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Source: *International Journal of Plant Sciences*, Vol. 174, No. 6 (July/August 2013), pp. 877-885
Published by: The University of Chicago Press
Stable URL: http://www.jstor.org/stable/10.1086/670366
Accessed: 09/07/2013 11:42

Int. J. Plant Sci. 174(6):877–885. 2013.

1058-5893/2013/17406-0003$15.00 DOI: 10.1086/670366

EFFECTS OF EARLY-LIFE ENVIRONMENT ON PHENOTYPE AND SELECTION IN *AGROSTEMMA GITHAGO*

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Premise of research. This study demonstrates that carryover effects from differences in early-life environmental conditions influence patterns of mortality, natural selection, and late-life phenotype.

Methodology. Using the annual species *Agrostemma githago*, an experimental design was established to create different early-life environments and a common late-life environment. Genotypes from a paternal cross design were replicated across three environmental treatments and two seasons, for a total of six different early-life environments, and then they all shared a common late-life environment.

Pivotal results. ANOVA revealed significant G × E interactions for size within early environment treatments. Effects of early-life environment were observed throughout the life cycle, and these effects extended to late life stages when all individuals were living in the common environment. Variation in phenotypic selection was also influenced by early-life environment, and this variation was caused by viability selection and a mismatch between early- and late-life environments.

Conclusions. Plastic responses to early-life environment explained differences in adult phenotype and determined the outcome of selection and survival in a common late-life environment.

Keywords: plasticity, selection, early environment, selective mortality, *Agrostemma githago*.

Introduction

An individual's phenotype and performance are determined by both genetics and environmental conditions, yet the life stage or age class of an individual when it is exposed to a particular environment may determine the influence of that environment on the phenotype (Weinig 2000; Donohue 2002). For example, environmental stress or disturbance imposed at different points in the life cycle can elicit different short-term responses in individuals (Ehrlen 2003; Juliano et al. 2004; Solberg et al. 2004; Marshall 2005; Jensen et al. 2006; Brody et al. 2007). The adult phenotype is a cumulative outcome of environmental effects across all life stages, and this may mask any life stage by environment interaction that occurred early in the life cycle (West-Eberhard 2003; Verdu and Traveset 2005). As a consequence, the effect of early environment by itself is poorly understood (Monaghan 2008).

Environmental conditions at early life stages can produce long-term effects in adult populations through either plastic responses or selective mortality. Plastic responses often have associated costs related to physiology (Nielsen and Nelson 1998), phenology (Galloway and Etterson 2007), or energetic resources (Marshall 2005). The consequences of these costs may be carried across life stages, allowing early-life environmental conditions to indirectly affect adult phenotypes and performance. Life stage by environment interactions may also be important in determining the capacity for and nature of a plastic response. For example, defoliation early in the life cycle of *Sesbania macrocarpa* resulted in a reduction in total reproduction, while defoliation at late life stages induced an overcompensatory response, increasing total reproduction (Marshall 2005). In soybeans, drought variously affected flower number, seeds per pod, and seed weight as a consequence of when the drought was imposed (Desclaux et al. 2000). This suggests that the timing of disturbance or stress is crucial in predicting the effects on an individual's phenotype and patterns of resource allocation.

Life stage by environment interactions may also influence selective events early in the life cycle and can affect adult populations by altering either the distribution of phenotypes in the surviving population or the relative frequency of genotypes (Lynch and Arnold 1988; Bennington and McGraw 1995; Sinervo and McAdam 2008; Mojica and Kelly 2010). A change in the phenotypic distribution results when a selective episode removes a subset of phenotypes from the population, resulting in a shift in the mean or variance of one or more traits. A change in genotypic frequencies is a consequence of differential survival among families, resulting in a change in the genetic structure of a population. If an early-life trait is correlated with a trait expressed at later life stages, selective mortality on the early trait will affect the distribution of the later-life trait (Stratton 1992). Bennington and McGraw (1995) examined the effect of size-dependent selective mortality by exposing two replicate populations to contrasting artificial selection regimes for seedling size. As a consequence of selective mortality at the seedling stage, they found that the two populations differed in their trait correlations, growth patterns, and selection gradients for late-life traits.

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Manuscript received July 2012; revised manuscript received February 2013; electronically published May 31, 2013.

To better examine the role played by early-life environment in trait distributions and patterns of selection, an experimental design that manipulates early-life environment while maintaining a common late-life environment is needed. One expectation would be that individuals exposed to a stressful (e.g., field) environment early in life might experience stronger selection for large seedling size, compared with individuals grown under benign (e.g., greenhouse) conditions during the same period. As a result of different early-life environments, selection at the late life stage may be very different in these two adult populations, despite the fact that they share a common late-life environment. This study is designed to determine the effects of early-life environment on trait distribution and selection at later life stages in a population of *Agrostemma githago*. Specifically, it addresses the following questions: (1) Do differences in early-life environment alter phenotype at later life stages? (2) How do early-life environments influence mortality through to reproduction? (3) Does selection vary for the same trait, size, between life stages for individuals experiencing different early-life environments?

Methods

Study Species

Agrostemma githago (Caryophyllaceae) is a small (30–120 cm high) annual herb that blooms in late summer. Native to Eurasia, *A. githago* has achieved a global distribution and can be found on all continents except Antarctica. It is a rare invader of winter grain crops and is usually found in waste spaces (Darbyshire 2003). Each plant produces ~200 seeds, 2–5 mm in diameter (Miller 2003), that mature 1 mo after fertilization (Firbank 1988). If given sufficient moisture, seeds will germinate immediately in the soil (Thompson 1973). Occasionally, seeds produced late in the season or during a dry year will germinate the following spring (Svensson and Wigren 1983), but seeds have little or no innate dormancy in the soil (Holzner and Numata 1982). After germinating in the fall, plants overwinter as a rosette and begin to gain height in early summer (Firbank 1988). Spring-germinating plants complete their life cycle in one summer. In natural populations, blooming is synchronous for both fall- and spring-germinated plants (Thompson 1973).

Genetic and Experimental Design

Seeds for this experiment were generated from a paternal half-sib crossing design. A total of 150 parental plants were randomly chosen from a natural population of >1000 *A. githago* plants near Newport, Virginia. These parental plants were transplanted from the roadside to 4-in pots in the greenhouse at Mountain Lake Biological Station (Giles County, VA) in mid-May, and crosses were done over 6 wk from July to mid-August. Plants were randomly assigned to function as either sire or dam and assigned to one of 35 nested paternal groups, each containing 3 dams. Dams were emasculated in the morning prior to anther dehiscence and pollinated by hand. Three paternal families failed to produce viable seed by more than one dam and were discarded. A total of 1802 seeds from the remaining 32 paternal families were collected, weighed, and evenly assigned to a season and an early environment treatment. Season refers to the season of germination, either fall germinants (FGs; 924 seeds) or spring germinants (SGs; 878 seeds). Within each season, seeds were further assigned to an early-life environmental treatment. Sample sizes for each season and treatment are given in table 1. Individuals assigned to early environment 1 (EE1) were planted directly into the field as seeds. Individuals assigned to early environment 2 (EE2) were germinated in the greenhouse environment and transplanted to the field at the early rosette stage, when plants had 4–6 leaves and were ~3 wk old. Individuals assigned to early environment 3 (EE3) were germinated in the greenhouse environment at the same time as EE2 and transplanted to the field at the late rosette stage, when plants had 10–14 leaves and were ~18 wk old for fall transplants and 7 wk old for spring transplants (fig. 1).

Table 1

Initial Number of Seeds Planted in Each Season and Early Environment (EE) Treatment

	Treatment			
Season	EE1	EE2	EE3	Total
Fall germinants	310	303	311	924
Spring germinants	303	272	303	878
Total	613	575	614	1802

Field Methods

The field portion of this experiment took place in a pasture less than 1 km from the parental population. Prior to planting the seeds in fall 2006, the area was mown to the ground, leaving root systems and meristems of the plant community intact. No further clipping or mowing was performed prior to planting or transplanting in the fall or spring. The mown area was divided into three spatial blocks (1 m × 7 m). Each block received two randomly chosen full-sib seeds, for a total of 18 half-sibs (2 seeds per dam × 3 dams per sire) per treatment-season combination. Planting sites within blocks were separated by 10 cm.

EE1 was planted in mid-September for FGs and in mid-March for SGs. Individual seeds were planted 0.5 cm deep and marked with swizzle sticks. Germination in the field was recorded every 3 d for 1 mo following planting in the fall and spring. Date of germination was standardized to the number of days after planting. After 1 mo, 93% of the FG seeds and 94% of the SG seeds had germinated. Plants in the field were then censused every 13–38 d until death. For ~6 wk during the winter, the field site was covered with snow, and no data were collected. Survival, leaf number, and length of longest leaf were recorded at every census. In addition, date of first flower and seed number were recorded for all individuals.

Greenhouse Methods and Transplanting

In both fall and spring, seeds assigned to EE2 and EE3 were planted singly into 2.4 × 2.4 × 15-cm ConeTainers filled with Metro-Mix soil. To closely synchronize germination in the field and greenhouse, EE2 and EE3 were planted a few days after EE1 had begun to germinate in the field. As expected, growth

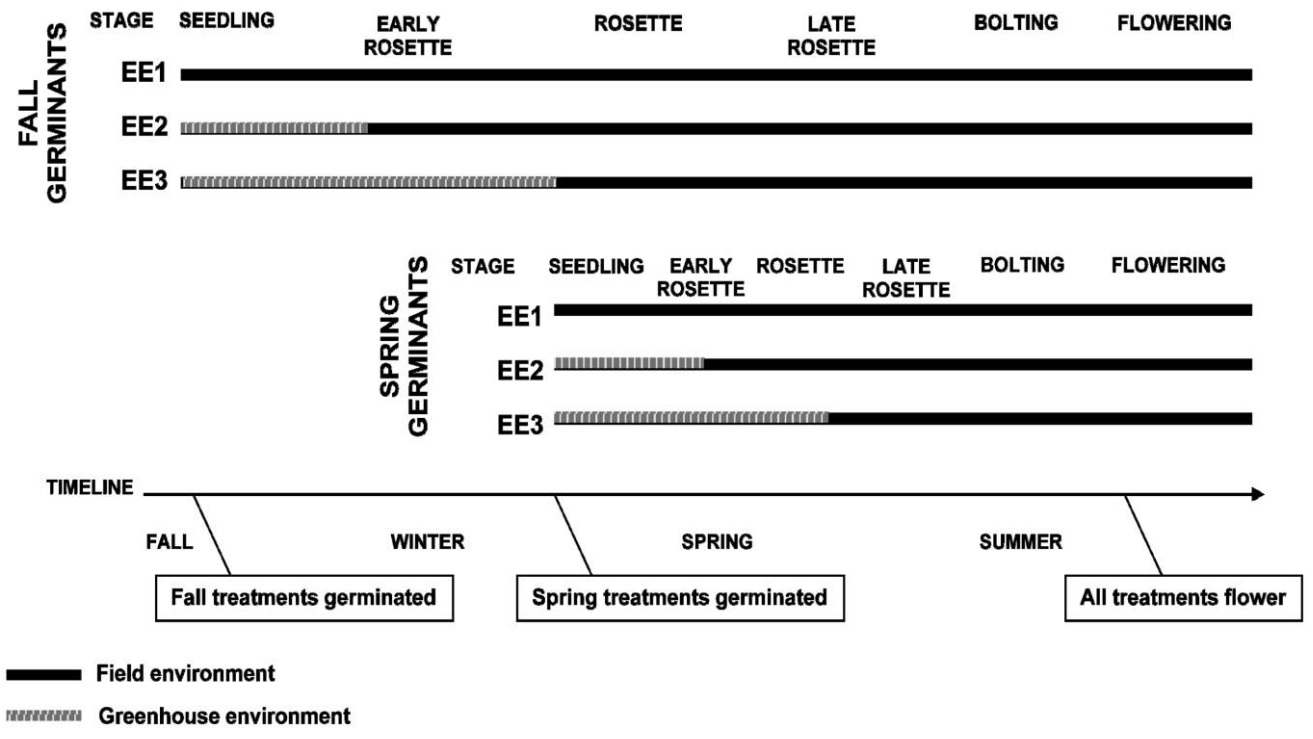

Fig. 1 Diagram of experimental design and plant phenology. Fall germinants were planted in October, and spring germinants were planted in March. EE*X* refers to early environment treatment (see "Methods"). Seeds receiving EE1 treatment were planted directly into the field environment (solid bars) in the fall and spring. In both fall and spring, seeds receiving EE2 and EE3 treatments were planted into the greenhouse environment (dashed bars) and transplanted to the field environment at the early rosette stage and the late rosette stage, respectively. Phenology is indicated above the EE1 bar for both seasons. All six treatments shared a common late-life environment, and late life stages were contemporaneous.

conditions were optimized, and the environmental conditions were less variable in the greenhouse relative to the field. Individuals in the greenhouse were censused daily for germination and, after germination, were censused monthly for mortality.

For field planting, each rosette was removed from its ConeTainer and inserted into a 3 × 3 × 15-cm hole. To reduce stress associated with transplanting, plants were hand-watered twice a day for 3 d following transplantation. For FGs, EE2 was transplanted in early November, and EE3 was transplanted in mid-March. For SGs, EE2 was transplanted in early May, and EE3 was transplanted in early June. Any mortality occurring within 2 wk of transplanting was attributed to transplant shock, and these individuals were omitted from the analysis.

Analysis

At each census, aboveground biomass (hereafter, size) was estimated by multiplying leaf number by length of the longest leaf. This calculation provided an estimate of total dry biomass in previous greenhouse experiments that employed destructive sampling techniques (biomass $= -1.78 + 0.88$(leaf length × leaf number); $r^2 = 0.88$, $P < 0.0001$, $n = 45$). Mean size varied between treatments and ontogeny; thus, the phenotypic variances in size were compared using the CV. Mortality rate, $q(x)$, was calculated by dividing the number of deaths at the end of a census interval by the number alive at the beginning of the census interval. All estimates of mortality were standardized to 28 d to account for uneven census intervals. The calculation of mortality is independent for each census interval; thus, a contingency analysis was used to determine significant differences in mortality between treatments. Fitness was defined as the number of seeds a plant produced.

To evaluate patterns of selection across the life cycle, directional and quadratic selection gradients (β and γ, respectively) were calculated for time to germination, seedling size, early rosette size, late rosette size, and time to flower, using multivariate partial regression analysis (Lande and Arnold 1983). Seed number was used to calculate relative fitness for each season and treatment. All traits except fitness were standardized to a mean of 0 and a standard deviation of 1. All standardized traits and relative fitness met the assumptions required for parametric significance tests. Differences in phenotypic selection gradients on size were estimated in an ANCOVA that included season and treatment as fixed effects. To keep quadratic terms from biasing linear terms (Wade and Kalisz 1989), quadratic gradients were estimated in a separate model that included both linear and quadratic terms. Genotypic selection gradients were estimated in the same manner as phenotypic selection gradients but used paternal family

means as observations. Plots of quadratic selection gradients were examined to determine whether selection was stabilizing, disruptive, accelerating, or decelerating. For each model, the variance inflation factor (VIF) option in SAS was used to test for multicollinearity. The highest VIF value was 6.489, which indicates that multicollinearity was not an issue in this analysis. To determine the effect of season and treatment on the strength or direction of selection, data were analyzed with an ANCOVA. The model included relative fitness as the dependent variable and season, cohort, and all interactions between fixed effects and size (Callahan and Pigliucci 2002; Bell and Galloway 2007).

The multivariate selection analysis evaluates fecundity selection; to evaluate the relationship between size and survival, we used a logistic regression (Janzen and Stern 1998). We ran three models in which each trait was used to predict survival at the subsequent life stage. For example, we used seedling size to predict survival to the early rosette stage, early rosette size to predict survival to the late rosette stage, and late rosette size to predict survival to flowering. The effects of early environment and season were then evaluated by including early environment and season along with interaction terms in each of these models. All data were analyzed using SAS for Windows (ver. 9.1; SAS Institute 2000).

Results

Variation in Phenotype Mean and Variance

Early-life environment contributed to variation in phenotype across the life cycle of *Agrostemma githago*. For both fall and spring treatments, early environment affected the number of days from planting to germination (table 2). Time to germination was longest in the field, with a mean of 10.7 ± 3.08 (SE) d after planting (EE1) and was shorter in the greenhouse (EE2 and EE3, with means of 4.2 ± 0.06 and 4.3 ± 0.05 d, respectively). Early environment also affected variation in size (tables 2, 3). At the seedling stage plants sown directly in the field (EE1) were smallest, while the largest were in EE2. Seedling sizes for EE2 and EE3 were not different. Similarly, at the late rosette stage, when all plants were in the same environment, EE1 plants were smaller than EE2 and EE3 plants. The CVs for size traits were approximately equal among early environment treatments and decreased slightly across life stages (table 3). Early environment influenced date of first flower such that plants that had been in the field longer flowered earlier (table 2; treatment mean was calendar date 150.37 ± 2.55, 172.07 ± 6.06, and 188.96 ± 2.55 for EE1, EE2, and EE3, respectively).

Seasonal germination treatments also had direct effects on phenotype at both early and late life stages (table 2). Time to germination was affected by season, with a mean of 5.2 ± 0.29 d for FG plants versus 8.6 ± 2.64 d for SG plants. Season of germination was found to affect size across the life cycle. At the seedling stage SG plants were smaller than FG plants, and these differences in size between seasons were even more pronounced at the early rosette stage (table 3). Season affected date of first flower, with FG plants blooming on a mean calendar date of 164.84 ± 0.14, compared with 198.77 ± 0.30 for SG plants ($P < 0.001$). In addition to variation in mean phenotype between seasons, there were also differences in the coefficient of variation in these traits such that the variance in seedling size and early rosette size was higher for SG plants but lower for late rosette size (table 3).

Family had a significant effect on phenotype across life stages (table 2). The range for family means was as follows: germination date, 3.4 ± 0.24 to 9.75 ± 1.88 d; seedling size, 8.26 ± 1.92 to 15.10 ± 0.66 cm; early rosette size, 9.57 ± 1.83 to 18.11 ± 2.16 cm; late rosette size, 11.71 ± 3.04 to 20.57 ± 1.72 cm; and date of first flower, from a calendar date of 136.40 ± 1.16 to 194.86 ± 49.11. Both early environment and season influenced the trait expression of families (EE × family and season × family interactions) for date of germination, seedling size, early rosette size, and date of first flower (table 2). Seasonal differences in the environment at early life stages (EE × season interaction) also affected phenotype at later life stages (table 2). Finally, a significant season × treatment × family interaction for date of first flower indicates that the seasonal quality of early-life environment affected family trait expression at late life stages (table 2).

Variation in Mortality and Selection

Mortality varied among the different early environment treatments and between seasons (fig. 2). Mortality for early

Table 2

ANOVA for Traits across Season, Early Environment (EE) Treatment, and Family

	Date of germination		Seedling size		Early rosette size		Late rosette size		Date of first flower	
Source	df	*F*	df	*F*	df	*F*	df	*F*	df	*F*
Season	1	15.87**	1	78.99***	1	190.69***	1	63.85***	1	4.18***
EE	2	97.46***	2	5.43**	2	1.39	2	15.59***	2	203.3***
Family	31	2.93*	31	4.00***	31	2.58***	31	1.55*	31	3.64**
Season × EE	2	2.08	2	1.58	2	6.68***	2	130.04***	2	71.5***
Season × family	32	5.45**	32	9.09**	32	1.54*	32	1.28	31	1.70**
EE × family	64	1.35***	64	6.58***	64	2.33***	64	1.23	63	1.43**
Season × EE × family	61	.68	61	1.82	61	1.22	61	.96	61	1.71**

Note. Numerator degrees of freedom are listed in the table for each source. Denominator df = 194, and error df = 1386.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

Table 3

Means and Variances for Size Traits through the Life Cycle

Treatment	Seedling size	Early rosette size	Late rosette size
Season:			
Fall germinants	14.70 ± .17^{A} (34.4)	21.77 ± .28^{A} (38.8)	27.04 ± .32^{A} (28.8)
Spring germinants	11.03 ± .23^{B} (59.1)	12.22 ± .23^{B} (47.6)	23.76 ± .62^{B} (14.0)
Early environment (EE):			
EE1	12.80 ± .26^{A} (47.5)	16.63 ± .36^{A} (49.7)	24.62 ± .66^{A} (37.1)
EE2	13.07 ± .27^{B} (47.1)	18.12 ± .39^{A} (48.3)	27.29 ± .41^{B} (30.2)
EE3	13.00 ± .26^{B} (46.1)	17.29 ± .39^{A} (49.9)	28.91 ± .48^{B} (28.9)

Note. Values are means (cm) ± 1 SE, with CVs for each trait given in parentheses. Letters denote significant differences in means between treatments.

environment treatments was highest for EE1 at early life stages, including the seedling stage ($\chi^2 = 14.04$, df = 2, $P < 0.001$) and the rosette stage ($\chi^2 = 13.97$, df = 2, $P < 0.001$). At the early rosette stage, EE3, which was still in the greenhouse, had significantly lower mortality than EE1 and EE2, which were in the field ($\chi^2 = 12.41$, df = 2, $P = 0.002$). At later life stages EE2 and EE3 had higher mortality than EE1 as late rosettes ($\chi^2 = 14.53$, df = 2, $P < 0.001$), and at bolting EE1 again had lower mortality than EE3 ($\chi^2 = 8.43$, df = 2, $P = 0.0148$), but EE2 did not differ from EE1 or EE3. Survival to flowering was significantly lower in EE1 than in EE2 and EE3 (63.3%, 74.8%, and 72.1%, respectively; $\chi^2 = 323.4$, df = 2, $P < 0.0001$). For season of germination treatments, mortality was higher for SG plants than for FG plants at the rosette ($\chi^2 = 49.81$, df = 1, $P < 0.0001$) and late rosette ($\chi^2 = 71.82$, df = 1, $P < 0.0002$) stages (fig. 2). For the FG treatment 78.8% of individuals survived to the reproductive stage, but only 61.2% of the SG plants survived ($\chi^2 = 67.58$, df = 1, $P < 0.0001$). There was variation in survival between families for both season of germination ($\chi^2 = 54.91$, df = 32, $P = 0.0071$) and early environment treatments ($\chi^2 = 50.6$, df = 33, $P = 0.0253$). In neither case was there a treatment × family interaction for survival.

Overall, larger size significantly increased the odds of survival to the subsequent life stage. The effect of larger size was greatest at the seedling stage, where an increase of one unit of size conferred a 52% increase in survival to the early rosette stage (survival = $-5.0 + 0.10 \times$ size; $\chi^2 = 7.61$, $P = 0.005$). In contrast, larger size at the early rosette stage increased the odds of survival to the late rosette stage by 14% (survival = $-2.36 + 0.07 \times$ size; $\chi^2 = 8.94$, $P = 0.002$). At the late rosette stage, larger size increased the odds of survival to bolting by 7% (survival = $0.88 + 0.13 \times$ size; $\chi^2 = 28.30$, $P < 0.0001$). Early-life environment had a significant effect on the relationship between size and survival at the seedling stage. In the field environment (EE1), larger size increased the odds of survival to the early rosette stage by 22.1% and 30.4%, compared with EE2 and EE3, respectively (model estimates vary by interaction; $\chi^2 = 17.45$, $P = 0.0002$). There was no difference in size advantage among seedlings in EE2 and EE3, and at later life stages early environment did not influence the relationship between size and survival. For season of germination treatments, larger size was more important to survival in SG plants than in FG plants at the seedling and early rosette stage. Among SG seedlings, larger size conferred a 21% increase in the odds of survival to the early rosette stage (survival = $5.72 + 0.14 \times$ size; $\chi^2 = 20.05$, $P < 0.0001$). In comparison, the odds of survival among FG seedlings increased by only 6% (survival = $5.14 + 0.12 \times$ size; $\chi^2 = 18.00$, $P < 0.0001$). Similarly, larger size at the early rosette stage resulted in an 8% increase in the odds of survival to the late rosette

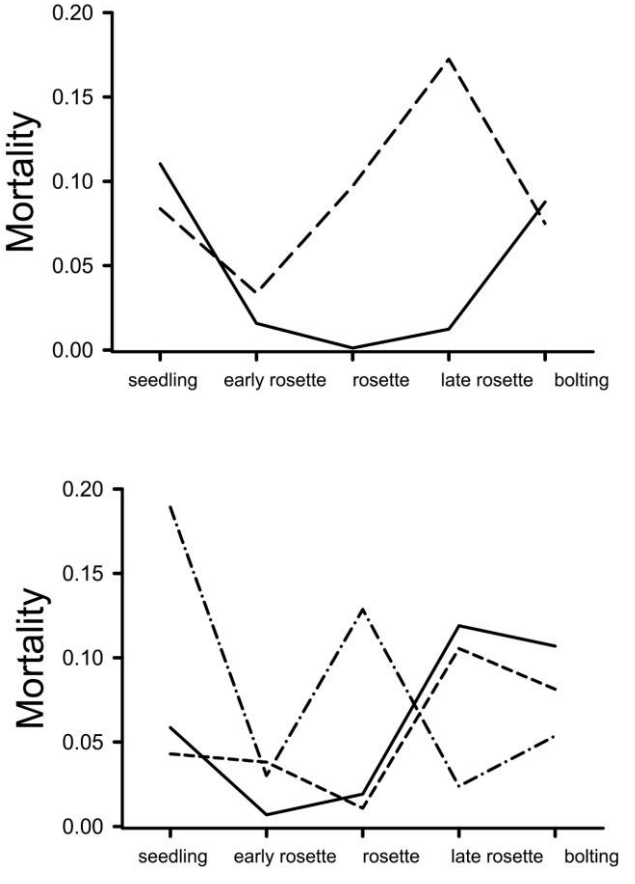

Fig. 2 Mortality at different life stages for the different treatments. The top panel shows mortality for fall germinant (solid line) and spring germinant (dashed line) treatments. Between seasons, mortality differed significantly at the rosette and late rosette stages (see text). The bottom panel shows mortality for early environment (EE) treatments (dashed-dotted line = EE1, dashed line = EE2, solid line = EE3). Between early-life environments, morality differed significantly across treatments at all life stages (see text).

stage in SG plants (survival = $-2.77 + 0.20 \times$ size; $\chi^2 = 22.65$, $P < 0.0001$), compared with 3.8% in FG plants (survival = $-0.17 + 0.13 \times$ size; $\chi^2 = 14.67$, $P < 0.0001$). Season of germination did not influence the relationship between size and survival at the late rosette stage.

At the phenotypic level both linear and quadratic selection was significant at the early and late rosette stages, whereas at the genetic level only linear selection was significant for these same traits (table 4). When selection differentials were estimated for seasons and early-life environments, there was evidence for positive linear selection for early and late rosette size (table 4). When the treatments are analyzed separately, the results show that early-life environment modified the strength of phenotypic selection across the life cycle such that at the seedling stage selection for larger size was significantly stronger in the field environment, EE1, and weaker in EE2 and EE3 (fig. 3). At the early rosette stage larger plants were favored in EE1 and EE2, but then at the late life stages, when all plants were in a common environment, larger late rosette size was strongly favored in EE3 and was favored to a lesser degree in EE1 and EE2. Stabilizing selection was significant for both early and late rosette size. These gradients were significantly less than 0 when tested within the regression model. Early environment affected quadratic phenotypic selection and varied between treatments for early rosette size: early rosette size was stabilizing in EE2 ($\gamma = -0.30$) and insignificant in EE1 and EE3. At the late rosette stage there was significant stabilizing selection in EE2 and EE3 ($\gamma = -1.69$ and -1.80 respectively) but not in EE1. There were no differences in genotypic quadratic selection between early environment treatments.

Seasonal effects on selection gradients for size were less frequent, but there was strong positive phenotypic selection for early rosette size on FG plants ($\beta = 0.87$), compared with insignificant selection on SG plants. Phenotypic quadratic selection for early rosette size differed significantly between the FG ($\gamma = 1.07$) and SG ($\gamma = 0.02$) plants as well. At later life stages a season × treatment interaction explained differences in selection gradients. For late rosette size, there was significant phenotypic selection for FG EE2 ($\gamma = 1.69$, $P < 0.05$). No other selection gradients differed between seasons.

Discussion

The results of this study show that early-life environment can alter phenotype, mortality, and selection at later life stages in *Agrostemma githago*. This experimental design made it possible to evaluate the effect of early-life environment on two levels. First, it examined how natural differences in seasonal germination time affected phenotypic change across the life cycle. Second, it manipulated the quality of early-life environment within seasons by keeping some cohorts in the greenhouse until later life stages. For both manipulations, variation

Table 4

Linear and Quadratic Selection Gradients for Size

Trait	s	β	β × season (F)	β × EE (F)	β × season × EE (F)
A. Phenotypic selection on size:					
Linear selection gradients:					
Seedling size	.013	−.061	.82	2.71*	.05
Early rosette size	.307***	.221**	2.78*	6.68***	.04
Late rosette size	.371***	.526***	.04	3.26**	2.15
		γ	γ × season (F)	γ × EE (F)	γ × season × EE (F)
Quadratic selection gradients:					
Seedling size	...	−.387	.84	2.52*	.06
Early rosette size	...	2.271**	4.42**	.74	.11
Late rosette size	...	.945**	.05	2.63*	3.91*
		β	β × season (F)	β × EE (F)	β × season × EE (F)
B. Genotypic selection on size:					
Linear selection gradients:					
Seedling size	.090	.092	1.54	.70	.02
Early rosette size	.319**	.175	.09	.01	.26
Late rosette size	.308***	.189	1.94	1.13	.01
		γ	γ × season (F)	γ × EE (F)	γ × season × EE (F)
Quadratic selection gradients:					
Seedling size	...	.000	1.26	.96	.64
Early rosette size	...	.000	.76	.80	1.05
Late rosette size	...	.000	.08	1.11	1.48

Note. Selection gradients are calculated for phenotype (pt. A) and genotype (pt. B). Selection differentials (s) are reported, along with linear (β) and quadratic (γ) selection gradients, pooled across seasons and early environmental treatments. F values from ANCOVA report interactions with season and early environment (EE). For phenotypic analysis, linear df = 938 and quadratic df = 968; for genotypic analysis, linear df = 35 and quadratic df = 38.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

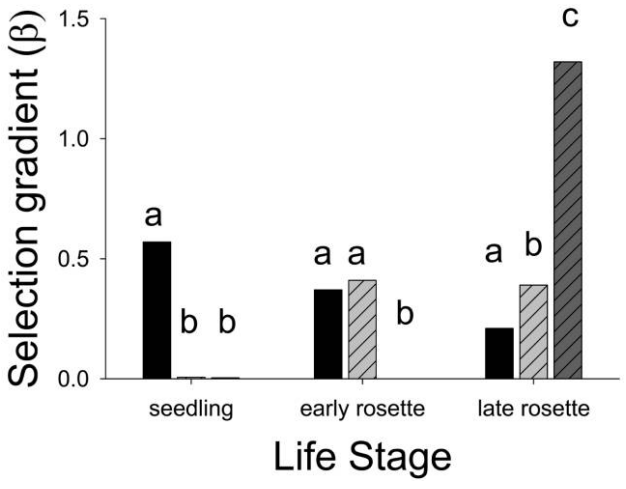

Fig. 3 Linear phenotypic selection gradients for size by early environment (EE) treatment. Black bars represent EE1, light gray bars represent EE2, and dark gray bars represent EE3. Different letters indicate significantly different selection gradients ($P < 0.01$).

in early-life environment had consequences for mortality and selection gradients across the life cycle. Many studies of natural populations have found cohort effects that persist to adult life stages (Albon et al. 1987; Gaillard et al. 2000; Forchhammer et al. 2001; Metcalfe and Monaghan 2001; Van de Pol et al. 2006). Yet unless the interaction between adult environment and cohort age (G × E × A; Wilson et al. 2008) is controlled for, the unique history of each cohort makes it difficult to attribute the relative contributions of past versus present conditions to an individual's phenotype. In this experiment, treatment groups were exposed to unique G × E × A combinations with respect to the environment at time of transplant and the timing of transplant with respect to life stage. However, late-life environment was shared among all individuals; thus, variation in the effect of the environment on the adult phenotype and selection was due to responses to early-life conditions.

Mortality at an early life stage can change the distribution of traits expressed at later life stages. This can bias observed trait correlations and later estimates of selection (Grafen 1988; Bennington and McGraw 1995; Hadfield 2008; Sinervo and McAdam 2008; Wilson et al. 2008). In our study, mortality influenced selection gradients across season treatments. This species naturally experiences two contrasting environments for germination and establishment, fall and spring. The temporal variation in the ecological conditions experienced by individuals germinating at these times is very distinct. Our results, across seasons, showed that FG plants were always larger and that SG plants had higher mortality, most likely due to their smaller size. Spring is a time of year when other species are growing rapidly, and competition is intense. Larger size was more important to survival early in life among SG plants, and high mortality may have influenced our ability to detect phenotypic selection for the SG plants. Our results showed strong positive selection for early rosette size for FG plants but not for SG plants. This was unexpected because there was more phenotypic variance for size and a stronger relationship between larger size and higher survival at the earliest stages for the SG plants. Fewer SG individuals survived to flower; thus, many individuals in this cohort would be part of the "invisible fraction" (Grafen 1988). Our results are consistent with the findings of a field study with *Mimulus guttatus* where genotypes of different flower sizes were used to evaluate selection on flower size (Mojica and Kelly 2010). The results showed that viability selection on the invisible fraction can deceptively reverse the overall direction of selection. Failure to account for prereproductive viability selection can critically influence our understanding of selection in field populations.

Variation in mortality across different early environment treatments resulted in variation in selection, but only in the field environment. EE2 and EE3 plants spent the earliest stages of their life in the greenhouse, and this was beneficial for late-life phenotype because the plants in these treatment groups were larger at both the earliest and the late life stages. Early-life mortality was also lower for these greenhouse-raised individuals, most likely because smaller individuals growing under greenhouse conditions escaped the increased risk of mortality experienced by individuals growing in the field. It was only after these greenhouse-raised plants had been moved into the more stressful field environment later in life that their mortality increased. Likewise, for all early environment treatments selection was significant only when the cohorts were in the field environment. At the earliest life stage, for example, selection on seedling size was significant only for EE1, which was already in the field. The selection gradient for EE3 size was not significant until the late rosette stage, after EE3 individuals had been moved to the field. Nonlinear selection showed an intermediate size optimum for fitness for EE2 and EE3, but again only after these cohorts were in the field environment. When environmental conditions changed, phenotypes were differentially favored (DeWitt et al. 1998). These results show that changing environments during ontogeny can influence selection on a trait across life stages (Weinig and Delph 2001). An individual's phenotypic trajectory is a function of its genotype and the temporal sequence of external environments to which it is exposed (Schlichting and Pigliucci 1998; Sultan 2000; Fusco and Minelli 2010). In this study with *A. githago*, both late-life phenotypes and selection on these phenotypes was influenced by early-life environment, and this variation was caused by viability selection and a mismatch between early- and late-life environments.

Life stage by environment interactions may influence the distribution of phenotypes in the surviving population or the relative frequency of genotypes (Lynch and Arnold 1988; Bennington and McGraw 1995; Sinervo and McAdam 2008). In this study with *A. githago*, the initial genetic structure of all cohorts was the same. Our results showed that there was a genetic component to size across the life cycle and that there was an interaction such that genotypes differed in size across early environments and seasons. With respect to survival, however, there was variation between families in the early-life treatments but no interaction between genotype and treatment. Because selection is on phenotype rather than genotype, it appears that a plastic response was important in determining the influence of early environment on the phenotype at late life stages. This suggests that although plastic responses to early-life environment are commonly found to have carryover

effects on adult phenotype (Sultan 2000; Donohue 2002; Marshall 2005; Brody et al. 2007), this may not result in differences in mortality across genotypes.

We had hypothesized that phenotype, mortality, and selection would vary between early-life treatments. Our results confirm this in some circumstances. Between season of germination treatments, prereproductive viability selection can bias our understanding of phenotypic selection for size across life stages. On the other hand, as shown with early environment treatments, changing environmental conditions during ontogeny can influence selection through the life cycle. Although the short-term effects of environmental variation on individual performance have been extensively studied, long-term effects of early environmental conditions are only just beginning to receive attention (Monaghan 2008). While many studies of cohort effects find that early environment is a critical factor in explaining adult performance (Beckerman et al. 2003), further studies that control for the effects of age and unique histories are needed. Likewise, although the effect of environment during early development has been linked to adult reproductive output in several studies (Metcalfe and Monaghan 2001; Gaillard et al. 2003; Van de Pol et al. 2006), a clear understanding of how early environmental conditions affect population growth and structure at multigenerational timescales is needed.

Acknowledgments

We thank L. Galloway, J. Antonovics, H. Wilbur, and D. Carr for their helpful comments on this research; Mark Givens for providing the field site; Jane Gurnick and Marta Tomic for assistance in the field; and helpful comments from the reviewers. This experiment was supported by the Mountain Lake Biological Station, a National Science Foundation Dissertation Improvement Grant (DEB-0608422), and a Walton Fellowship (to S. H. Goodrich).

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