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Parental environmental effects on life history traits in *Arabidopsis thaliana* (Brassicaceae)

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SUMMARY

Environmentally induced maternal effects on offspring phenotype are well known in plants. When genotypes or maternal lineages are replicated and raised in different environmental conditions, the phenotype of their offspring often depends on the environment in which the parents developed. However, the degree to which such maternal effects are maintained over subsequent generations has not been documented in many taxa. Here we report the results of a study designed to assess the effects of parental environment on vegetative and reproductive traits, using glasshouse-raised maternal lines sampled from natural populations of *Arabidopsis thaliana*. Replicates of five highly selfed lines from each of four wild populations were cultivated in two abiotic environments in the glasshouse, and the quality and performance of seeds derived from these two environments were examined over two generations. We found that offspring phenotype was strongly influenced by parental environment, but because the parental environments differed with respect to the time of seed harvest, it was not possible to distinguish clearly between parental environmental effects and the possible (but unlikely) effects of seed age on offspring phenotype. We observed a rapid decline in the expression of ancestral environmental effects, and no main environmental effects on progeny phenotype persisted in the second generation. The mechanism of transmission of environmental effects did not appear to be associated with the quantity or quality of reserves in the seeds, suggesting that environmental effects may be transmitted across subsequent generations via some mechanism that generates environment-specific gene expression.

Key words: *Arabidopsis thaliana*, environmental effects, maternal effects, non-Mendelian inheritance, parental effects, phenotypic plasticity, seeds.

INTRODUCTION

Parental effects on offspring phenotype in general, and maternal environmental effects in particular, are of interest to population biologists and evolutionary ecologists since it has become clear that these effects have ecological and evolutionary impacts on flowering plant populations (Alexander & Wulff, 1985; Mazer, 1987, 1992; Roach & Wulff, 1987; Stratton, 1989). Even small differences among the environments in which parental (or even grandparental) plants are raised can generate large phenotypic differences among their progeny with respect to both size and performance (Austin, 1966a,b; Gutterman *et al.*, 1975; Pet & Garretsen, 1983; Alexander & Wulff, 1985; Wulff & Alexander, 1985; Wulff, 1986; Miao *et al.*, 1991; Schmitt *et al.*, 1992; Wulff & Bazzaz, 1992; Curtis *et al.*, 1994; Delesalle & Blum, 1994; Hume, 1994; Mazer & Gorchov, 1996). Because such maternal environmental effects can result in the transmission of phenotypic effects across

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generations independently of the genes expressed by the maternal nuclear genotype, maternal environmental effects may mask additive genetic variation. When additive genetic variation is overwhelmed by maternal environmental effects on offspring phenotype, the response to selection on targeted traits may be retarded or may progress in unexpected directions (Kirkpatrick & Lande, 1989; Stratton, 1989). For these reasons, and because environmental heterogeneity is a common feature of natural populations, it is important to take parental effects into account when attempting to predict the evolutionary trajectory of fitness-related traits in wild species.

Difficulties in predicting the response to selection on traits that influence plant survivorship and reproduction are particularly intense if parental environmental effects on offspring performance persist over many generations (Alexander & Wulff, 1985; Miao *et al.*, 1991). Few studies have evaluated in detail the specific factors that may induce maternal environmental effects within a single species, and the degree to which such effects may be expressed over more than one generation.

Mazer & Gorchov (1996) proposed three mechanisms that might generate maternal effects on offspring phenotype or performance. Firstly, the availability of resources: because angiosperm seeds remain attached to the maternal parent throughout their development, the resource base accessible to a maternal plant can influence the provisioning of offspring. Secondly, environmental effects on maternal gene expression: the environment experienced by a maternal plant may affect gene expression within its seed coats, egg cells, embryos and developing endosperm. This environment-specific gene expression may influence progeny phenotype. Thirdly, environment-specific selection: different external environments may impose distinct selective regimes on populations of developing ovules, pollen tubes or seeds, leading to a generation of progeny differing both genetically and phenotypically among the environments in which they were produced.

Here we report results of a glasshouse study designed to assess the effects of parental environment on offspring phenotype, and to determine which of the above mechanisms may be responsible for the observed patterns of transmission. In addition, we evaluate the degree to which environmentally induced effects on progeny phenotype endure for more than one generation. We used field-collected maternal seed families (maternal lines) of *Arabidopsis thaliana*. This small annual herb was highly suitable for this study due to its short life cycle (2–3 months seed to seed) and small size (10–50 cm in height). Because of its high selfing rate ($<0.3\%$ outcrossing; Abbott & Gomes, 1989), maternal and paternal effects cannot be distinguished, as the maternal environment is by definition the same as the paternal environment for selfed progeny. Thus we refer to the environmental effects detected in this experiment as 'parental' environmental effects.

Homozygous lines of seeds sampled from wild populations were grown in two different combinations of sowing date, ambient temperature and time of harvest. The seeds produced by these lines were then cultivated simultaneously for two generations in a uniform environment to investigate the effects of initial parental environment on progeny phenotype for several vegetative and reproductive characters. Because two generations of descendants were evaluated, both parental and grandparental environmental effects on offspring phenotype could be measured.

The following questions are addressed: (1) what are the immediate and cross-generational effects of the parental environment on the expression of vegetative and reproductive traits? (2) does the magnitude of parental environmental effects persist across generations? and (3) what are the characteristics of the seeds produced under different environmental conditions?

MATERIALS AND METHODS

Plant material

Four wild populations of *Arabidopsis thaliana* (L.) (Brassicaceae) were chosen for study on the basis of seed availability and abundance. One population was sampled from southern France (population A: St-Jean-Cap-Ferrat, Alpes-Maritimes, collected May 1993), one from England (population B: Oxford University Botanic Garden, collected November 1993) and two from central France (Malsherbes, Loiret): population C sampled from a shaded site, and population D sampled from a sunny site, collected April 1991). From each field population, seeds from five mature maternal plants were collected and then cultivated in the glasshouse. From one glasshouse-raised offspring per field-collected plant, seeds were collected at maturity. The seeds produced by this plant represent a maternal line in this experiment, and because of the high selfing rate of this species we believe the seeds of the maternal line to be highly homozygous. The seeds produced by these glasshouse-raised plants were collected in February 1994 from population A, in March 1993 from population B, and in July 1993 from populations C and D. Seeds were removed from each maternal plant and stored in a seed cabinet at 4°C, 35% r.h.

Cultivation

For all the treatments described, plants were raised from seed in a glasshouse at the Université Paris-Sud XI (Orsay, France) and provided with supplementary lighting by mercury lamps to ensure a

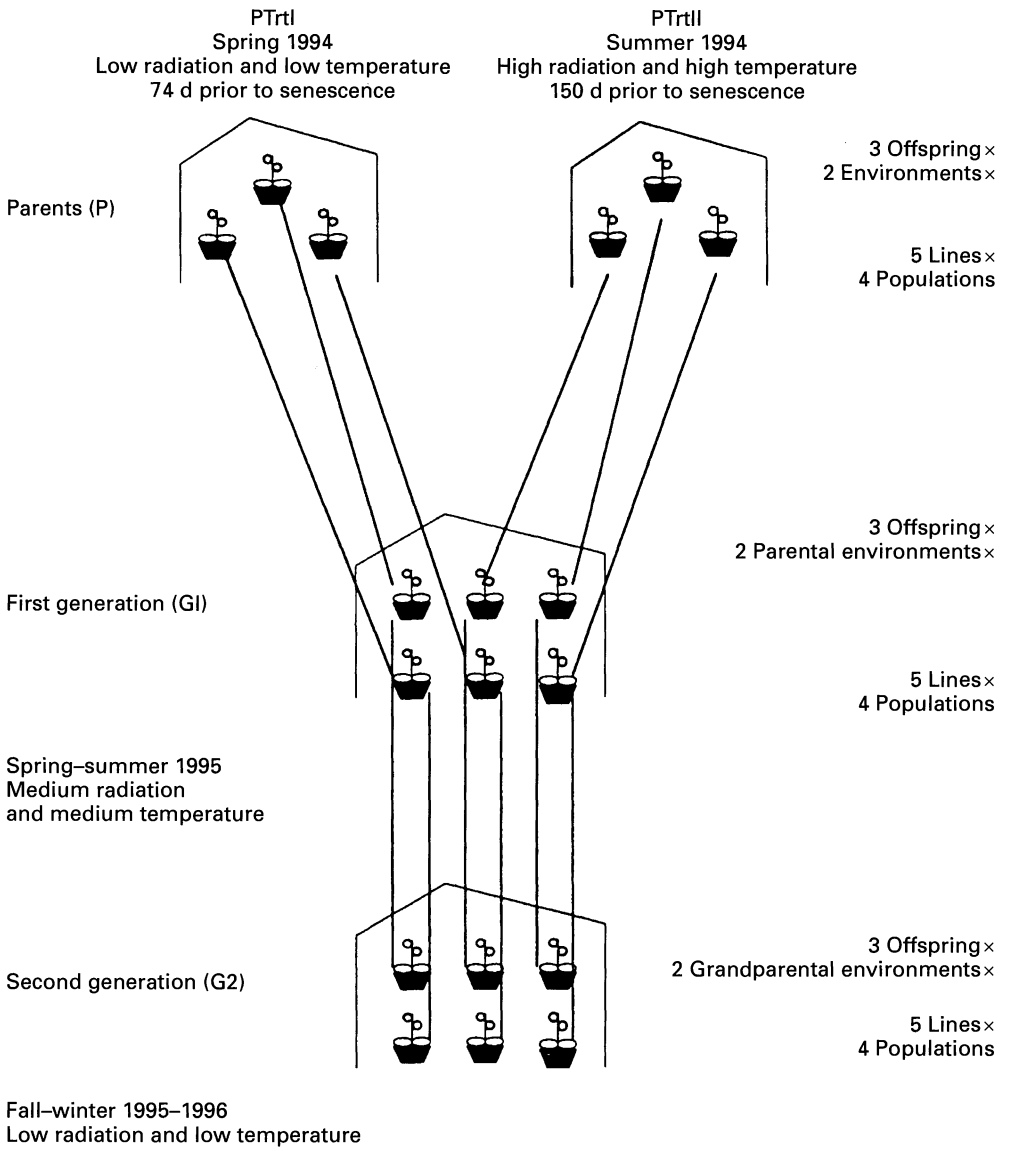

Fig. 1. Experimental design. In each generation, 120 plants were cultivated. In the parental generation, three offspring representing each of five maternal lines sampled from each of four field populations were raised in two environments. In the first generation (G1), one offspring was raised from each of the 120 plants in the parental generation. In the second generation (G2), one offspring was raised from each of the 120 plants in the first generation.

16-h day length. Although there was some temperature regulation, the glasshouse temperature was closely correlated with the outdoor ambient temperature. Throughout the duration of a similar experiment conducted during spring and summer 1996, temperatures were recorded inside and outside the glasshouse each minute to provide hourly mean temperatures. When outdoor temperatures were above 17°C, the correlation between outdoor temperatures and those inside the glasshouse was strongly positive ($R = 0.86$, $P < 0.0001$, $N = 775$). When outdoor temperatures were below 17°C, the correlation was also significantly positive, but somewhat weaker ($R = 0.60$, $P < 0.0001$, $N = 1809$).

In each treatment, the same cultivation procedure was used. A large number of seeds was sown in 6 cm diameter pots, with one maternal line sown in each pot. Each pot was filled with a mixture of peat and compost and covered with a Petri dish. The pots were pre-incubated in a cold dark chamber at 4°C for 7 d to break dormancy, and pots were then transferred to the glasshouse. For the purpose of recording date of flowering, day 1 was taken to be the day on which seeds were sown.

At day 21, seedlings were transplanted into individual pots (6 cm diameter). All the pots were watered daily as needed, received no additional fertilizer, and were spatially randomized every 2 wk. For each plant, when the primary floral stem reached 15 cm in height, an arasystem (a transparent cylinder (Betatech)) was placed on each plant to collect all the seeds. At the end of each generation, as plants senesced and dried, each plant was cut at the soil surface and the seeds were collected. In each

Table 1. *Summary of ANOVA conducted on parental generation plants to determine the effects of the environment, population, line and the interactions between them on two vegative or reproductive parameters (see Fig. 2 for treatment means)*

Source of variation	F/R[a]	df	Total seed mass MS	*P*	Shoot mass MS	*P*
Parental environment	F	1	25536	*	287096	**
Population	F	3	170241	***	142320	***
Line (population)	R	16	3432	ns	15234	ns
Parental environment × population	F	3	20491	**	49998	ns
Parental environment × line (population)	R	16	3599	ns	21112	*
Residual			2832		9675	
Residual df[b]			75		69	

[a]F, fixed effect; R, random effect.
[b]Because of missing data points, residual df are not the same for all tests.
***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

generation, a total of 120 plants was cultivated (six plants for each original maternal lineage × five lineages × four populations).

Experimental design

The experiment was initiated in 1994 by sowing replicate maternal lines in the glasshouse in each of two environments (the two environmental treatments were sown 3 months apart); we refer to this generation as the parental generation (Fig. 1).

Parental generation (P). The first treatment (PTrtI) was initiated in spring 1994: during the first 30 d of the experiment, the mean daily radiation outdoors was 9.6 MJ m^{-2}, and the mean ambient outdoor temperature was 9.1°C.

In the glasshouse, many seeds of each line were sown as described, and 3 wk later three seedlings per line were transplanted into separate 6 cm diameter pots. In this treatment, watering ceased at day 74 (at which time almost all plants had completely senesced), and all plants were harvested (three plants per maternal line × five lines × four populations).

The second treatment (PTrtII), begun July 1994, was a repetition of the first (the same five maternal lines per population were used), except that plants grew in a summer environment. During the first 30 d the mean daily radiation outdoors was 20.4 MJ m^{-2} and the mean outdoor temperature was 22.4°C. In this treatment, watering continued until all the plants had completely ripened seeds.

Because the abiotic environments differed between these two treatments, plants developed at different rates. Thus the time required for full fruit maturation was shorter during the summer (PTrtI) than during the autumn (PTrtII). In PTrtI, most plants (except those of population A) had completely ripened seeds when harvested (at day 74). The amount of time between sowing and harvesting was much longer in PTrtII (at day 150). In both treatments, however, seeds were counted and harvested when plants had reached their final reproductive output. Because the seeds were produced at different times, it was not possible to distinguish formally between the abiotic parental environmental conditions and the time of harvest (which determined the length of seed storage prior to cultivation of the next generation) as the proximal cause of phenotypic differences observed among the offspring of plants raised in these two environments (see the Discussion section).

First and second generations (G1 and G2). The offspring of the plants of both parental treatments were grown simultaneously under the same environmental conditions in order to investigate potential parental environmental effects on progeny phenotype. To generate the first (G1) generation following the parental generation, on 6 April 1995 seeds from each parental plant were separately sown in individual 6 cm diameter pots, and 3 wk later one seedling representing each parental plant was transplanted into another pot (Fig. 1). When mature (July–August 1995), seeds from these plants were harvested. Thus this first generation offspring of the same seed parent was studied that had been generated in the two parental environments.

Next, in order to study possible grandparental environmental effects on performance, the offspring of this first generation were grown simultaneously in a uniform environment. To produce this second generation (G2), seeds produced by each G1 plant were sown in separate pots (the seeds of one G1 plant

Table 2. *Summary of ANOVA conducted on plants of the first generation (G1) to determine the effects of parental environment, population, line and the interactions between them, on six vegetative or reproductive parameters (see Fig. 5 for treatment means)*

			Total seed mass		Shoot mass		Number of leaves at day 50		Number of leaves at flowering		Number of days until flowering		Number of days before first silicula	
Source of variation	F/R[a]	df	MS	*P*	MS	*P*	MS	*P*	MS	*P*	MS	*P*	MS	*P*
Parental treatment (PTrtI versus PTrtII)	F	1	52501	**	538680	***	0.2	ns	508	ns	32	ns	175	**
Population	F	3	156839	***	56779	ns	502	***	17080	***	2076	***	1840	***
Line (population)	R	16	2704	ns	17781	ns	51	ns	83	ns	55	ns	12	ns
Parental treatment × population	F	3	9860	ns	65823	**	86	ns	483	ns	266	*	107	**
Parental treatment × line (population)	R	16	4595	ns	11826	ns	63	*	218	ns	69	*	20	ns
Residual			3659		16189		29		140		31		19	
Residual df			80		80		80		77		77		76	

[a]F, fixed effect; R, random effect.
***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

Table 3. *Summary of ANOVA conducted on plants of the second generation (G2) to determine the effects of the grandparental treatment, population, line and the interactions between them on four vegative or reproductive parameters*

			Total seed mass		Shoot mass		Number of leaves at day 35		Number of leaves at flowering	
Source of variation	F/R[a]	df	MS	*P*	MS	*P*	MS	*P*	MS	*P*
Grandparental treatment	F	1	1773	ns	114	ns	29	ns	76	ns
Population	F	3	156611	***	52166	**	8	ns	13491	***
Line (population)	R	16	1191	ns	9708	ns	13	ns	634	ns
Grandparental treatment × population	F	3	1531	ns	7404	ns	14	ns	216	ns
Grandparental treatment × line (population)	R	16	1672	ns	16660	**	21	**	766	ns
Residual			1359		6144		8		688	
Residual df			71		71		71		71	

[a]F, fixed effect; R, random effect.
***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

in each pot) on 17 October, 1995, and 3 wk later one seedling representing each G1 plant was transplanted into a separate pot. When mature (February 1996), these plants were harvested.

Measured characters

Parental generation. When the plants of PTrtI and PTrtII were harvested in each environment, the seeds were collected and weighed (total seed mass), and for five seeds per individual plant, length and width were measured and the seed volume was calculated. In addition, the nitrogen content of seeds was measured for each of three individuals of each maternal line representing population C: 4 mg seed was collected from each plant and the nitrogen content was measured from the combustion of seed samples in a Carlo Erba elemental analyser (model NA1500NCS, Thermoquest, Courtaboeuf, France). Except for the roots, all vegetative parts (stems, leaves and empty siliculas) of the adult plants produced in PTrtI and PTrtII were dried at 80°C for 3 d and their dry mass was recorded as shoot mass. The data from the two treatments (PTrtI and PTrtII) were pooled for statistical analysis.

First generation (G1). The following traits were recorded or estimated for each individual: total seed mass, total volume of seeds, shoot mass, number of leaves at day 50, number of leaves at flowering, number of days until flowering, and number of days until production of the first silicula. The criterion for determining first silicula production was the protrusion of the silicula from the corolla.

Table 4. *Summary of ANOVA conducted on seeds produced by parental plants cultivated in two treatments (PTrtI and PTrtII) to determine the effects of the parental treatment, population, line, individual and the interactions between them on seed volume (see Fig. 4 for treatment means)*

			Volume of one seed	
Source of variation	F/R[a]	df	MS	*P*
Treatment (PTrtI or PTrtII)	F	1	735675830	**
Population	F	3	1070811354	**
Line (population)	R	16	150786984	ns
Individual (population × line)	R	39	81795343	ns
Treatment × population	F	3	447121561	**
Treatment × line (population)	R	16	69610931	ns
Treatment × Individual (population × line)	R	26	72476145	**
Residual		420	38901724	

[a]F, fixed effect; R, random effect.
**, $P < 0.01$.

Second generation (G2). The following traits were recorded for each individual: total seed mass, shoot mass, number of leaves at day 35, and number of leaves at flowering.

Statistical analyses

Normality of the residuals was tested for all traits and, when necessary, the data were transformed. In no case did transformation result in any qualitative changes in the statistical significance of the results. We present here the ANOVAs performed on the untransformed variables (SAS Institute, 1990).

In the parental generation, the analysis tested for significant differences among population means and among maternal lines nested within populations. The pooling of the data from the two parental treatments allowed us to test for a direct environmental effect on the phenotype of parental plants, in addition to environment × population and environment × line interactions.

Using the first generation (G1) data, the experimental design allowed us to test for the effects of the parental (P1) environment, population, maternal line, and the interactions between them on the phenotypes of G1 plants. In the second generation, we tested for the effects of the grandparental (P1) environment, population,. maternal line, and the interactions between them on the phenotypes of G2 plants. Details concerning the models are presented in the legends to Tables 1–4.

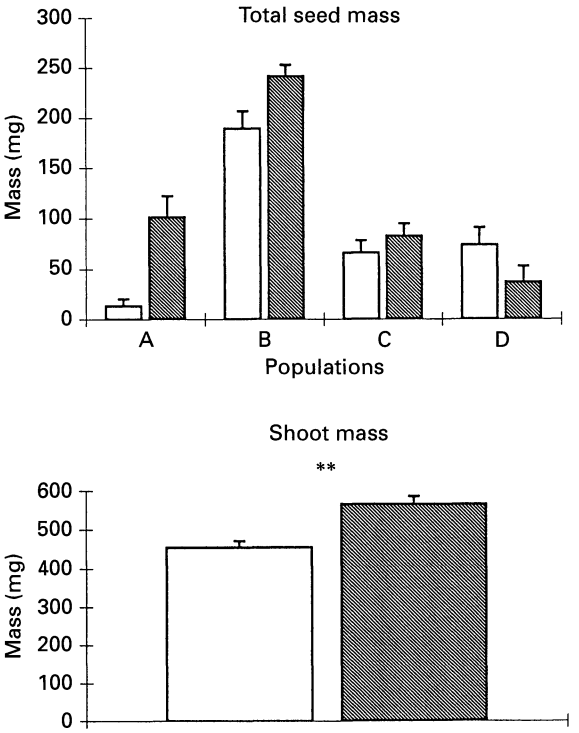

Fig. 2. Analysis of the parental generation of plants. Mean total seed mass produced by plants and mean shoot dry mass are plotted with standard errors. When the treatment × population interaction is significant, the results are plotted for each of the four populations (A–D) in each treatment (PTrtI (open columns) or PTrtII (hatched columns)), for which the sample size used to estimate each mean is 15 (five maternal lines × three plants per line). When the interaction is not significant, the results are plotted by treatment, in which the sample size used to estimate the mean is 60 (four populations × five maternal lines × three plants). ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

RESULTS

Parents TrtI and TrtII

The two treatments (PTrtI and PTrtII) included the same maternal lines, but differed with respect to their time of initiation and abiotic conditions. For total seed mass and shoot mass, significant differences were detected among the means of the four

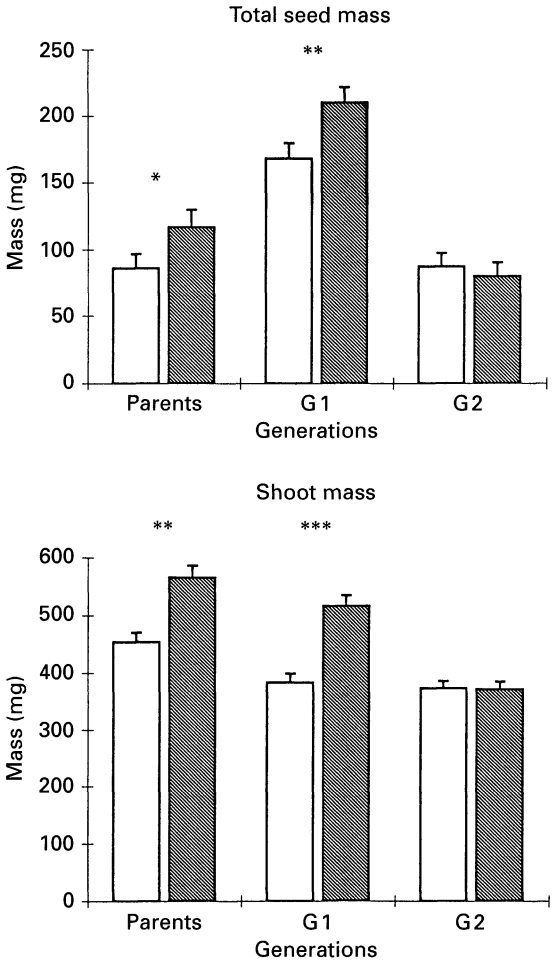

Fig. 3. Analysis of the magnitude of the initial (Parents), parental (G1) and grandparental (G2) environmental effects. PTrtI (open columns), PTrtII (hatched columns). Mean values of total seed mass and shoot mass are plotted along with standard errors. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

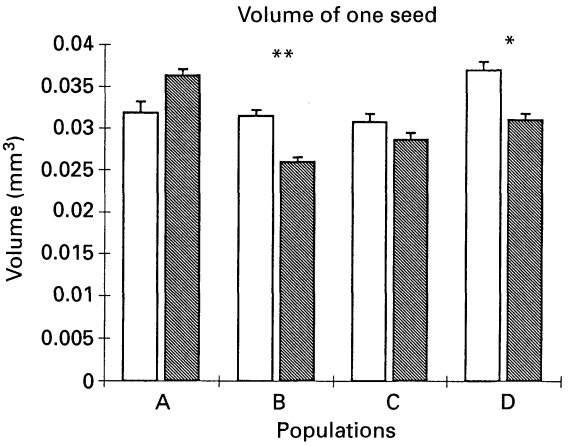

Fig. 4. Analysis of the parental generation. Mean values of the volume of one seed are plotted along with standard errors for the four populations (A–D) in each treatment (PTrtI (open columns) or PTrtII (hatched columns)), and the sample size used to estimate each mean is 75 (five maternal lines × three plants × five seeds measured). ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

populations, but within populations no significant line effect was seen.

On average, the plants raised in the second treatment (PTrtII) produced more vegetative and reproductive material than those of the first treatment. The growing conditions strongly influenced shoot mass (Fig. 2: PtrtII, mean $\pm$ SD = 566.6 $\pm$ 138.4 mg, $N = 49$; PTrtI, mean $\pm$ SD = 454.9 $\pm$ 128.1 mg, $N = 60$) in the same way for all the populations. The same pattern was observed for total seed mass per plant (Fig. 3: PTrtII, mean $\pm$ SD = 117.1 $\pm$ 94.9 mg, $N = 55$; PTrtI, mean $\pm$ SD = 85.7 $\pm$ 83.3 mg, $N = 60$), but was population-dependent (there was a significant environment × population interaction). Lines representing populations A, B and C produced higher total seed mass (and more seeds) in PTrtII than in PTrtI, while population D showed the opposite pattern. Although plants in PTrtII had higher total seed mass than plants in PTrtI, the former also produced smaller individual seeds. The higher total seed mass produced by PTrtII plants was therefore due to higher fecundity.

Parental environments also differed significantly with respect to the size of seeds produced (Fig. 4). Plants from the second treatment (PTrtII) produced smaller seeds than plants from PTrtI (0.033 versus 0.031 mm^3, measured with an ocular micrometer). Seed size appears also to have a genetic basis, as there were significant differences between populations with respect to mean seed size. The possibility that the observed differences among populations result from maternal environmental effects induced in the field and transmitted to the glasshouse-raised progeny cannot, however, be ruled out.

The significant population × parental environment interaction term indicates that the effect of parental environment on seed size differed among populations. In particular, for population C no significant difference in seed volume was observed between plants raised in the two parental environments. At a finer level, the significant parental environment × individual (nested within lines and populations) interaction term indicates that the magnitude of seed size variation between siblings also depends on the parental environment.

PTrtI and PTrtII did not differ significantly with respect to the nitrogen content of seeds produced by the lines representing population C (the only population measured for this trait). Mean nitrogen contents were 4.47 and 4.85%, respectively, for PTrtI and PTrtII.

First generation (*G*1)

For all characters measured, the population effect was significant either as a main effect or as a component of a significant interaction term (Fig. 5). However, significant parental environmental effects were detected for only some of the characters.

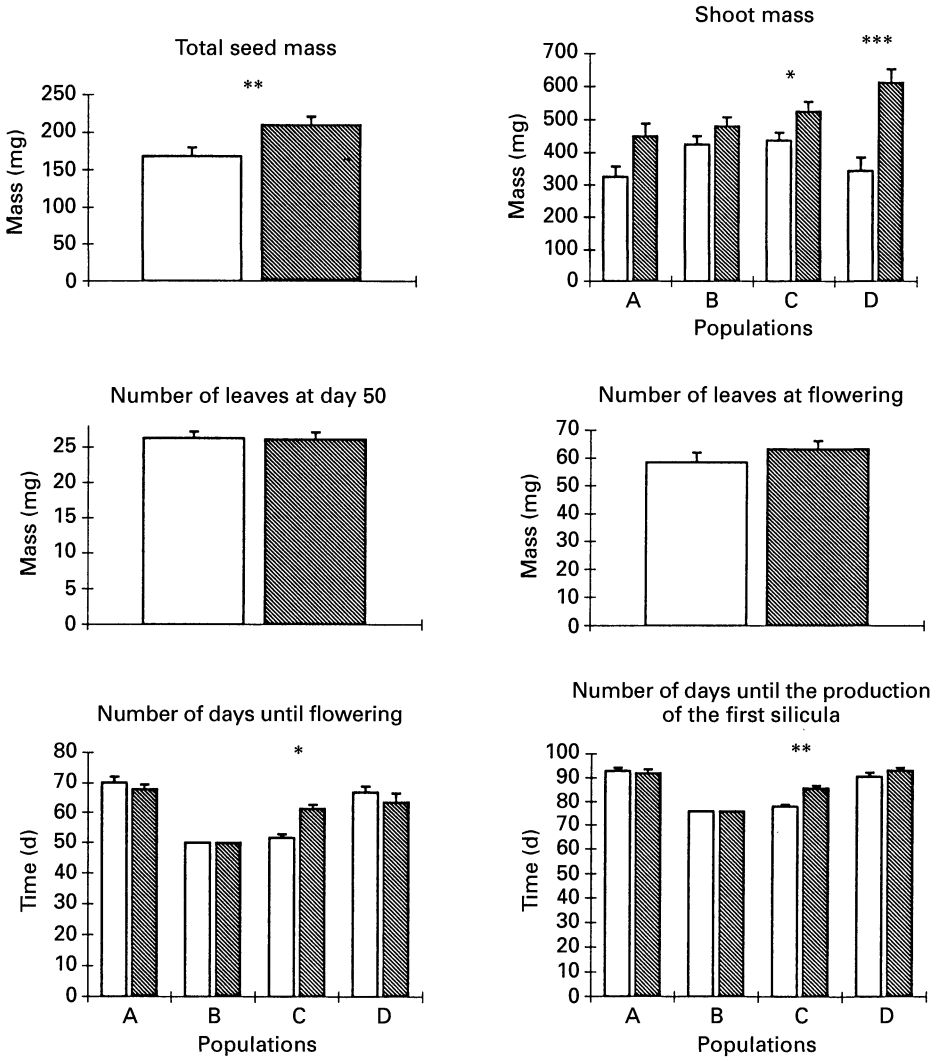

Fig. 5. Analysis of the first generation. Mean values of six parameters are plotted with standard errors. When the treatment × population interaction is significant, the results are plotted for each of the four populations (A–D) in each treatment (PTrtI (open columns) or PTrtII (hatched columns), for which the sample size used to estimate each mean is 15 (five maternal lines × three plants). When the interaction is not significant, the results are plotted by treatment, in which the sample size used to estimate the mean is 60 (four populations × five maternal lines × three plants). ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

Even though the seeds produced in PTrtII were relatively small, total seed mass per plant was higher for plants raised from seed produced in PTrtII (mean ± SD = 210 ± 87.4 mg per plant, $N = 60$) than for those raised from seed produced in PTrtI (mean ± SD = 168 ± 88.1 mg, $N = 60$). Within populations, there was no significant line effect and no interactions between factors were observed for this trait.

The shoot mass was also higher for the G1 plants derived from PTrtII than for those derived from PTrtI. This difference appeared in all four populations, although not to the same degree. This accounts for the observation that there was no significant main effect of population, although there was a significant population × parental environment interaction.

The number of leaves at flowering was not influenced by the parental environment. However, the number of days to flowering and to silicula production, and the number of leaves at day 50, depended on both the parental environment (as either a main effect or part of the interaction term) and the genotype (maternal line and/or population). In particular, population C individuals derived from PtrtII delayed flowering and fruiting in G1.

The seeds produced by the first generation (G1) plants showed no differences in seed size associated with their grandparental environment. Differences in seed size were observed among populations, however, as for the other characters reported (results not shown).

Second generation (G2)

As in the previous generations, there were significant differences among population means for all traits except for the number of leaves at 35 d. By contrast,

no significant effect of the grandparental environment was detected, except with respect to the environment × line (nested within population) interaction term for two traits: the number of leaves at 35 d, and the shoot mass. This means that the effect of the grandparental environment depended on the line observed or that the magnitude (or rank) of the differences between the lines depended strongly on which grandparental environment was considered (Table 3, Fig. 3).

DISCUSSION

Direct and persistent environmental effects

In PTrtII, plants were subjected to higher temperatures and higher levels of solar radiation than the plants cultivated in PTrtI. Environmental effects on plant performance appeared in both parental and first generations. Plants produced in PTrtII (sown in summer 1994) in general exhibited higher vegetative and reproductive biomass than plants produced in PTrtI (sown in spring 1994). In addition, the maturation period was longer in PTrtII. Because environmental factors were not controlled, however, these observed differences between treatments cannot be attributed to a specific environmental factor. The highly statistically significant effect of the parental environment on plant performance indicates that almost all maternal lines were influenced by this effect (Table 1), although the significant line × parental environment interaction indicates that the strength of the parental environmental effect varied among lines and/or populations. This may mean that there is genetically based variation in the expression of environmental effects.

The parental environment (PTrtI versus PTrtII) had a stronger effect on the vegetative growth of individuals in the first generation (G1) than it had on total seed production. Although the parental environment had no effect on the number of leaves at day 50, the number of leaves at flowering, or the number of days until flowering expressed by individuals in the first generation (G1), it did have a significant effect on shoot mass and on the number of days before production of the first silicula. The shoot mass of the offspring produced by PTrtII exceeded that of the offspring produced by PTrtI by a factor of 1.35. The number of days before producing the first silicula was higher for the offspring of PTrtII than for the offspring of PTrtI by a factor of 1.03. By comparison, the total seed mass produced by the offspring of PTrtII exceeded that produced by the offspring of PTrtI by a factor of 1.25.

The seeds produced by PTrtI and PTrtII differed in two ways: firstly, with respect to the abiotic conditions in which they developed, and secondly, with respect to the length of time they were stored prior to being sown in the G1 generation. This means that the cause of phenotypic differences between the progeny produced by the two parental treatments cannot be attributed with certainty to environmental differences between them. If there is an effect of the duration of seed storage before sowing on offspring phenotype or performance, this could have generated the parental environmental effects on offspring phenotype detected in the first and second generations. There are a few studies that report the effects of seed age on performance. Several studies have found that seed age reduces (or otherwise influences) germination rate or increases germination time (*Datura stramonium*: Pawlak *et al.*, 1990; *Festuca altaica*: Romo, 1996; *cucumber*: Rab *et al.*, 1996; *tomato*: Alsadon *et al.*, 1996; *Crambe maritima*, *Eryngium maritimum*, *Glaucium flavum*, *Honckenya peploides*, *Lathyrus japonicus*: Walmsley & Davy, 1997). The single study that detected a clear effect of seed age on adult performance was conducted by Lysgaard (1991), who found that the final yield of plants derived from 4–7-yr-old seeds of the cultivar *Brassica napus* var. *napobrassica* was 4 and 12% less than that of new seeds. In these studies, the duration of seed storage was relatively high (4–10 yr of artificial ageing), but its effect on germination or growth was rather weak. In the present experiment, seeds were stored for 10 and 5 months, respectively. If the duration of seed storage contributed to the parental environmental effects observed in our experiment, its effect was probably very minor.

All populations produced a higher shoot mass in G1 when their parents had been cultivated in PTrtII (Fig. 5), reflecting the effects of parental growing conditions on progeny performance. The environment of PTrtII seemed to promote more vigorous growth in *A. thaliana* than that of PTrtI (although the mean volume of individual seeds was generally higher for seeds produced in PTrtI), and this advantage seemed to be transmitted to the offspring. It might be expected that the transmission of these parental effects into the second generation would mirror the extent and direction of the parental effects observed in the first generation; however, this was not demonstrated.

The analysis of the second generation suggested that environmentally induced effects become undetectable after two generations, at least for the traits observed here (Fig. 3). A rapid decrease was observed in the expression of ancestral environmental effects, as no main environmental effect persisted in the second generation. There were, however, significant interactions between the grandparental environment and the maternal line involving two vegetative traits: the number of leaves in the early growth phase, and the shoot mass at the end of the growth period. These interactions demonstrated that the occurrence or the direction of transmission of the environmental effect depends on the maternal line. Such transmission over several

generations has been found in other taxa: for example, Miao *et al.* (1991) found that the maternal nutrient environment in *Plantago major* influences competitive ability over at least two generations. Similarly, Case *et al.* (1996) report that the temperature of the grandparental environment in *Plantago lanceolata* had a significant effect on all adult traits measured (including flowering time and male sterility). Interestingly, they also found that this effect was independent of seed weight, a result similar to the present observations in *A. thaliana*.

Since shoot mass was measured over three generations in the current study, it was possible to compare the magnitudes of direct environmental effects, parental environmental effects, and grandparental environmental effects for this trait (Fig. 3). In the parental generation (among the adult plants represented in PTrtI and PTrtII), both environment and line contributed to shoot mass, the environmental effects being expressed both directly and in the environment × line interaction. Plants in the PTrtII environment generally produced a higher shoot mass. In the first generation (G1), a significant parental environmental effect on this trait was detected, as was a significant parental environment × population interaction. By the second generation (G2), the initial parental environment no longer had a significant effect on shoot mass (as a main effect). Interestingly, in G2 the grandparental environment × line (nested within population) interaction was significant, indicating that the initial environmental effects were not transmitted equally across generations by all lines.

The present results suggest that the grandparental environment can influence phenotypic variation in *A. thaliana*, but these effects are much smaller than parental environmental effects, are population-specific, and are unlikely to have a major effect on plant fitness under field conditions.

The meaning of population effects

The populations represented in this experiment differed in two ways. Firstly, they were sampled from geographically separated sites, such that phenotypic differences between their progeny may have been the result of genetic differentiation among the populations. However, the possibility cannot be ruled out that maternal environmental effects were induced at each field site and persisted throughout the course of this experiment, accounting for significant population effects in many of the analyses of variance. The value of controlling for population origin in the analyses was not to draw conclusions concerning the existence of genetic differentiation among populations of *A. thaliana*, but rather to partition the variance caused by this factor in the primary effort to detect significant effects of parental (and grandparental) environments on progeny performance. If the maternal lines had been cultivated in the glasshouse for several generations prior to establishing the parental environments, this possibility would have been largely eliminated.

Possible mechanism for the transmission of parental effects

In previous studies, it has been proposed that parental effects on progeny performance are ultimately due to the effects of the maternal environment on seed size (e.g. Schaal, 1984; Stanton, 1984) or seed chemistry (e.g. Parrish & Bazzaz, 1985). In *A. thaliana*, the mechanism of transmission of parental effects seems not to be associated with the quantity of reserves in the seeds. In fact, the observed effect of the parental environmental on offspring growth was opposite to that expected based on the observed environmental effects on seed size. The seeds produced by PTrtII were smaller than those produced by PTrtI, but they developed into relatively large plants in G1, with high values of shoot mass and total seed mass. That is, plants raised in PTrtII produced relatively small seeds that nevertheless developed into plants with relatively high vegetative and reproductive biomass. This effect of seed size contrasts with that observed previously in *Raphanus raphanistrum* (also in the mustard family), in which larger seeds develop into plants with higher fitness components than small seeds (Stanton, 1984; Mazer, 1987).

We considered that parental environment effects might be caused by environmental effects on protein content. For example, a low proportion of protein in the seed reserves could act as a limiting factor during embryo development, since nitrogen is necessary for enzyme synthesis. For example, an embryo may not be able to use all the available carbohydrate reserves if it cannot synthesize a sufficient quantity of enzymes. However, no significant effects of parental environment on seed N content were found. It is possible that the observed parental effects were due to some other aspects of seed quality, such as hormone content or the thickness or permeability of the seed coat. In this case, investigation of the seed coat would be desirable, but such studies could be extremely difficult due to the small size of *A. thaliana* seeds. In addition, seed quality differences between PTrtI and PTrtII could have been caused initially by differences in the duration of seed storage before seeds were planted to generate the first generation.

Another possible explanation for parental environmental effects such as those observed here, which is doubtful in this study, is that there were differences between the selection regimes (among developing gametes or seeds) acting in PTrtI and PTrtII. Such environment-specific selection could have led to genetic differences between PTrtI and

PTrtII with respect to their surviving progeny and their descendants (Mazer & Gorchov, 1996). However, since the highly autogamous lines of seeds used here were likely to be highly homozygous, the opportunity for environment-specific selection to create environment-specific genotypes was extremely low.

A final explanation for the environmental effects observed here, which we suggest is the most convincing, is that the parental treatments resulted in environment-specific gene expression which was then passed on to G1 and G2 (as proposed by Mazer & Gorchov, 1996). The present experiment demonstrated environmental effects that were transmitted over two subsequent generations, at least in some lines. Transmission might involve interactions between the parental (and/or grandparental) environment and genotype, as detected for the lines sampled within populations. It has been proposed that the influence of such interactions is via gene expression (Stanton, 1984; Alexander & Wulff, 1985), whereby the expression of genes in the grandparental environment leads to differences in gene expression in one or more subsequent generations. This kind of parental environmental effect is consistent with that discussed by Lacey (1996).

Seed storage duration as a confounding variable

If it is true that the duration of seed storage (especially the relatively short delay in our experiment) has a strong effect on seedling performance and phenotype, then there are dozens of publications that ignore this potential effect. Every study of geographic or ecotypic variation, in which seeds are collected from each of several distinct populations at the end of their reproductive season (the timing of which can vary among conspecific populations by several weeks or months in a given year) and then sown in a common garden, could be subject to this kind of effect. In a separate literature review (S. J. Mazer & G. L. Lebuhn, unpublished), it is clear that common garden experiments conducted to detect genetically based ecotypic or geographic variation seldom consider the fact that the seeds represented by different populations matured in the field at different times and were subsequently stored for different amounts of time before sowing. Just as we surmise that such differences in storage age were not a cause of the differences among populations and parental treatments detected in this study, we suspect that such differences in storage age have a minimal effect on the detection of population differentiation in common garden experiments. Nevertheless, the potential importance of seed storage effects on experimental and field studies that include seeds of different age should no longer be ignored in studies that include seeds harvested at different times.

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