstrated by Tukey's pairwise comparisons. Stress treatment abbreviations are as follows: Hboron, high boron; Hsalt, high salt; Lwater, low water; Llight, low light; Lnutrient, low nutrient.

Fecundity responses to stress.—Stress reduced both the mean and the variance in fecundity (Fig. 2). We used a natural-log transformation of fitness to ameliorate this effect. However, Levene's test (in which the absolute value of the residuals from the overall model are used as dependent variables in an identical model) indicated significant heteroscedasticity (Levene 1960), so we used weighted ANOVA to eliminate heteroscedasticity (Neter and Wasserman 1974). Each observation was weighted by the inverse of the variance of the residuals for the highest-order interaction indicated to be significant in Levene's test (Welch 1951). For example, in our analysis of fecundity, Levene's test was significant for the three-way interaction between current environment, selection history, and replicate. The variances of the residuals from the original model were calculated at this level and their inverse was used as a weight for each observation in that combination of effects.

Did stress-selected lineages adapt to stress environments? Adaptation to a specific stress is demonstrated when stress-adapted lineages achieve greater fitness than nonadapted lineages in stressful environments. Our 6 × 6 factorial design was not the most efficient one for evaluation of this question, but we were able to test for this special case of interaction between selection history and current environment in determining fecundity. Based on the a priori null hypothesis of no adaptation to stresses, we compared the performance of control and stress selection histories in each relevant stress environment. For example, we asked whether the low-light selection history outperformed the control selection history in a low-light environment. The ANOVA models, tested separately for each current stress environment, included selection history (stress selection history from that same stress environment vs. the control selection history), replicate, and their interaction, with the fixed effect selection history tested over the interaction. As for the full analysis of fecundity, total seed weight was log transformed and weighted ANOVA was required to eliminate heteroscedasticity.

Responses of other phenotypic traits to stress.—The assumptions of MANOVA include multivariate normality, homogeneity of variances for each dependent variable, and equality of correlations among dependent variables. In the case of the six phenotyic traits other than fecundity, our dependent variables were nearly normally distributed and there was no evidence for heteroscedasticity. Examining the correlations among dependent variables for each current environment and selection history did not reveal any changes in sign. Together, these preliminary analyses suggest that the assumptions of MANOVA were not violated drastically. We determined significance in the MANOVA using Pillai's trace, which is most robust to departures from these assumptions when sample sizes are equal among groups (Bray and Maxwell 1985).

Opportunity for selection in different environments.—Extreme or novel environmental conditions may influence the potential for evolution (Johnson and Frey 1967; Holloway et al. 1990; Yampolskii 1992; Gavrilets and Scheiner 1993; Posthuma et al. 1993; Merila 1997; Hoffman and Schiffer 1998). One component of this potential is the opportunity for selection, which is defined as variation in relative fitness (Arnold and Wade 1984). To determine the effect of stress on opportunity for selection, we calculated the variance in relative fitness within each lineage based on eight plants, each from a different full-sib family belonging to a particular selection history within that replicate. Individual relative fitness was calculated as log-transformed seed weight, divided by the mean log seed weight in that lineage. Three-way ANOVA with current environment, selection history, and replicate as main effects and all two-way interactions indicated which factors influenced the variance in relative fitness. *F*-tests were constructed using the Scheffé model. To determine whether the opportunity for selection differed between novel environments and environments to which selected populations had been exposed previously, we conducted a second ANOVA with environment type (novel vs. previously experienced) as the main effect.

Environmental effects on phenotypic trait variances.—The response to selection depends on the extent to which variance in relative fitness is determined by genetically based variance in the phenotype. Because phenotypic variation is one component of evolution by natural selection, assessing the effects of stress on phenotypic variance can reveal whether stresses constrain or facilitate adaptive evolution. Without replication of family within environments, we were unable to compare genetic variance between stressful and near-optimal environments; however, our split-family design has the advantage of exposing similar genotypes to all environments. Much as for the analysis of opportunity for selection, we calculated variances for six phenotypic traits within each lineage in each environment (see previous section). As in previous phenotypic analyses, traits were untransfomed. Within-lineage variances for six traits were used as the outcome variables in a three-way MANOVA with current environment, selection history, and replicate as main effects. Following MANOVA, six one-way ANOVA models of the same structure were conducted for each trait. To determine whether stress regimes collectively altered phenotypic variances, we conducted a priori contrasts with respect to both current environmental treatment and selection history, in which the near-optimal control regime was compared to all five stresses pooled.

Phenotypic selection within each stress environment.—To determine what individual traits influenced relative fitness in each of the six environments, we used the standard multiple regression approach for analyzing phenotypic selection (Lande and Arnold 1983). The strength of selection can be described by two measures, the selection differential and the selection gradient, both of which predict the change in the mean of a trait between generations. The selection differential, which is calculated as the covariance between relative fitness and the phenotypic value, reflects the change in the mean due both to selection operating directly on a given trait and indirectly on phenotypically correlated traits. The selection gradient, the vector of partial regression coefficients for a number of phenotypic traits included in the same multiple regression model, estimates the change in the mean due only to direct selection acting on a particular phenotypic trait. Selection acting on phenotypically correlated traits is incorporated by including other traits in the regression model. Here, we are interested in using selection analysis to predict how traits will evolve in a given stress and then comparing our selection histories to see if traits have changed in a way that is consistent with predictions. Because we could not account for all phenotypically correlated traits, we used the selection differential for this purpose. Of course, evolutionary response to phenotypic selection also depends on the additive genetic variances and covariances of traits expressed within an environment. Given our experimental design, we are only able to make conservative estimates of cross-environment heritabilities.

For our analysis of phenotypic selection, we included six growth and phenology traits: plant height at week 2, rate of change in plant height between weeks 2 and 3, length of longest leaf at week 2, rate of leaf expansion between weeks 2 and 3, number of leaves at week 3, and the Julian date of first flower. We considered this set of traits because it allows us to explicitly evaluate whether adaptation to stress is conferred by small stature and/or lower growth rates, traits commonly inferred to promote stress tolerance, or by earlier flow-

ering, a trait often associated with avoidance of harsh conditions. All phenotypic traits were transformed to standard normal variables prior to these analyses. Relative fitness was calculated as total seed weight for each maternal plant, relative to mean seed weight for all plants in a given environment and replicate. In each environment we pooled individuals from all selection histories because fitness did not vary among selection histories within an environment (Table 1) and selection histories did not differ phenotypically within environments (Table 2).

Significance of selection differentials and gradients was determined by bootstrapping (Efron 1982; Dixon et al. 1987; Dixon 1993). The covariance between relative fitness and each trait (selection differentials) and regression coefficients from multiple regression models of six traits on relative fitness (selection gradients) were calculated for the original data and for an additional 999 datasets generated by randomly resampling families with replacement from the original data. Resampling families maintained a common genetic background in each stress environment. Ninety-five-percent confidence intervals were determined following the shift distribution method in which the distribution of resampled values is adjusted back to the original value by subtracting the overall mean and adding back the real value to each observation prior to calculating confidence intervals (Noreen 1989).

Responses to selection under stresses.—Three generations of artificial selection in each stress environment allowed us to test whether phenotypic changes matched those predicted by the phenotypic selection analysis. In the final test generation, we tested for phenotypic response in two ways. In the multivariate analysis of variation in six phenotypic traits already described, significant main effects of selection history and interactions involving selection history indicate significant phenotypic differentiation among selection regimes. In a second, more general approach for testing evolutionary responses to stress, we considered selection in all stress environments simultaneously. Using linear regression, we asked whether the phenotypic difference between control and stress lineages in a given stress (Y) was predicted by the selection differential in that stress environment (X) for all six traits. Considering the six traits and five stress environments together yields 30 samples for the analysis. The regression tests whether genetic changes in phenotype are consistent with the magnitude of selection on that trait in five stress environments, and the slope indicates the average rate of response across three generations of selection.

Results

Phenotypic Response to Environmental Stresses: Fecundity

A highly significant main effect of current environment indicated that, over all replicates, the experimental stress environments reduced individual female fecundity (Fig. 2, Table 1A). The significant preplanned contrast of the five stress environments to the control environment confirmed, as expected from preliminary studies, that reduction in fecundity is a general phenomenon observed across these diverse types of stress (Table 1B). However, the effect of current environment on fecundity varied significantly among selection histories and replicates, as indicated by the three-way interaction

TABLE 1. The effect of current environment, selection history, replicate, and family on individual fecundity. (A) The overall model includes all replicates, whereas subsequent analyses were done by replicate. (B) Results of preplanned contrasts between five pooled stresses and the control environment. Significant sources of variation, after sequential Bonferroni adjustment, are indicated in bold.

				Overall	By replicate							
					1	2	3	4	5	6	7	8
(A) Source of variation	df	MS	*F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*
Current environment	5	608.73	12.62	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Selection history	5	1.53	0.53	0.7547	**0.0014**	0.1088	**0.0039**	0.0167	**0.0007**	0.0816	**0.0001**	0.8874
Replicate	7	53.74	61.65	**0.0001**	—	—	—	—	—	—	—	—
Current × selection history	25	0.81	0.59	0.9381	**0.0035**	0.6230	**0.0005**	0.0358	**0.0001**	0.3887	**0.0035**	0.5607
Current environment × replicate	35	48.23	59.91	**0.0001**	—	—	—	—	—	—	—	—
Selection history × replicate	35	2.91	3.34	**0.0001**	—	—	—	—	—	—	—	—
Current × selection × replicate	175	1.37	1.71	**0.0001**	—	—	—	—	—	—	—	—
Family (replicate × selection)	336	0.87	1.08	0.1681	0.7524	0.0478	0.8814	0.1217	0.2837	0.0116	0.9999	0.2817
Error	1845											
(B) Preplanned contrasts												
Source of variation in fecundity	df	MS	*F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*
Current environment:												
Control vs. all stresses	1	2055.89	42.62	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Selection history:												
Control vs. all stresses	1	0.01	0.00	0.9532	0.1059	0.5166	0.9894	**0.0004**	0.4874	0.7620	**0.0015**	0.6153

TABLE 2. MANOVA for the effect of current environment, selection history, replicate, and family on six growth and phenology traits: height at week 2, relative rate of height growth (weeks 2–3), length of the longest leaf at week 2, relative rate of leaf expansion (weeks 2–3), leaf number in week 3, and Julian flowering date. (A) The overall model includes all replicates, whereas subsequent analyses were done by replicate. (B) Results of preplanned contrasts between five pooled stresses and the control environment. Significant sources of variation, after sequential Bonferroni adjustment, are indicated in bold.

		Overall	By replicate							
		MANOVA	1	2	3	4	5	6	7	8
(A) Source of variation	df	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*
Current environment	5	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Selection history	5	0.5423	**0.0097**	**0.0004**	0.1015	0.4949	**0.0042**	0.0376	0.0115	0.6878
Replicate	7	**0.0001**	—	—	—	—	—	—	—	—
Current × selection history	25	0.4080	0.8665	0.1902	**0.0072**	0.2722	0.0891	**0.0040**	**0.0007**	0.8123
Current environment × replicate	35	**0.0001**	—	—	—	—	—	—	—	—
Selection history × replicate	35	**0.0001**	—	—	—	—	—	—	—	—
Current × selection × replicate	175	**0.0003**	—	—	—	—	—	—	—	—
Family (replicate × selection history)	336	**0.0001**	**0.0012**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	0.0173	0.1758
Error	1875									

(B) Preplanned contrasts

		Overall	By replicate							
		MANOVA	1	2	3	4	5	6	7	8
Source of variation	df	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*	Pr > *F*
Current environment:										
Control vs. all stresses	1	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Selection history:										
Control vs. all stresses	1	0.2780	0.0123	0.0068	0.0598	0.7124	0.4555	0.0984	0.4951	0.4754

in the overall model (Table 1A). The unique temporal sequence of greenhouse environments experienced by each replicate generated remarkable variability in the phenotypic effects of current environment and in the evolved responses of selection histories. To explore the nature of these effects, we examined these responses in each replicate separately. In four replicates, only the current environment accounted for any of the observed variability in fecundity. In the other four replicates, individual female fecundity depended both on the current environment and on the selection history, as indicated by the significance of this interaction (Table 1A).

Effects of Selection History and Family on Fecundity

We found little evidence for genetically based variation in fecundity, associated either with selection history or family membership. Selection history had no impact on fecundity overall and its effects were inconsistent across replicates and current growth environments (Table 1B). Family membership had no significant effect on fecundity over the entire experiment or in any replicate, following the sequential Bonferroni adjustment. In contrast, the effect of replicate was highly significant. Taken together, these results suggest that variation in fecundity in this experiment was strongly influenced by environmental variation within and among replicates.

We were unable to detect adaptation to the five stress environments used in this experiment. Overall, the interaction between selection history and current environment was not significant, indicating that the effects of the six current environments on female fitness were not altered by three generations of diversifying selection (Table 1A). This interaction includes all combinations of selection history and current environment (a 6 × 6 design), whereas a test for adaptation to specific stresses involves a small subset of these combinations. In preplanned tests, we dissected the overall statistical model into five separate tests for adaptation to specific environmental stresses, asking whether plants selected under a given stress outperformed plants from the control selection history under that same stress regime. In no case did the effect of selection history on fecundity under stress approach statistical significance (P = 0.25–0.82 for the five stress regimes, for selection history tested over the replicate by selection history interaction). The difference between selected and control lineages for log-transformed seed weight ranged from 0.023 to 0.0061, and retrospective power analysis (SAS 1994) revealed that our sample sizes for these specific comparisons (n = 123–128) were much too low to detect significance (across five stresses, average power was 0.179).

Phenotypic Response to Environmental Stresses: Growth and Phenology

The results of MANOVA conducted on six individual traits indicate that overall growth and phenology were significantly influenced by the current environmental regime (Table 2A). Follow-up tests indicated that all traits were significantly influenced by the current environment (Table 3A; Table 4A). As for fecundity, the interaction between current environment and replicate was significant for all traits (Table 3A), suggesting that the consequences of experimental manipulations varied among positions in the greenhouse.

Preplanned contrasts between the current control environment and the pooled stress environments showed that environmental stresses, taken together, had several consistent effects on plant phenotype (Table 2B, Table 3B, Fig. 3A). Plants grown under the five stress treatments showed significantly decreased seedling height growth, leaf size, and leaf number, compared with plants in the near-optimal control

TABLE 3. The contribution of six traits to significant variation in overall growth and phenology, as determined by MANOVA (see Table 2). Tablewide significance is indicated in bold, based on the sequential Bonferroni adjustment for the six independent tests.

(A) Source of variation	df	Phenotype: Height	Height RGR	Leaf length	Leaf length RGR	Leaf number	Flowering date
Current environment	5	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Selection history	5	0.4918	0.7565	0.6959	0.4428	0.4099	0.1019
Replicate	7	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Current environment × selection history	25	0.9829	0.3771	0.0822	0.5702	0.4619	0.1717
Current environment × replicate	35	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Selection history × replicate	35	**0.0018**	**0.0001**	0.1068	0.1341	**0.0023**	**0.0019**
Current × selection × replicate	175	0.6104	0.1841	0.2325	0.0182	0.0995	**0.0049**
Family (replicate × selection history)	336	**0.0001**	**0.0001**	**0.0009**	0.9164	**0.0030**	**0.0001**
Error	1875						

(B) Preplanned contrasts

Source of variation	df	Height	Height RGR	Leaf length	Leaf length RGR	Leaf number	Flowering date
Current environment:							
Control vs. all stresses	1	0.0818	**0.0001**	**0.0001**	0.7740	**0.0001**	**0.0001**
Selection history:							
Control vs. all stresses	1	0.8342	0.2558	0.2120	0.6934	0.5021	**0.0077**

environment. On average, plants responded phenotypically to stress by flowering significantly later, although plants grown under the low-nutrient regime had the earliest flowering dates and plants grown under the low light regime had the latest flowering times (Table 4A).

Effects of Selection History and Family on Growth and Phenology

MANOVA also demonstrated that the effect of selection history on plant phenotype varied significantly among replicates and current environments (Table 2A). In three of eight replicates, selection history alone influenced phenotypic expression. In three different replicates, the phenotypic effects of selection history depended on the current environment, suggesting that lineages varied in their phenotypic plasticity in response to treatment conditions. For the full model, no single trait varied significantly among the six selection histories. Preplanned contrasts between the control selection regime and all stress selection histories combined showed that selection under environmental stress did not consistently alter the set of six phenotypic traits taken together (Table 2B). However, examining specific traits showed that plants from stress selection histories flowered consistently earlier than those from the control selection history (Table 3B; Fig. 3B; Table 4B).

Patterns of phenotypic variation among families support the hypothesis that genetic variation for plant traits is expressed, even when this species is exposed to a range of environments (Table 2A). In addition to the substantial effects of environmental treatments on phenotype, family identity accounted for a significant proportion of phenotypic variance in seedling height, relative rate of seedling height growth, length of the longest leaf, leaf number, and flowering date (Table 3A). Broad-sense heritability for these traits, which were estimated as the partial R^2 for the effect of family, ranged from 5.9% to 12.9%.

Phenotypic variance is a prerequisite for evolution by natural selection. Phenotypic variance overall and for three of the six growth and fecundity traits was strongly dependent on current environment (Table 5, Table 6). Although all five stresses considered together significantly increased phenotypic variation overall (Table 5B), this effect was highly variable among environments and for different traits. Phenotypic variance for leaf length (Table 6) was greatest in the two environments that suppressed fecundity the least, control and high boron (Fig. 2). Low-nutrient stress reduced phenotypic variance for most traits, but variance in seedling height was greatest under low-nutrient stress (Table 6). Variance in seedling leaf number was relatively insensitive to current environmental treatments. Variance in flowering date was most dramatically influenced by current environment, being least in the low-nutrient treatment and by far the greatest in the low-light treatment (Table 6). These results suggest that the ability of selection to discriminate among phenotypes is likely to depend on environmental conditions.

Opportunity for Selection in Control versus Stressful Environments

The environmental stress regimes used in our experiment strongly influenced the opportunity for selection, as measured by variation in relative fecundity within lineages (Table 7). Potential for selection was greatest in two stress environments (high salt and low light), lowest in control and low-nutrient environments, and intermediate in high-boron and low-water environments (Fig. 4). A preplanned contrast between the control environment and all five stress regimes demonstrated that, on average, the opportunity for selection was significantly greater under stresses than under near-optimal conditions for singly grown *S. arvensis* (Table 7B). Opportunity for selection was influenced both by current environment and by replicate, indicating that variation in relative fitness was greater in some replicates than in others. Furthermore, after three generations of selection, the opportunity for selection also varied among selection histories in a given stress environment, suggesting that artificial selection, inbreeding, and/or drift may have influenced fitness variation in some environments.

Lineages grown in novel environments did not show great-

TABLE 4. Growth and phenology traits expressed in: (A) six current growth environments, pooled across selection histories; and (B) plants from six environmental selection histories, pooled across current growth environments. Least-squares means for the main effects of current environment (A) and selection history (B) derived from the MANOVA in Table 2 are shown, along with standard errors in parentheses. $N = 2162$.

(A)

	Current environmental regime					
Trait	Control	High boron	High salt	Low water	Low light	Low nutrients
Height	3.546	3.492	2.802	2.953	5.660	4.735
	(0.192)	(0.194)	(0.198)	(0.205)	(0.254)	(0.191)
Height RGR	1.888	1.474	0.981	1.439	0.818	1.643
	(0.064)	(0.065)	(0.066)	(0.069)	(0.085)	(0.064)
Leaf length	6.034	6.079	4.466	3.928	4.379	3.279
	(0.165)	(0.167)	(0.170)	(0.176)	(0.218)	(0.165)
Leaf length RGR	0.278	0.438	0.212	0.273	0.539	−0.028
	(0.028)	(0.028)	(0.029)	(0.030)	(0.037)	(0.028)
Leaf number	4.081	3.984	3.776	3.923	2.890	3.874
	(0.069)	(0.070)	(0.072)	(0.074)	(0.092)	(0.069)
Julian flowering date	266.548	268.056	266.890	266.449	289.761	265.760
	(0.251)	(0.253)	(0.257)	(0.265)	(0.319)	(0.250)

(B)

	Selection history					
Trait	Control	High boron	High salt	Low water	Low light	Low nutrients
Height	3.887	3.823	3.907	3.899	3.674	3.999
	(0.117)	(0.125)	(0.118)	(0.116)	(0.116)	(0.113)
Height RGR	1.321	1.422	1.376	1.405	1.348	1.371
	(0.050)	(0.053)	(0.051)	(0.050)	(0.050)	(0.048)
Leaf length	4.761	4.676	4.648	4.682	4.661	4.736
	(0.057)	(0.061)	(0.058)	(0.057)	(0.057)	(0.055)
Leaf length RGR	0.280	0.288	0.300	0.297	0.264	0.282
	(0.013)	(0.014)	(0.013)	(0.013)	(0.013)	(0.013)
Leaf number	3.722	3.718	3.734	3.782	3.720	3.853
	(0.054)	(0.057)	(0.054)	(0.053)	(0.053)	(0.052)
Julian flowering date	271.698	270.878	270.354	270.190	270.102	270.241
	(0.434)	(0.462)	(0.438)	(0.431)	(0.429)	(0.418)

er opportunity for selection compared with lineages experiencing environments in which they had evolved. When selection history-by-environment combinations were coded as either ''previously experienced'' or ''novel,'' the effect of environmental novelty on the opportunity for selection was not statistically significant ($F_{1,286} = 0.31$, $P > 0.576$).

Phenotypic Selection within Each Stress Environment

Direct selection under stress, as estimated by linear selection gradients, consistently favored early flowering. The selection gradient on flowering time was significant or very nearly significant in all five stress environments (Fig. 5). The magnitude of direct selection on flowering time varied among stresses; it was the strongest in low-light and weakest in the low-nutrient environment. Rapid early height growth was directly favored in three stress environments: high boron, low water, and low nutrients. Statistically significant selection on other traits was less consistent across environmental stresses. For example, greater leaf expansion rate (leaf RGR) was favored in high-salt and low-water treatments, whereas larger leaves were favored in low-water and low-nutrient environments. These same two traits, larger leaves and rapid leaf expansion, were also favored in the control environment. The low-water environment exerted direct selection on all traits except the number of leaves. The multiple regression model estimating linear selection gradients explained 19.6% of the variance in plant fecundity in the low-water treatment. In other environments, where fewer traits influenced fecundity, R^2 values ranged from 3.8% to 6.7%, suggesting that unmeasured traits strongly influenced plant fitness in this experiment. Nonlinear multiple regression models that included quadratic terms and cross-trait products produced little evidence of nonlinear selection in any environment.

Response to selection predicted by the selection differential will depend on the magnitude of selection acting directly on particular traits, as well as selection acting indirectly on phenotypically correlated traits. In the near-optimal control environment, no evolutionary changes are predicted in the six measured traits because none of the selection differentials were different from zero (Fig. 6). Although there was direct selection favoring larger leaves and greater rates of leaf expansion in the control environment (Fig. 5: linear gradients), the strongly negative phenotypic correlation between these traits eliminates the direct effects of selection. In all stress environments, rapid height growth (height RGR) and early flowering are expected to evolve (Fig. 6). The negative phenotypic correlation between these two traits reinforces patterns of direct selection (Fig. 5). Increases in leaf length are expected in the low-water and low-nutrient environments and increases in leaf expansion are expected in high-salt environments. In general, plants with accelerated development (rapid height growth, rapid leaf expansion, and early flow-

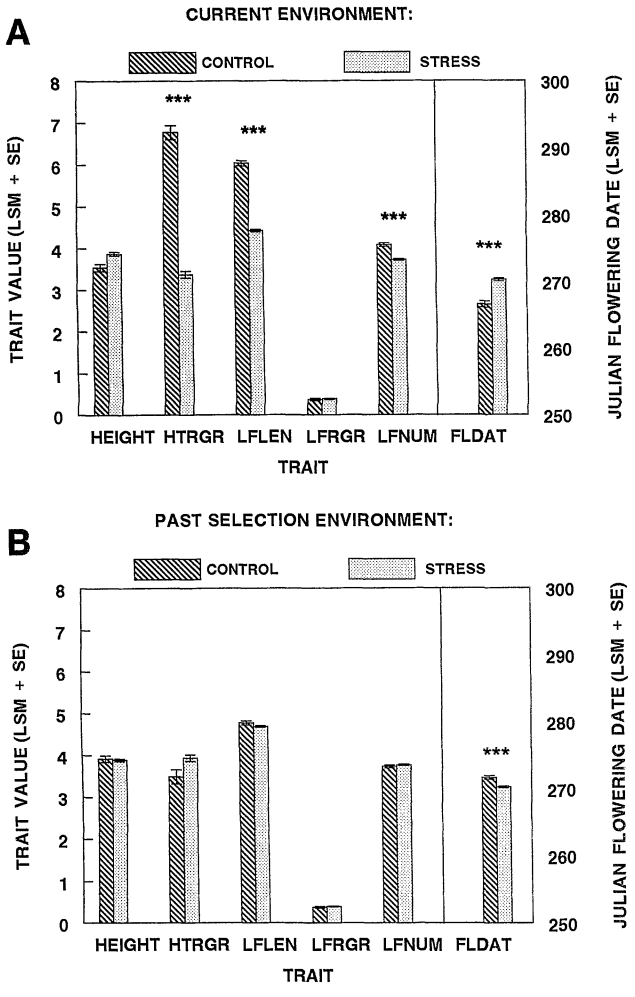

FIG. 3. Effects of environmental stresses on growth and phenology. (A) Phenotypic responses to the control environments, compared to the five pooled stress environments. (B) Evolutionary responses to the control selection history, compared to the five pooled stress selection histories. Least-square means for the main effects of current environment (A) and past selection environment (B) from the MANOVA model in Table 2 are shown for each trait. Significant contrasts between control and the pooled stresses are shown as follows: *** $P < 0.0001$; * $P < 0.05$. Trait abbreviations are as follows: HEIGHT, height in cm on 13 September; HTRGR, relative rate of height growth, 13–20 September; LFLEN, length of longest leaf on 13 September; LFRGR, relative rate of increase in leaf length, 13–20 September; LFNUM, number of expanded leaves on 13 September; FLOWDAT, Julian date of the first flower.

ering) appear to perform best in stressful environments. In some environments individual size also appears to be important, as reflected by positive selection differentials for height and leaf length in low-water and low-nutrient environments.

Evolved Responses to Artificial Selection in Each Stress Environment

Regression analysis of variation in phenotype across all stress environments provides striking evidence of evolved phenotypic changes between stress and control selection histories. For each of the six traits, the selection differential observed in the test generation within a given stress regime closely predicts the mean divergence in that trait between selected and control lineages over three generations of selection (Fig. 7). The least-squares linear regression between trait divergence (trait value in the stress-selected minus the control lineages) and selection differential in each of five stress regimes has a slope of nearly one ($Y = 1.135X - 0.064$, $F_{1,28} = 27.839$, $P < 0.0001$). This analysis makes it clear that selection for earlier flowering resulted in all stress selection histories flowering earlier than control selection histories. Selection for more rapid height growth was associated with an increase in this trait in the high-boron, high-salt, low-water, and low-light stress selection histories. Weak phenotypic selection on seedling height resulted in no character change for most stress selection regimes. In contrast, selection under low light was predicted to reduce seedling height, and this response was observed. Variation in seedling leaf length among selection histories was not well predicted by selection differentials. This may be due to low levels of genetic variation in this trait, the only one for which variation among full-sib families was not statistically significant in the test generation (Table 3).

DISCUSSION

Although adaptation to environmental stress plays a key role in some models of life- history evolution, it has been difficult to make generalizations about the evolutionary impact of stresses based on currently published experiments (reviewed in Hoffman and Parsons 1991). Two factors make it especially difficult to compare studies that have used different species, experimental protocols, and types of stress. First, the physiological effects of stress and modes of adaptation to stress may change dramatically with the levels of the stress factor to which populations are exposed (e.g., Ernst 1993; Blum 1994). Compared with mild stresses, intense selection under severe stress may involve fewer genes of large effect (Lande 1983), occur via ''hard'' rather than ''soft'' selection (Wallace 1981; Brown 1984), and result in the evolution of specialist rather than plastic phenotypes (Abrams 1994; Van Tienderen 1997). Second, the potential responses to stress are likely to be dictated by the life history and genetic background of the study population (e.g., Chapin and Shaver 1985; Bradshaw and McNeilly 1991; Chapin et al. 1993). Finally, the effects of abiotic stresses per se are likely to be confounded with the effects of competition and/or natural enemies in field studies (McGraw and Chapin 1989; Campbell et al. 1991; Grubb 1992; Oksanen 1993; Turkington et al. 1993).

In the highly controlled experiment described here, we addressed some of the limitations of previous studies to rigorously examine some generalizations that have been made about the phenotypic and genetic impacts of environmental stresses on plant life-history traits. Rather than comparing different ecotypes or species characteristic of contrasting habitats, we exposed random samples of a single, naturalized population of wild mustard to contrasting stress selection regimes, each of which differed from the near-optimal control environment by a single factor. Instead of focusing on one or two types of environmental stress, lineages experienced

TABLE 5. MANOVA for the effect of current environment, selection history, and replicate for the within-lineage phenotypic variance of six growth and phenology traits (also see Table 2). Phenotypic variance was measured for 288 lineages, consisting of eight plants from the same selection history and replicate, grown in a single environment. (A) The overall MANOVA includes all traits, whereas subsequent analyses were done separately for each trait. (B) Results of preplanned contrasts between five pooled stresses and the control environment. Tablewide significance for all sources of variation are indicated in bold. For univariate models, we used the sequential Bonferroni adjustment to account for the six independent tests.

Within-lineage phenotypic variance	df	MANOVA: all traits	Height	Height RGR	Leaf length	Leaf length RGR	Leaf number	Flowering date
(A) Source of variation								
Current environment	5	**0.0001**	**0.0001**	0.0776	**0.0072**	0.1929	0.5190	**0.0001**
Selection history	5	0.1769	0.8404	0.0795	0.4152	0.6066	0.3534	0.2489
Replicate	7	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0062**	**0.0001**
Current environment × selection history	25	0.1725	0.8995	0.5171	0.5329	0.5360	0.3770	**0.0046**
Current environment × replicate	35	**0.0001**	**0.0002**	**0.0001**	**0.0001**	**0.0001**	0.2542	**0.0001**
Selection history × replicate	35	**0.0016**	**0.0025**	0.0344	0.0130	0.4127	0.0920	0.1301
(B) Preplanned contrasts								
Current environment:								
Control vs. all stresses	1	**0.0078**	0.1490	0.3553	0.0487	0.9793	0.5628	0.1861
Selection history:								
Control vs. all stresses	1	0.0735	0.3577	0.0415	0.8692	0.2251	0.3219	0.0149

one of five different stresses, including both abiotic deficiencies and excesses. To standardize the intensity of stress experienced by different selection lineages, we made an effort to choose levels of stress that severely reduced fecundity, but had minimal impact on mortality. This effort was largely successful, although plants achieved greater fitness in the high-boron treatment than in other stress treatments. Finally, we minimized the potentially confounding effects of competition and natural enemies by growing plants individually within containers spaced along a greenhouse bench. The admittedly artificial experimental conditions we used allowed us to identify, unambiguously, the phenotypic and selective effects of environmental stresses on our study population. In companion studies, we examined the effects of stress on leaf hair density (Roy et al. 1999) and fluctuating asymmetry (Roy and Stanton 1999). Ongoing experiments are investigating ways in which competition and natural enemies interact with abiotic stress in determining the fitnesses and phenotypes of individual plants (M. L. Stanton, D. A. Thiede, and B. A. Roy, unpubl. data; B. A. Roy, T. Steinger, and M. L. Stanton, unpubl. data).

Phenotypic Effects of Environmental Stresses on Phenology

It is commonly assumed that weedy or ruderal species respond to stress by flowering earlier (Grime 1993). However, the phenotypic response we observed to the five environmental stresses used in this study did not strictly conform to this prediction. As anticipated based on our experimental trials, the five stress regimes consistently decreased fecundity (Fig. 2). On average, plant size and relative growth rate (Fig. 3A) also decreased, as expected under stressful conditions. However, the effects of different stresses on phenology were highly variable. Plants exposed to low-nutrient stress flowered earliest, those exposed to low light flowered latest, and overall, stresses delayed flowering compared to the control environment (Fig. 3A). Other ruderal plants also show a range of phenological responses to abiotic stress (Aronson et al. 1992; Kelly 1992; Andersson and Shaw 1994; Zhang and Lechowicz 1994; Pigliucci et al. 1995b; Steyn et al. 1996; Van Tienderen et al. 1996; Pigliucci and Schlichting 1998), suggesting that stress avoidance through accelerated reproduction may not be a general, plastic response to poor environmental conditions in such species.

TABLE 6. Effects of the current growth environment on phenotypic variance within lineages. Each cell of the table shows the least squares mean of a trait's within-lineage variance, for the main effect of current environment from the MANOVA in Table 5, along with the standard errors in parentheses. $N = 288$ lineages.

Trait	Current growth environment					
	Control	High boron	High salt	Low water	Low light	Low nutrients
Height	1.450	1.433	0.887	1.199	2.305	3.558
	(0.263)	(0.263)	(0.263)	(0.263)	(0.268)	(0.263)
Height RGR	0.313	0.362	0.305	0.294	0.091	0.133
	(0.074)	(0.074)	(0.074)	(0.074)	(0.075)	(0.074)
Leaf length	1.044	1.149	0.477	0.520	0.826	0.282
	(0.176)	(0.176)	(0.176)	(0.176)	(0.179)	(0.176)
Leaf length RGR	0.052	0.102	0.025	0.042	0.069	0.020
	(0.024)	(0.024)	(0.024)	(0.024)	(0.025)	(0.024)
Leaf number	0.409	0.495	0.363	0.422	0.443	0.521
	(0.063)	(0.063)	(0.063)	(0.063)	(0.063)	(0.063)
Julian flowering date	11.966	13.763	12.159	9.798	143.405	2.294
	(16.450)	(16.450)	(16.450)	(16.450)	(16.729)	(16.450)

TABLE 7. Effects of current environment, selection history, and replicate on the opportunity for selection. Opportunity for selection is estimated as the variance in relative fitness within a lineage.

Selection opportunity	df	MS	F	Pr > F
(A) Sources of variation				
Current environment	5	60.869	8.22	**0.0001**
Selection history	5	1.685	1.52	0.2087
Replicate	7	2.785	3.22	**0.0031**
Current environment × selection history	25	1.558	1.80	**0.0154**
Current environment × replicate	35	7.402	8.56	**0.0001**
Selection history × replicate	35	1.108	1.28	0.1523
Error	175			
(B) Preplanned contrasts				
Current environment:				
Control vs. all stresses	1	44.715	6.04	**0.0191**
Selection history:				
Control vs. all stresses	1	3.449	3.16	0.0843

Effects of Environmental Stress on Variation in Relative Fitness and Phenotype

The debate over the evolutionary consequences of environmental stress has focused on the effects of stress on trait variation and heritability, rather than on variation in relative fitness (reviewed in Hoffman and Parsons 1991; Travisano and Lenski 1996; but see Hoffman and Schiffer 1998). In some cases, stress has been shown to decrease trait heritability, whereas the opposite pattern has been observed in other traits or study species (e.g., Richards 1978; Bull et al. 1982; Blum 1988; Yampolskii 1992; Kawecki 1995; Hoffman and Schiffer 1998). Environmental stresses can influence evolutionary potential by affecting the varience in relative fitness and variation in phenotype. However, the evolutionary response to stress depends on the presence of genetically based phenotypic variation that is associated with variation in fitness.

For the weedy mustard *Sinapis arvensis,* we found that the opportunity for selection under most (but not all) environmental stresses was greater than that in the near-optimal control environment. That is, variation in relative fitness among plants from a similar genetic background was, on average, enhanced by unfavorable growth conditions (Fig. 4B). We emphasize that this pattern was observed for singly grown plants experiencing minimal competition. In dense field populations, asymmetric competition may greatly en-

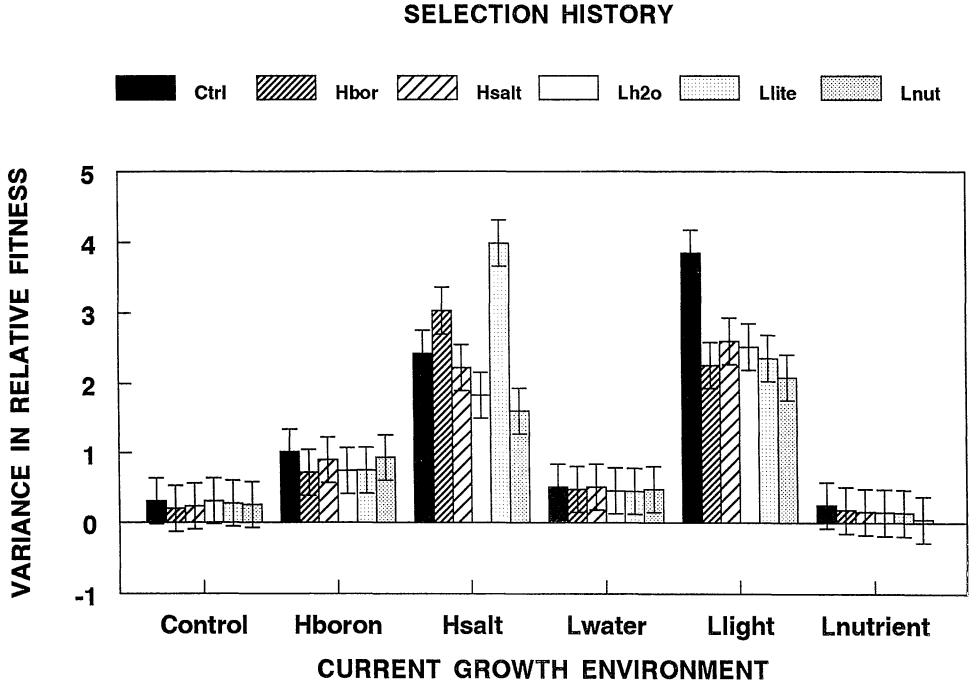

FIG. 4. Opportunity for selection in six environmental growth regimes, as estimated by the variance in fecundity within lineages. The figure illustrates the significant interaction between current environment and selection history. Least-square means and standard errors are shown for relative fitness (individual plant fecundity, relative to mean fecundity within each current environment and replicate). N = 48 for each bar in the histogram.

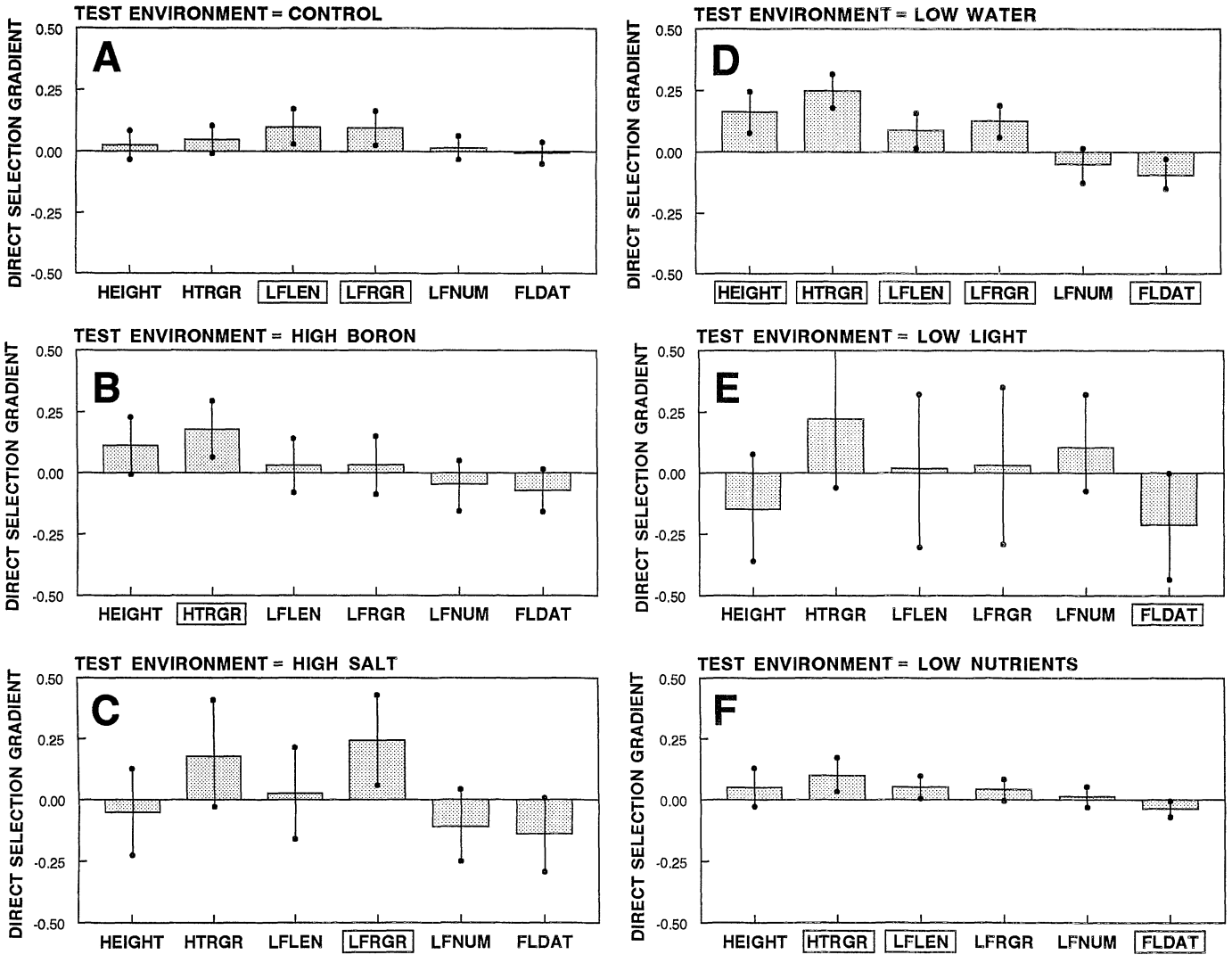

FIG. 5. The magnitude of direct selection acting on growth and phenology traits in the control (A) and five stress environments (B–F). Linear selection gradients and 95% bootstrapped confidence intervals were derived from separate multiple regression models for each current environment. Sample sizes for each model ranged from 279 to 382. Boxed traits (below the x-axis) show selection gradients that are significantly different from zero at $P < 0.05$.

hance variation in relative fitness, even in fertile environments (Obeid et al. 1967; Weiner 1990).

It may be that harsh conditions per se increase variation in fitness among individuals. Alternatively, stress regimes used in this experiment may have increased fitness variation among individuals because these represent novel environments to which the study population had rarely been exposed (Hartl and Dykhuizen 1981; Holloway et al. 1990). Our results support the first of these possibilities, in that we found no significant effect of environmental novelty on the opportunity for selection. Variance in relative fitness among *S. arvensis* plants was increased under stress, but was statistically independent of whether a given subpopulation had been selected in the same environment or in a different environment. Previous experimental studies using laboratory-selected organisms have shown that genetic variation for traits or fitness is enhanced in novel environments (Holloway et al. 1990; Silva and Dykhuizen 1993; Travisano and Lenski 1996), but interpreting these studies is problematic because novel environments are also likely to impose stress on experimental populations. In an experiment designed to resolve this ambiguity, Kawecki (1995) found that additive genetic variation for most traits in cowpea weevil was increased in low-quality environments, independent of environmental novelty. Our analysis lends support to his finding, but because our lineages of *S. arvensis* had been exposed to only three generations of diversifying selection, ours is a less powerful test. Further studies are needed to distinguish the impacts of environmental quality and environmental novelty on evolutionary potential.

Although we found that overall phenotypic variance was increased by stresses, on average, compared with the favorable control environment (Table 5), closer inspection of the data revealed great variation in this pattern among stress

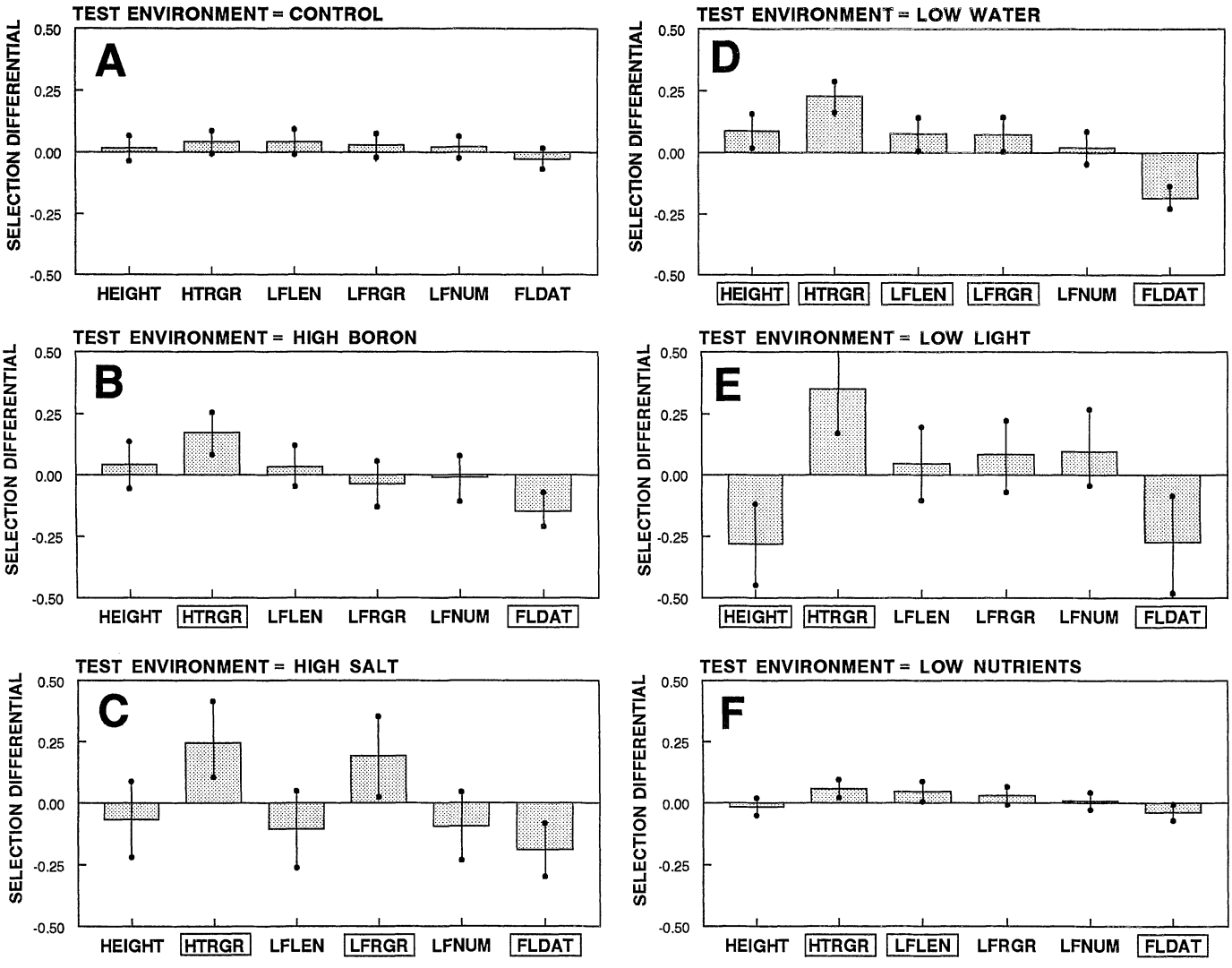

FIG. 6. The magnitude of selection acting both directly and indirectly on growth and phenology traits in the control (A) and five stress environments (B–F). Selection differentials and 95% bootstrapped confidence intervals are shown; boxed traits (below the x-axis) show selection differentials that are significantly different from zero at $P < 0.05$. Sample sizes for covariance calculations ranged from 279 to 382.

regimes and among traits (also see Pigliucci et al. 1995a). For *S. arvensis,* low-nutrient stress tended to reduce phenotypic variance in most traits, but increased phenotypic variance in seedling height. Variance in flowering time was 60 times greater in low-light stress than under low-nutrient stress, but low light tended to reduce phenotypic variance in the rate of seedling height growth. Our results corroborate previous experimental studies in which variation in different traits responded idiosyncratically rather than consistently to unfavorable environmental conditions (e.g., Johnson and Frey 1967; Bennington 1996; Hoffman and Schiffer 1998). It may prove difficult to make generalizations about the effects of environmental stresses on phenotypic variation. However, our data imply that the magnifying effect of stresses on the opportunity for selection need not be counteracted by reduced variation in growth and phenology.

Patterns of Phenotypic Selection under Environmental Stress

To test adaptive hypotheses, it is critically important to manipulate putative agents of selection (Wade and Kalisz 1990; Dudley 1996). For *S. arvensis,* we found that patterns of direct selection on some phenotypic traits were consistent across treatments, whereas others differed between stress and control regimes. The direct selection gradients for traits related to size and growth rate, when significant, were positive in both control and stress treatments. This finding contradicts a basic prediction of Grime's triangle model (Grime 1977) and some other models of adversity selection (Odum and Pinkerton 1955; Sibly and Grime 1986; Iwasa and Cohen 1989; Poorter 1990; Arendt and Wilson 1997), namely, that abiotic stress will select for metabolic conservatism. Results

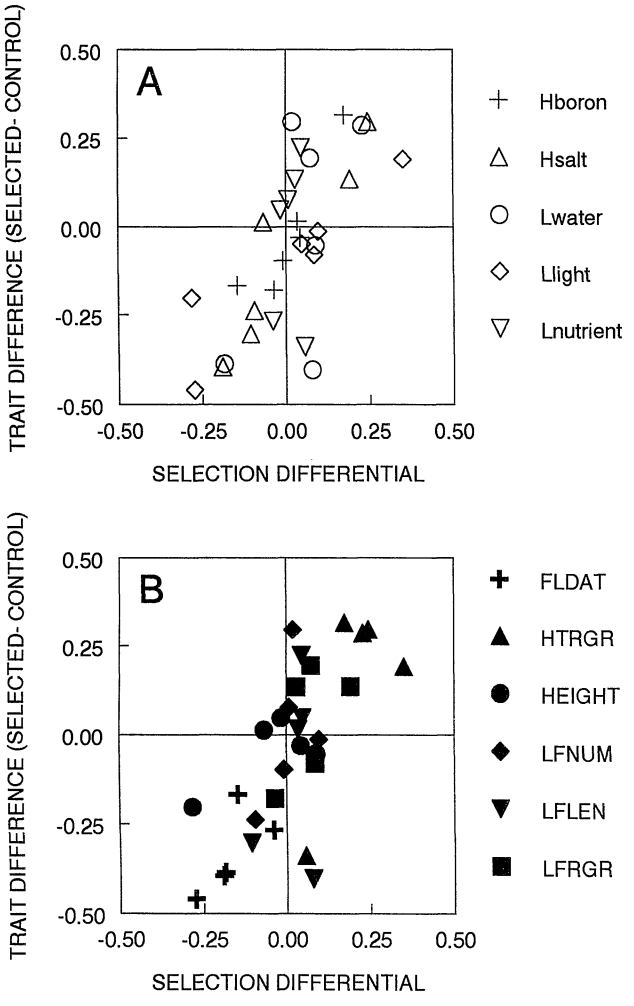

FIG. 7. The relationship between evolutionary response and phenotypic selection. Least-squares linear regression was used to test for the relationship between the phenotypic difference between stress and control selection histories (Y), the evolutionary response, and the selection differential on a given trait (X) for each of the growth and phenology traits in five stress environments. In (A) different symbols are used to indicate relationships observed in the five current stress environments. In (B) different symbols represent each of the six measured traits. See legend of Figures 2 and 3 for treatment and trait abbreviations, respectively.

from previous selection experiments have been mixed; some have found that stress tolerance is inversely related to growth or metabolic rate (McNab 1986; Service 1987; Hoffman and Parsons 1989), whereas others have not (Djawdan et al. 1997). In contrast to quite consistent selection for larger size and/or higher growth rates in our study, direct selection on flowering date contrasted markedly between stress and control environments. Direct selection on flowering time was negligible in the control environment, but selection for earlier flowering was significant or nearly significant in all five stress treatments (Fig. 5). Moreover, correlations between life-history traits in stress environments reinforced direct selection for rapid height growth and early flowering (Fig. 6). Although stresses were applied uniformly to experimental plants throughout their lifetime, our results suggest that direct selection under environmental stress favors early flowering, a stress avoidance strategy, in this ruderal species. Phenotypic selection for early flowering may represent counter-gradient selection (Berven and Gill 1983) to compensate for the observed phenotypic delay in flowering under stress. In low light, where flowering was most delayed, we observed the strongest selection for early flowering and the greatest evolutionary response (Fig. 7).

Evolutionary Responses to Environmental Stress

In stress environments consistent selection in favor of rapid early height growth and early flowering, as well as increased opportunity for selection, worked together to generate a significant phenotypic shift toward early flowering (Table 3; Fig. 7) and a tendency toward more rapid early height growth (Fig. 7) in lineages selected under stress. This response to selection is consistent with the evolution of stress avoidance rather than stress tolerance. Accelerated development may not be an adaptation unique to stressful environments. Artificial selection on another ruderal mustard under intense competition also resulted in more rapid growth and earlier flowering time (Miller 1995). Taken together, these studies would seem to conflict with Grime's triangle model (Grime 1977), which suggests that competition and stress should impose divergent selection pressures on life-history traits. However, no single study has yet made a direct comparison of patterns of selection in stressful and competitive environments.

We were unable to demonstrate that this phenotypic response to our selection regimes was adaptive, in that the fecundity of stress-selected lineages was not significantly greater than that of control lineages when grown under stress. This may be due to three factors. First, our experimental design had limited statistical power in testing for environment-specific adaptation (via five pairwise comparisons) within the context of the 6 × 6 factorial experiment. Second, genetic responses to selection under stress may have been generated, in part, by trait-associated variation in male fertility because our selection regime allowed stresses to generate variation in both fecundity (female fitness) and paternity (male fitness). More likely, the absence of an evolutionary response in fecundity may be explained by low levels of genetically based variation in this fitness component (Table 1). Comparative studies have shown that life-history traits have lower heritabilities (Mousseau and Roff 1987; Roff and Mousseau 1987) and more negative intertrait genetic correlations (Roff 1996) than traits less closely related to fitness. In addition, Price and Schluter (1991) have argued that fitness-related traits are subject to greater environmental variation. Given these limitations on evolutionary potential, three generations of selection in our experiment may not have been sufficient to generate a significant response in fecundity.

Alternative Effects of Stress on Life-History Evolution

Life-history evolution under severe environmental stress may proceed along two very different trajectories, depending on the nature of the population under selection. For long-lived perennials, selection under environmental stress may lead to a metabolically more conservative phenotype characterized by reduced maximal growth rate, increased allo-

cation to storage, and delayed reproduction, as envisioned by the triangle model (Grime 1977). Many perennial plant species or ecotypes characteristic of stressful environments have slower maximal growth rates than related taxa adapted to fertile environments (Clarkson 1967; Grime and Hunt 1975; Davies and Snaydon 1976; Veerkamp et al. 1980; Chapin et al. 1982; but see Shaw 1988; Wilson 1988; McGraw and Chapin 1989; Rice and Bazzaz 1989; Poorter and Remkes 1990; Chapin and Shaver 1996; Walters and Reich 1996). In contrast, when stress imposes selection on shorter-lived pioneer species, a stress avoidance strategy may evolve. As we observed in *S. arvensis,* selection on opportunistic species under stress can lead to the evolution of increased progenesis, rather than to the delayed reproduction of classic stress tolerators. In annual plants, marginal or unfavorable habitats are often occupied by faster-developing species or ecotypes (e.g., Ritland and Jain 1984; Wyatt 1986; Rice and Mack 1991a; Aronson et al. 1992). Similarly, breeding crop varieties for high yield under environmental stresses may result in accelerated flowering and early height growth, rather than in delayed flowering and metabolic conservatism (Cregan and Busch 1978; Blum 1988).

Environments that are both highly disturbed and highly stressful may not be too extreme for successful adaptation, as Grime (1977; 1979) has suggested. In fact, alternative life-history models recognize the possibility that severe environmental stress and disturbance may act in concert to select for unique life-history attributes (Greenslade 1983; Loehle 1988; Southwood 1988; Taylor et al. 1990). Our analysis suggests that adaptation to stress in disturbed habitats dominated by ruderal species may proceed differently than in less-disturbed habitats. Evolution of stress tolerance or stress avoidance in response to stress could be driven by the contrasting life histories of affected species, distinct responses to different stresses, and differences in the selective regime imposed by the interaction of stress and disturbance.

Although our results suggest that a population's response to selection under environmental stress may depend on its life history, experiments based on a single model system cannot determine whether the evolutionary consequences of stress differ between long-lived and annual lineages. If it were possible to do multigeneration selection experiments on long-lived perennials, the effects of life history on response to selection under stress could be measured directly. Alternatively, phenotypic selection and/or response to selection under imposed stress could be compared in pairs of sister taxa with contrasting life histories. A third comparative approach for disentangling the effects of life history and environmental stress would be to contrast traits associated with tolerance or avoidance in many pairs of sister taxa occupying habitats of contrasting fertility. Such phylogenetically independent contrasts (Felsenstein 1985) could show that stress-avoidance traits are favored in annual lineages, whereas stress-tolerance traits are favored in perennial lineages.

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