

# How important are environmental maternal effects in plants? A study with *Centaurea maculosa*

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## Summary

**1** Previous studies have reported significant influences of maternal environment on offspring fitness in plants. We investigated the early plant development from seeds of *Centaurea maculosa* plants grown in four environments of increasing severity: (1) control; (2) herbivory; (3) herbivory + nutrient shortage; and (4) herbivory + nutrient shortage + grass competition.

**2** Although these treatments had huge effects on the size of the mother plants and the number of seeds they produced, there was no evidence that the treatments affected the weight of the seeds they produced. There was, however, significant variation in seed weight among maternal plants within treatments.

**3** We grew individually weighed seeds from 33 of these maternal plants in three competitive regimes (individually, with one conspecific neighbour, with three *Festuca pratensis* neighbours) to test if maternal environment and seed weight influenced first-year growth, and if these effects were more pronounced in the presence of competition.

**4** There were a few weak but significant environmental maternal effects on offspring performance. Seed weight was positively correlated with initial growth, but its influence decreased over time and disappeared after 8 weeks. The presence of one conspecific neighbour or three *Festuca pratensis* neighbours did not influence growth during the first few weeks, but strongly suppressed growth after 9 weeks. Competition did not accentuate the influence of seed weight on offspring performance.

**5** Differences among individual maternal plants were a major source of variation in seed weight and early offspring growth. Some of the evidence that has been cited in support of environmental maternal effects in plants may be the result of confounding maternal identity and maternal environment.

**6** Our results support the generalization that seed size is one of the least plastic of plant characters. Plants express great plasticity in reproductive output, but this occurs primarily in terms of the number of seeds produced, and only secondarily, if at all, in terms of seed size or quality.

**7** Effects of a plant's maternal environment on its performance can be detected, but they appear to be small compared to other factors that influence a plant's fitness, such as its genotype and the environment in which it grows.

*Keywords:* *Centaurea maculosa*, maternal effects, offspring quality, seed size, seed weight  
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## Introduction

The phenotype of an organism can be influenced by maternal effects as well as by its genotype and environ-

ment (Futuyma 1986). Maternal effects are influences of the mother plant on the phenotype of her offspring via mechanisms other than the genetic information carried on the chromosomes. For example, the environment of the maternal tissues within which the zygote develops into a sporophyte can influence the phenotype of the resulting plant after it has germinated (Roach & Wulff 1987; Schmid & Dolt 1994). Seeds are provisioned with energy and nutrients by the mother plant, and the importance of the amount

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of provisioning is supported by evidence that seed size is correlated with subsequent growth and survivorship, especially when competition is important (Black 1957; Lush & Wien 1980; Gross 1984; Stanton 1984; Wulff 1986a,b; Kromer & Gross 1987; Aarssen & Burton 1990).

Maternal effects can be divided into genetic and nongenetic effects. The former includes genetic differences among mother plants that result in differences in the provisioning or other aspects of the environment of seeds as they develop within flowers and fruits. Genetic maternal effects can occur due to the genetic material in mitochondria, chloroplasts or plastids, which are contributed only by the mother (Roach & Wulff 1987; Platenkamp & Shaw 1993). Non-genetic maternal effects can occur because the environment in which the mother plant grows may influence her ability to provision seeds. For example, the nutrient content of seeds can be influenced by the soil nutrient level in which the mother plant is growing (Parrish & Bazzaz 1985). Environmental maternal effects on individual growth often appear to be transitory (Miao, Bazzaz & Primack 1991; Wulff & Bazzaz 1992; Schmid & Dolt 1994), but they could still play a role if the period of their influence is important for plant fitness, e.g. germination time (Alexander & Wulff 1985; Platenkamp & Shaw 1993). In the most commonly described scenario, environmental maternal effects are mediated by seed size: the environment in which the maternal plant is growing influences the size of seeds produced, and there are numerous studies showing a positive relationship between seed size and the probability and speed of germination, and subsequent seedling size (e.g. Harper, Lovell & Moore 1970; Weis 1982; Hendrix 1984; Schaal 1984; Stanton 1984; Crawley & Nachapong 1985; Schmid & Dolt 1994), although some studies did not find these effects (Schmitt & Antonovics 1986; Dolan 1984). Environmental maternal effects mediated by seed weight are possible only to the degree that the seed weight distribution produced by a plant is plastic, and seed weight is considered to be one of the least plastic of plant reproductive characters (Harper *et al.* 1970; Schmid & Dolt 1994). An individual plant will produce a distribution of seed masses, but this distribution is relatively insensitive to the environment in which the plant grows. One of the most robust generalizations in plant reproductive ecology is that plants express huge differences in reproductive output primarily through variation in seed number, rather than in individual seed weight. Plants seem to vary mean seed weight only when plasticity in seed number is unavailable or limited (Hodgson & Blackman 1957a,b; Harper *et al.* 1970; Schmid 1992).

We studied the influence of four maternal environments of increasing severity on the phenotype offspring in *Centaurea maculosa*, to ask the following questions:

1 Do plants growing in more severe environments produce smaller seeds?

2 Do offspring of maternal plants that were grown in more severe environments grow more slowly than offspring from those in less severe environments? If so

(a) does the influence of maternal environment on offspring growth decrease over the course of growth, and

(b) does competition, intraspecific or interspecific, extend or amplify the influence of the maternal environment on offspring growth?

## Materials and methods

*Centaurea maculosa* Monnet de la Marck (Asteraceae), spotted knapweed, is a monocarpic biennial that inhabits dry, calcareous grasslands in Europe, and has invaded North American rangeland (Müller 1989; Müller-Schärer & Schroeder 1993). During the first year it forms a rosette and a tap root. In the second year the stem bolts and produces up to 60 flowering heads that each produce  $\approx 20$ –30 seeds (Müller 1991a,b). In the native range in central Europe, flowering occurs in July, and the plant dies as the seeds ripen in August.

## MATERNAL TREATMENTS

In a previous study on the influence of four stress factors on the growth and development of *C. maculosa* (Müller 1991b; Steinger & Müller-Schärer 1992), seeds collected from a natural population in eastern Austria were grown under all combinations of the presence and absence of four factors: (1) nitrogen fertilization; (2) competition with the grass *Festuca pratensis*; (3) herbivory by the root-feeding moth *Agapeta zoegana* L.; and (4) herbivory by the root-feeding weevil *Cyphocleonus achates* Fahr. These treatments had major effects on the size, physiology and allocation patterns of the plants (Steinger & Müller-Schärer 1992), and resulted in huge differences in the number of seeds produced by the plants (Müller & Steinger 1990). We selected four of the 16 treatments, representing a series of increasing stress and therefore decreasing seed production: (1) Plants grown in the most favourable environment (i.e. without competition, without herbivory and with added nitrogen) produced, on average, 1412 seeds. (2) Herbivory by both insects reduced this to 730 seeds per plant. (3) Herbivory in the absence of nitrogen fertilizer resulted in an average of 274 seeds per plant. (4) Plants experiencing all three 'stress factors' (*Festuca* competition, herbivory and no added nitrogen) produced, on average, 117 seeds, and the seeds produced were on average smaller and had lower nitrogen content than plants grown in more favourable environments (Müller & Steinger 1990; Müller 1991b).

We collected 200 seeds from eight or nine randomly

selected mother plants from each of the four above-mentioned treatments:

- 1 no herbivory, fertilized with nitrogen, no competition; 8 maternal plants;
- 2 herbivory, fertilized with nitrogen, no competition; 8 maternal plants;
- 3 herbivory, unfertilized, no competition; 8 maternal plants;
- 4 herbivory, unfertilized, competition from *Festuca*; 9 maternal plants.

We use the term 'maternal treatment' for these four environments, and 'treatment' for the treatment in which the offspring were grown.

#### GERMINATION EXPERIMENT AND OFFSPRING TREATMENTS

Each of the 800 randomly collected seeds was given a number, and the identity of the mother plant and the treatment in which the mother plant grew was recorded. The seeds were individually weighed ( $\pm 0.001$  mg) and then stratified at 4 °C for 17 weeks and subsequently germinated in a heated glasshouse with 8 h of light at 20 °C and 16 h of darkness at 17 °C. On 15 April 1989 all 800 seedlings were planted individually in fine vermiculite. The substrate was kept moist with tap water. Plants were considered to have germinated as soon as cotyledons emerged above the soil.

On 27–28 April we randomly selected 120 seedlings from each of the four maternal treatments. Two hundred of 480 seedlings were transplanted into clay pots (400-mL, 8 cm diameter, 10 cm height) with a nutrient-poor soil mixture of 40% compost, 50% silica sand and 10% peat. Another 200 seedlings were used to provide a single neighbour in each pot, such that there were 10 replicates of each possible maternal treatment combination. The remaining 20 plants from each maternal treatment were used for two further treatments: (1) one *Centaurea* with three *Festuca* plants (as described in Steinger & Müller-Schärer 1992) and (2) one *Centaurea* alone. Thus, the experiment consisted of 200 pots with two *Centaurea* plants (of which only one 'target' plant was analysed), 40 pots with one *Centaurea* and three *Festuca* plants, and 40 pots with single *Centaurea* plants. The pots were arranged in 10 blocks with one of the 10 replicates of each treatment assigned to each block. On 3–4 May 1989 all the pots were placed outside in the Botanical Garden of the University of Basel, separated from each other by 4 cm.

The experimental design is summarized in Fig. 1, and the distribution of maternal and offspring treatments among the 33 maternal plants is shown in Table 1.

#### MEASUREMENTS

Plants were measured 14 times during the 22 weeks of the experiment. The interval between each of the

first eight measurements was four days, after which measurements were made every 14 days, except for the last measurement, which occurred 30 days after the previous one. After each measurement the blocks were rotated and the pots within each block were randomized. The number of leaves greater than 3 cm in length, and the length of the longest rosette leaf were recorded on each occasion.

On 8 July we cut the grass that extended beyond the top of the pots in those pots that contained grass. The experiment was concluded on 18 September 1989. We removed the plants from the pots and washed the soil from the roots. We harvested the tap root; fine roots could not be fully recovered. Above- and below-ground parts of the plants were dried for 50 h at 60 °C, and weighed ( $\pm 0.001$  g). We analysed plant total dry mass (above- and below-ground).

Two of the plants died shortly after transplantation, and two plants formed flowering stalks during this first year's growth. These four plants were not included in the analyses.

#### STATISTICAL ANALYSES

The influence of maternal environment and of maternal plant within environment on individual seed mass was analysed using analysis of variance models with maternal environment and maternal identity as factors. Size of the offspring on each measurement date and biomass at harvest were analysed with ANCOVA with offspring treatment, maternal treatment and maternal identity as factors and individual seed mass as a covariate. Individual mother plants were nested within the maternal treatments and considered as a random effect (Sokal & Rohlf 1981). To consider all the seeds produced by the mother plants in a given treatment as a single population without accounting for the variation due to differences among mother plants – for example because their identity was not recorded – would be a form of 'pseudoreplication' (Hurlbert 1984). When many analyses were performed, we calculated 'table-wide' probability levels using the 'sequential Bonferroni' correction (Rice 1989).

To compare the relative importance of different factors, we used 'effect sizes' ( $\eta^2 = \text{SS factor} / [\text{SS factor} + \text{SS residual}]$ ; Cohen 1977). The effect size is the squared (multiple) partial correlation coefficient, and is a useful measure of the contribution of a source of variation to the total variation in situations with mixed models. An alternative would be to use variance components, but their application for fixed effects is not generally accepted. We therefore decided to present (multiple) partial coefficients of determination ( $r^2$ ) instead of variance components for random as well as fixed effects. This also has the advantage that all factors could be compared for their relative influences on the same basis. Thus defined, an effect size indicates the proportion of variance in the dependent variable explained by a single independent

## Experimental Design

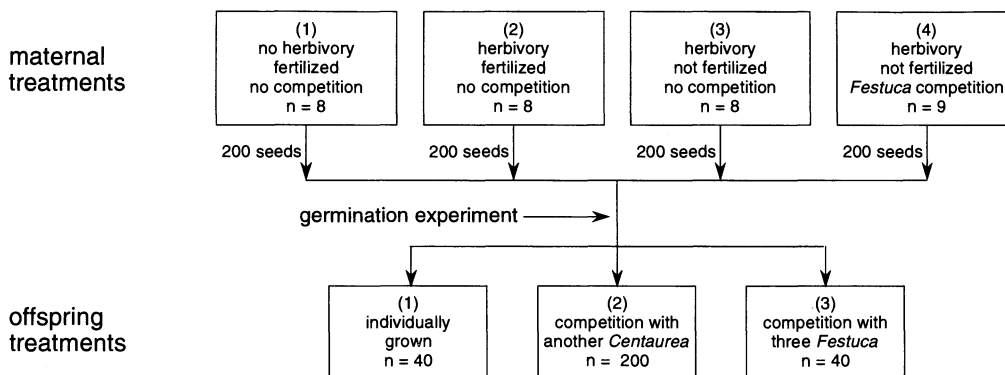


Fig. 1 Experimental design. All 800 seeds from the four maternal treatments were used in the germination experiment. Of those that germinated, 70 from each maternal treatment were randomly selected for the offspring treatments, and another 50 from each maternal treatment were randomly selected to be used as neighbours in the 'competition with another *Centaurea*' offspring treatment.

variable or factor when all other independent variables and factors are held constant.

To obtain more statistical power in detecting effects of the maternal treatment on offspring growth, we fitted simple growth curves to the repeated size measurements of each individual plant over all the measurement dates (see Meredith & Stehman 1991; Stoll, Weiner & Schmid 1994; Weiner 1995). We then analysed the parameter estimates for each plant using ANCOVA as described above. This is similar to a

repeated-measures ANCOVA, except that a more biologically meaningful growth curve is used rather than linear and polynomial terms to model growth over time. We modelled growth with the asymptotic function  $y = a - br^x$ , where the variables  $y$  and  $x$  represent size and time, and the parameters  $a$ ,  $b$  and  $r$  represent the asymptotic size, asymptotic minus initial size, and initial growth rate, respectively.

To normalize the residuals, seed mass was log transformed and the number of leaves on a plant

Table 1 Number offspring of each of the 33 maternal plants (plant number in *italic*) from each of the four maternal treatments receiving each offspring treatment (1 = individually grown; grown in competition with another *Centaurea*; grown in competition with three *Festuca*)

Maternal treatment	Offspring treatment	Maternal plant								Total
		1	2	3	4	5	6	7	8	
Treatment 1										
no herbivory	1	1	0	4	0	2	2	0	1	10
fertilized	2	9	7	5	6	8	7	3	5	50
no competition	3	1	1	3	0	2	1	0	2	10
		9	10	11	12	13	14	15	16	
Treatment 2										
herbivory	1	1	1	2	2	2	0	1	1	10
fertilized	2	6	9	4	7	6	9	2	7	50
no competition	3	2	3	2	0	1	1	1	0	10
		17	18	19	20	21	22	23	24	
Treatment 3										
herbivory	1	1	0	3	2	0	3	0	1	10
not fertilized	2	6	6	9	7	3	9	7	3	50
no competition	3	1	3	1	2	2	0	0	1	10
		25	26	27	28	29	30	31	32	33
Treatment 4										
herbivory	1	1	3	0	0	0	2	0	1	3
not fertilized	2	6	4	6	7	5	8	4	4	6
<i>Festuca</i> competition	3	2	0	1	1	2	2	0	2	0

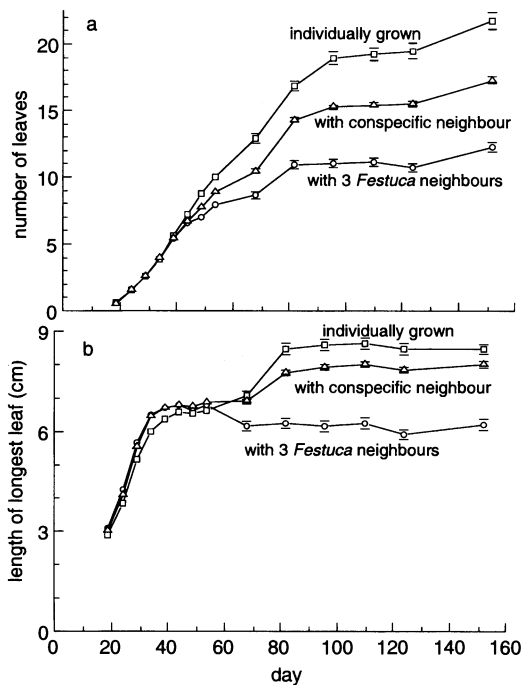
was square-root transformed. To avoid any possible nonindependence in the analysis of plants grown in pairs, we analysed only the left plant in each pot. All analyses were performed with the GENSTAT statistical package (Payne & Arnold 1989).

## Results

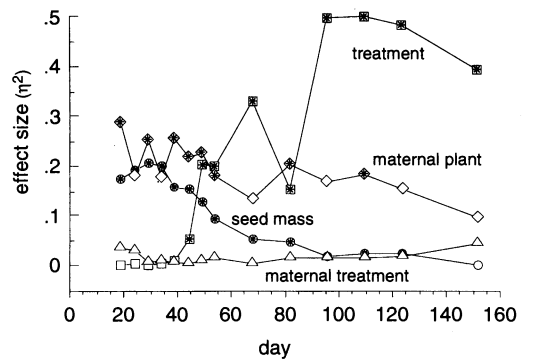
The mean mass of seeds was strongly influenced by the identity of the maternal plant (d.f. = 29, SS = 2.99,  $P < 0.0001$ ), but the differences among the four maternal treatments were not significant (d.f. = 3, SS = 0.121,  $P = 0.76$ ). Variance in seed mass among mothers receiving the same treatment was similar to the variance in seed mass produced by a maternal plant. Thus, maternal plant differences accounted for approximately half of the total variance in seed mass.

The offspring treatment had little influence on the number of leaves on a plant until day 44, but thereafter there were major effects of offspring treatment. Both conspecific and grass neighbours reduced the number of leaves, with grass having a much greater effect (Fig. 2). Maternal treatment never had a significant influence but the identity of the maternal plants was highly significant (Fig. 3; Table 2). Seed mass had a strong positive influence on subsequent growth up to day 34 but became less important thereafter until its effect vanished (Fig. 3; Table 2).

The offspring treatment also had a significant effect on the length of the longest leaf of the plants (Fig. 2, Table 3). As with leaf number, maternal treatment



**Fig. 2** Mean number of leaves greater than 3 cm in length (a) and mean length of longest rosette leaf (b) vs. time since sowing of *Centaurea maculosa* plants grown individually ( $\square$ ), with one conspecific neighbour ( $\triangle$ ) and with three *Festuca pratensis* neighbours ( $\circ$ ).



**Fig. 3** Effect sizes ( $\eta^2 = \text{SS factor} / (\text{SS factor} + \text{SS residual})$ ) for seed mass ( $\circ$ ), treatment ( $\square$ ), maternal treatment ( $\triangle$ ) and maternal plant ( $\diamond$ ) on the number of leaves over the 14 measurement dates in ANCOVA shown in Table 2. Symbols have an asterisk within when the effect is significant ( $P < 0.05$  with sequential Bonferroni correction).

did not significantly affect length of the longest leaf, whereas the identity of maternal plant had a very large effect over the first four weeks. The influence of seed mass on maximum leaf length similarly was large initially and then declined after day 54 (Table 3). There were no significant interactions between offspring treatment and maternal treatment (as can be seen from the means for the final measurement; Table 4), maternal identity or seed mass in their effect on number of leaves or on maximum leaf length.

The repeated-measures analysis was able to detect significant effects of maternal treatment on the value of the parameter estimates of the growth equation fitted to the two dependent variables (number of leaves and length of the largest leaf). Maternal treatment had a significant effect on the estimated asymptotic leaf number ( $P = 0.029$ ; Table 5), but not the initial growth rate (Table 6). Offspring treatment was always highly significant, and had the largest effect size of any factor. When length of the longest leaf was used as the dependent variable in the repeated-measures analysis, maternal treatment had a significant effect on both the asymptotic maximum leaf length ( $P = 0.036$ ; Table 7) and the initial growth rate ( $P < 0.001$ ; Table 8). However, even when significant, effect sizes for maternal treatment were relatively small. The range of  $r^2$  for the fitted growth curves was 0.81–1.0 (mean = 0.96) for leaf number and 0.11–0.99 (mean = 0.88) for maximum leaf length.

Only offspring treatment and maternal identity had significant effects on the total dry mass of plants at harvest (Table 9).

## Discussion

There were large and highly significant differences in the mean mass of seeds produced by different mother plants, but there was no evidence that the different environments in which the maternal plants grew con-

**Table 2** ANCOVA of the effects of treatment, maternal treatment, and maternal identity on the number of leaves greater than 3.0 cm long (square-root transformed) for each measurement date (after sowing), with initial seed mass (log transformed) as a covariate. Identity of the mother plant is nested within maternal environments and treated as a random effect (model II). Values are sum of squares; *F* values are shown in parentheses

	Source of variation					
	seed mass (d.f. = 1)	block (d.f. = 9)	treatment (d.f. = 2)	maternal treatment (d.f. = 3)	maternal plant (d.f. = 29)	Residual (d.f. = 234)
day 19	9.033 (49.63)***	2.315 (1.41)	0.065 (0.18)	1.682 (0.94)	17.316 (3.28)***	42.589
day 24	7.594 (56.42)***	2.116 (1.75)	0.205 (0.76)	1.041 (1.43)	7.019 (1.80)	31.495
day 29	1.474 (61.61)***	0.408 (1.89)	0.012 (0.26)	0.047 (0.24)	1.916 (2.76)***	5.597
day 34	1.271 (59.01)***	0.439 (2.26)	0.022 (0.50)	0.052 (0.45)	1.102 (1.76)	5.040
day 39	0.902 (43.91)***	0.575 (3.11)*	0.058 (1.42)	0.032 (0.19)	1.654 (2.78)***	4.809
day 44	1.331 (43.41)***	0.530 (1.92)	0.400 (6.53)*	0.026 (0.12)	2.022 (2.28)***	7.173
day 49	1.073 (34.98)***	1.141 (4.13)***	1.864 (30.37)***	0.088 (0.40)	2.107 (2.37)***	7.181
day 54	1.058 (24.4)***	0.511 (1.31)	2.553 (29.44)***	0.162 (0.69)	2.266 (1.80)*	10.147
day 68	0.960 (13.11)***	0.632 (0.96)	8.568 (58.53)***	0.096 (0.34)	2.702 (1.27)	17.127
day 82	0.949 (12.38)***	0.533 (0.77)	3.339 (87.04)***	0.325 (0.68)	4.605 (2.07)*	17.929
day 96	0.463 (4.94)	1.234 (1.47)	21.876 (116.91)***	0.368 (0.80)	4.440 (1.64)	21.894
day 110	0.601 (6.33)*	1.387 (1.62)	22.557 (118.69)***	0.368 (0.70)	5.068 (1.84)*	22.235
day 124	0.760 (6.27)*	2.140 (1.96)	26.729 (110.26)***	0.595 (1.10)	5.206 (1.48)	28.363
day 152	0.059 (0.34)	4.842 (3.11)*	26.553 (76.81)***	1.910 (4.19)	4.403 (0.88)	40.446

\*\*\*  $P < 0.001$ , \*\*  $P < 0.01$ , \*  $P < 0.05$  with 'table wide' sequential Bonferroni correction; all others not significant with sequential Bonferroni correction.

**Table 3** ANCOVA of the effects of treatment, maternal treatment, and maternal identity on the length of the longest leaf for each measurement date (after sowing), with initial seed mass (log transformed) as a covariate. Identity of the mother plant is nested within maternal environments and treated as a random effect (model II). Values are sum of squares; *F* values are shown in parentheses

	Source of variation					
	seed mass (d.f. = 1)	block (d.f. = 9)	treatment (d.f. = 2)	maternal treatment (d.f. = 3)	maternal plant (d.f. = 29)	Residual (d.f. = 234)
day 19	30.63 (70.85)***	11.85 (3.05)*	0.80 (0.92)	6.63 (1.12)	57.29 (4.57)***	101.15
day 24	29.77 (68.38)***	20.19 (5.15)***	4.17 (4.80)*	3.40 (0.72)	45.76 (3.62)***	101.86
day 29	40.10 (56.60)***	12.15 (1.91)	6.52 (4.06)	5.74 (1.09)	51.00 (2.48)***	165.77
day 34	34.76 (46.84)***	12.02 (1.80)	9.05 (6.10)*	10.41 (2.51)	40.17 (1.87)	173.78
day 39	24.18 (33.29)***	18.46 (2.82)*	4.13 (2.85)	11.92 (3.42)	33.34 (1.60)	169.96
day 44	25.42 (35.21)***	17.22 (2.65)*	2.13 (1.48)	11.19 (3.26)	33.21 (1.59)	168.90
day 49	18.52 (24.82)***	12.20 (1.82)*	2.05 (1.38)	10.84 (4.33)	24.19 (1.12)	174.61
day 54	16.34 (21.72)***	15.78 (2.33)	2.22 (1.48)	10.32 (3.39)	29.47 (1.35)	175.99
day 68	8.13 (10.11)*	16.93 (2.34)	23.67 (14.46)***	3.29 (1.30)	24.44 (1.05)	188.28
day 82	4.14 (3.57)	38.64 (3.702)**	108.45 (46.68)***	12.41 (2.89)	41.51 (1.23)	271.82
day 96	2.62 (2.33)	43.15 (4.25)***	132.81 (58.89)***	15.49 (2.89)	51.83 (1.59)	263.85
day 110	2.08 (1.69)	34.07 (3.07)*	131.97 (53.53)***	14.01 (2.73)	49.59 (1.39)	288.46
day 124	2.72 (2.38)	44.30 (4.32)**	150.47 (65.99)***	18.39 (3.81)	46.59 (1.41)	266.77
day 152	3.10 (2.53)	33.71 (3.06)*	128.66 (52.56)***	13.23 (2.65)	48.20 (1.36)	286.42

\*\*\*  $P < 0.001$ , \*\*  $P < 0.01$ , \*  $P < 0.05$  with 'table wide' sequential Bonferroni correction; all others not significant with sequential Bonferroni correction.

**Table 4** Mean final leaf number (a) and mean final maximum leaf length (b) for all combinations of offspring treatment (1–3) and maternal treatment (1–4)

Maternal treatment	Offspring treatment			mean
	1	2	3	
(a) final leaf number				
treatment 1	21.8	17.2	11.2	17.0
treatment 2	24.3	18.4	12.7	18.4
treatment 3	19.4	16.7	13.3	16.6
treatment 4	21.4	17.0	12.1	16.9
mean	21.7	17.3	12.3	17.2
(b) final maximum leaf length (cm)				
treatment 1	8.30	7.67	6.03	7.52
treatment 2	8.60	8.33	6.12	8.05
treatment 3	8.35	7.85	5.97	7.66
treatment 4	8.65	8.08	6.68	7.96
mean	8.48	7.98	6.20	7.80

**Table 5** ANCOVA of the effects of maternal treatment and maternal identity on parameter *a* (asymptotic size) in growth equation  $y = a - br^x$ , which was fit to repeated measures of leaf number for each plant. Initial seed mass (log transformed) is a covariate. Identity of maternal plant is nested within maternal treatment and treated as a random effect (model II). All interactions were highly nonsignificant.  $\eta^2$  is the effect size

Source of variation	d.f.	SS	F	P	$\eta^2$
Seed mass	1	1.06	0.05	0.829	0.0
Block	9	299.54	1.47	0.161	0.073
Treatment	2	3335.31	73.81	<0.001	0.466
Maternal treatment	3	280.99	3.45	0.029	0.069
Maternal plant	29	787.75	1.20	0.234	0.171
Residual	169	3818.31			

**Table 6** ANCOVA of the effects of maternal treatment and maternal identity on parameter *r* (initial growth rate) in growth equation  $y = a - br^x$ , which was fit to repeated measures of leaf number for each plant. Initial seed mass (log transformed) is a covariate. Identity of maternal plant is nested within maternal treatment and treated as a random effect (model II). All interactions were highly nonsignificant.  $\eta^2$  is the effect size

Source of variation	d.f.	SS	F	P	$\eta^2$
Seed mass	1	1.71	3.13	0.079	0.018
Block	9	7.43	1.51	0.147	0.075
Treatment	2	59.33	54.36	<0.001	0.391
Maternal treatment	3	2.76	1.53	0.219	0.029
Maternal plant	29	17.45	1.10	0.340	0.159
Residual	169	92.23			

**Table 7** ANCOVA of the effects of maternal treatment and maternal identity on parameter *a* (asymptotic size) in growth equation  $y = a - br^x$ , which was fit to repeated measures of length of the longest leaf for each plant. Initial seed mass (log transformed) is a covariate. Identity of maternal plant is nested within maternal treatment and treated as a random effect (model II). All interactions were nonsignificant.  $\eta^2$  is the effect size

Source of variation	d.f.	SS	F	P	$\eta^2$
Seed mass	1	3.07	2.44	0.120	0.014
Block	9	36.06	3.19	0.001	0.145
Treatment	2	86.68	34.50	<0.001	0.290
Maternal treatment	3	21.35	3.25	0.036	0.091
Maternal plant	29	63.49	1.74	0.016	0.230
Residual	169	212.33			

**Table 8** ANCOVA of the effects of maternal treatment and maternal identity on parameter  $r$  (initial growth rate) in growth equation  $y = a - br^x$ , which was fit to repeated measures of length of the longest leaf for each plant. Initial seed mass (log transformed) is a covariate. Identity of maternal plant is nested within maternal treatment and treated as a random effect (model II). All interactions were highly nonsignificant.  $\eta^2$  is the effect size

Source of variation	d.f.	SS	$F$	$P$	$\eta^2$
Seed mass	1	166.19	6.25	0.013	0.036
Block	9	679.44	2.84	0.004	0.131
Treatment	2	1450.69	27.27	<0.001	0.244
Maternal treatment	3	409.12	7.86	<0.001	0.083
Maternal plant	29	503.43	0.65	0.912	0.101
Residual	169	4495.13			

**Table 9** ANCOVA of the effects of maternal treatment and maternal identity on plant mass (rosette + root) at the end of the experiment, with initial seed mass (log transformed) as a covariate. Identity of maternal plant is nested within maternal treatment and treated as a random effect (Model-II).  $\eta^2$  is the effect size. No interactions were significant

Source of variation	d.f.	SS	$F$	$P$	$\eta^2$
Seed mass	1	0.177	2.408	0.122	0.010
Block	9	2.789	4.210	<0.001	0.139
Treatment	2	27.801	188.842	<0.001	0.617
Maternal treatment	3	0.385	1.049	0.386	0.022
Maternal plant	29	3.546	1.661	0.022	0.171
Residual	234	17.224			

tributed to the variation in seed mass. The generalization that seed mass is one of the least plastic plant characters (Harper *et al.* 1970) remains one of the most compelling in plant population biology. A given genotype produces a distribution of seed masses, but it will respond to the environment through the number of seeds it produces. Plants vary the mass distribution of seeds produced only when plasticity in the number of seeds produced is somehow limited by developmental constraints. For example, cultivated sunflowers (*Helianthus annuus*), which have been bred to bear a single apical capitulum vary seed weight greatly in response to density, whereas the highly branched wild sunflower shows very little variation in seed weight (Harper 1977). In the present study, differences among mothers within a treatment were probably due to genetic variation, rather than variation in environmental conditions within maternal treatments, which the design minimized. Some of this variation could be due to genetic maternal effects.

The competitive regime in which the offspring were grown had a large effect on all the measures of their performance. There were also significant effects of maternal plant identity, and these could not be explained by differences in seed mass. Although maternal treatment had major effects on the physiology, morphology, growth, and resultant size of the maternal plants, and huge effects on the number of seeds they produced, these effects did not result in major changes in the mass or quality of seeds and offspring produced. Even when maternal treatment was significant, the effect sizes were quite small. The

absence of significant interactions between offspring and maternal treatment implies that, even under competition, offspring of mothers from stressful environments did not perform worse than offspring of mothers from less stressful environments. Our results suggest that, even though environmental maternal effects on offspring quality do occur, they may not be very important compared with other influences acting on plant fitness. The importance of a plant's environment on its fitness is manifest primarily through the number offspring it produces, and only secondarily, if at all, through their quality (Sultan 1996).

The differences in offspring performance due to variation among mothers within maternal treatments were much larger than the nonsignificant differences due to the differences among maternal treatments. Had the identity of mother plants not been recorded and included in the analysis, the effects of maternal treatment would have appeared to be highly significant (e.g.  $P < 0.001$  for final plant mass). The failure to recognize maternal identity as a potential type of pseudoreplication (Hurlbert 1984) may account for the apparent evidence for environmental maternal effects in plants in some of the earlier studies. When variation due to maternal identity is included in the analysis, evidence for environmental maternal effects is usually absent, weak, or transitory (e.g. Alexander & Wulff 1985; Schmitt, Niles & Wulff 1992; Platenkamp & Shaw 1993; Wulff, Cáceres & Schmitt 1994; Sultan 1996).

Environmental effects on seed mass represent the strongest cases of environmental maternal effects, although even these effects are often transitory. It



has been argued that competition can exacerbate the influence of seed size, and thus increase the importance of environmental maternal effects (Roach & Wulff 1987; Stratton 1989; Schmid & Dolt 1994). The absence of interactions between initial seed mass and the competitive regime under which the offspring grew in the present study suggests that competition did not accentuate the effects of seed mass. If competition is symmetric, i.e. if the competitive effect of a neighbour is proportional to its size, a larger seedling (from a larger seed) will have an absolute, but not an increasing, advantage over the course of competition. The extreme initial advantage due to size occurs only when competition is asymmetric, and this seems to occur when competition for light is very important (Weiner 1990).

Although environmental maternal effects on offspring quality are detectable, they are quite weak compared with many of the other influences on an offspring's fitness, such as its genotype and the environment in which it grows.

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