

THE EFFECT OF MATERNAL AND PATERNAL ENVIRONMENTS ON SEED CHARACTERS IN THE HERBACEOUS PLANT *CAMPANULA AMERICANA* (CAMPANULACEAE)<sup>1</sup>

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Maternal environments typically influence the phenotype of their offspring. However, the effect of the paternal environment or the potential for joint effects of both parental environments on offspring characters is poorly understood. Two populations of *Campanula americana*, a woodland herb with a variable life history, were used to determine the influence of maternal and paternal light and nutrient environments on offspring seed characters. Families were grown in the greenhouse in three levels of light or three levels of nutrients. Crosses were conducted within each environmental gradient to produce seeds with all combinations of maternal and paternal environments. On average, increasing maternal nutrient and light levels increased seed mass and decreased percentage germination. The paternal environment affected seed mass, germination time, and percentage germination. However, the influence of the paternal environment varied across maternal environments, suggesting that paternal environmental effects should be evaluated in the context of maternal environments. Significant interactions between family and the parental environments for offspring characters suggest that parental environmental effects are genetically variable. In *C. americana*, the timing of germination determines life history. Therefore parental environmental effects on germination timing, and genetic variation in those parental effects, suggest that parental environments may influence life history evolution in this system.

**Key words:** *Campanula americana*; genotype-by-environment interaction; germination; maternal effects; parental effects; paternal effects; seed mass.

An individual's phenotype or its growth environment may influence the expression of traits in its offspring. The maternal phenotype and the maternal environment have been assumed to underlie parental environmental effects for several reasons (Roach and Wulff, 1987; Schmid and Dolt, 1994; Mazer and Gorchov, 1996; Rossiter, 1996). First, the maternal parent provisions the offspring, and the quantity and quality of resources available for provisioning are strongly environment dependent. Second, two-thirds of the endosperm genetic material and the cytoplasmic DNA are typically of maternal origin and environmentally influenced gene expression may therefore disproportionately reflect the maternal environment (Mazer and Gorchov, 1996). Last, the thickness of the seed coat may be affected by the maternal environment, influencing dormancy and timing of germination (Sultan, 1996; Lacey, Smith, and Case, 1997; Baskin and Baskin, 1998). Empirical studies in plants support the expectation that maternal environments commonly influence their offspring's phenotype (Roach and Wulff, 1987; Rossiter, 1996; Donohue and Schmitt, 1998).

In contrast, we know very little about paternal environmental effects. The paternal environment's influence on the offspring is only prezygotic (Lacey, 1996). As a consequence, the mechanisms by which paternal environments may influence the offspring phenotype are less direct than those of maternal environments (Schmid and Dolt, 1994; Mazer and Gorchov, 1996). Until recently paternal environmental effects

have frequently been assumed to be negligible (Roach and Wulff, 1987). However, there is mounting evidence that the paternal environment may influence both pollen quantity and quality (e.g., Young and Stanton, 1990; Delph, Johannsson, and Stephenson, 1997; Aizen and Raffaele, 1998). But it is not known whether these environmental effects on pollen influence the offspring generation. The few studies to date have found equivocal evidence for paternal environmental effects (Young and Stanton, 1990; Schmid and Dolt, 1994; Lacey, 1996). In natural populations, the environment of pollen production, ovule production, and seed production will vary among individuals reflecting small-scale spatial environmental heterogeneity (cf. Antonovics, Clay, and Schmitt, 1987; Bell, Lechowicz, and Schoen, 1991; Stratton, 1994). As a result, pollen produced in one environmental condition will likely fertilize ovules produced in another. Due to this pattern of natural variation, it is important to determine whether both parental environments influence offspring characters. Moreover, it is possible that the expression of paternal environmental effects depends on the maternal environment. There are no studies to date that have tested whether the maternal and the paternal environments jointly affect the progeny phenotype (but see Lacey, 1996).

*Campanula americana* is a woodland herb that grows as either an annual or a biennial. Germination time determines life history schedule (Baskin and Baskin, 1984). Seeds may germinate immediately after dispersal in the late summer or fall, the following spring, or they may enter the seed bank, germinating in either season in the future (Wardle, 1998). The vegetative rosettes have a vernalization requirement for flowering. Therefore, seeds that germinate in the fall are winter annuals and flower the following summer, whereas those that germinate in the spring exhibit a strict biennial life history and flower the summer of their second year. Because parental ef-

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fects are typically strongest for juveniles, especially seed characters (Roach and Wulff, 1987; Schmitt, Niles, and Wulff, 1992; Platenkamp and Shaw, 1993; Donohue and Schmitt, 1998), they may influence life history in this system. If genetically based, parental environmental effects may play a role in the evolution of this polymorphic life history.

To determine the potential for parental environmental effects to influence life history in *C. americana*, a controlled environment study was conducted in which families from two populations were grown under a series of nutrient and light environments. Nutrient and light were chosen as environmental factors because *C. americana* is typically found in nutrient-rich soils on roadsides, stream cuts, and relatively open understory habitats where light is patchily distributed (personal observation). As a result these two resources are variable for *C. americana* and appear to limit its distribution (personal observation). This study addresses the following questions: (1) Do maternal or paternal light and nutrient environments affect seed characters in *C. americana*? (2) If so, do the maternal and the paternal environments influence offspring characters independently? (3) Is there variation between populations or among families within populations for parental effects suggesting that they are genetically variable?

## MATERIALS AND METHODS

Two populations of *Campanula americana* that differ in elevation were used to evaluate the presence of parental effects in response to controlled light and nutrient environments. One population ("Mountain") is located on Bean Field mountain in Giles County, Virginia, USA (elevation 1143 m), 3.5 km from the Mountain Lake Biological Station. At this site, *C. americana* grow in the understory of an oak-hickory forest and extend into a large light gap. The second population ("River") is located on a railroad embankment along the New River at Eggleston, Virginia (elevation 488 m), 9 km from the Mountain site. At the River site, *C. americana* is shaded part of the day by deciduous trees growing along the river. Rates of fall germination, and hence the annual life history, are greater at the River site than the Mountain Site (unpublished data). In addition, dates of flowering and seed production are almost a month earlier at the River site (personal observation). Seeds were collected by plant from both populations when mature. Seeds from a single plant are likely a mix of full and half-sibs since *C. americana* is protandrous and insect pollinated (Richardson and Stephenson, 1992; Johnson, Delph, and Elderkin, 1995). I will refer to a group of siblings as a "family." Seeds were germinated later in the fall in a growth chamber (25°/15°C 12-h days). Two weeks after germination, seedlings were cold stratified for 6 wk at 5°C to induce flowering (Baskin and Baskin, 1984).

**Parental light environments**—Following cold stratification, seedlings were moved to the greenhouse and transplanted into 15.5-cm pots in a soilless potting mix (2:1 Promix BX:Perlite). At this time, eight families from the River site and eight from the Mountain site were arbitrarily selected to evaluate the influence of parental light on offspring seed and germination characters. Two to three individuals from each family were randomly assigned to each of the following light treatments: full sun ("high"), 30% neutral shade ("medium"), and 73% neutral shade ("low"). The treatment levels were chosen to span the light gradient found in natural populations (personal observation). In total, 84 individuals were grown. Groups of 4–5 individuals in the same light level were clustered and surrounded by a shade enclosure if necessary. Individuals were fertilized every 2 wk with 20:20:20 NPK fertilizer and supplemental lighting increased day length to 18 h to stimulate flowering.

**Parental nutrient environments**—Ten different families from the River site and eight from the Mountain site were selected to evaluate the effect of parental nutrient environment on seed and germination characters. Two or three individuals from each family were randomly assigned to each of the following

nutrient treatments: weekly addition of Peters NPK 20:20:20 fertilizer ("high"), no nutrient addition ("medium"), or no supplemental nutrients and the addition of 50% nutritionally inert fritted clay to the potting medium ("low"). The potting medium contained some nutrients and provided a limited nutrient supply to the medium- and the low-nutrient plants. With the exception of the low-nutrient individuals, potting conditions were identical to those for the light experiment. All plants were fertilized twice in the first 2 wk following removal from the cold. However, the fertilizer was diluted in the limited nutrient treatments to one-quarter strength for the medium and one-eighth strength for the low-nutrient individuals. The 90 plants were grown in randomized locations in a separate bay of the greenhouse from the light experiment. Supplemental lights increased day length to 18 h.

**Experimental crosses**—For each environmental gradient, individuals were crossed to create seeds with all combinations of maternal and paternal environments. Once most plants had started flowering, crosses were conducted separately for each population. Flowers were emasculated in the bud prior to anther dehiscence to prevent self-pollination. On each day of pollinations, plants were arbitrarily divided into two groups; the specific members of each group varied from day to day. In each group, pollen was collected from one newly open flower per plant. Pollen from flowers in the same treatment and population was mixed and applied to open stigmas on plants in all three treatments in the other group. A maximum of three pollinations for each paternal environment were conducted on each maternal plant. Dividing the plants into two groups permitted mixed-donor pollinations while avoiding the potential for self-pollination. However, sib-pollinations were not controlled and because on average eight families contributed to the pollen pool, one of every eight pollen donors was a sibling. Pollinations resulted in nine types of seed for each environmental gradient (3 maternal environments  $\times$  3 paternal environments). Because the same families were grown in each level of the environmental treatment, on average the paternal genetic contribution was constant across the paternal environments.

The fruits were collected when ripe, and seeds were counted in each fruit. After the fruits were collected, the aboveground biomass of each plant was harvested and weighed when dry.

**Test of parental effects**—Parental environmental effects were evaluated for seed characters. A single fruit was chosen for each paternal treatment on each maternal plant and six typical seeds were selected from the ~40 present. This resulted in ~15 seeds for each maternal-paternal combination for each family (6 seeds/paternal treatment/maternal plant  $\times$  2–3 maternal plants/maternal treatment), and on average of 45 seeds for each maternal and each paternal treatment for each family. Seeds were individually weighed on a microbalance. Seeds from all families and treatments were randomized and placed on damp filter paper in tissue culture trays. The trays were placed in an environmental chamber with 12-h days at 21°/14°C, close to optimum conditions for *C. americana* (Baskin and Baskin, 1984). The filter paper was kept moist, and seeds were checked daily for germination (extension of the radicle beyond the seed coat). Germination was scored for 100 d, although the number of days to germination through day 50 (on average 94% of the total) will be presented as inconsistent moisture influenced later germination. Percentage germination was calculated for each treatment combination for each family at day 10 for the River population and day 20 for the Mountain, near their respective germination peaks. The percentage of seeds that had germinated by the peak germination date was analyzed since total percentage germination for some treatments in the River population was not sufficiently variable for analysis (83% on average). Total germination in the Mountain population was only 43%, but the correlation between peak germination and total germination in this population was  $R = 0.90$ , therefore peak germination approximates total germination. The influence of parental environments on four characters will be presented: seed number per fruit, individual seed mass, number of days to germination, and percentage germination.

**Statistical analysis**—Response to the light and nutrient environments in the parental and offspring generations was evaluated using analysis of variance (ANOVA). For parental biomass, ANOVA included the maternal environment

and population as fixed effects and family (nested within population) as a random effect. For seed number/fruit, seed mass, and days to germination, ANOVA was conducted with maternal environment, paternal environment, and population as fixed effects. Family (nested within population) and maternal plant (nested within population, family, and maternal environment) were included as random effects. Analyses included all possible interactions except at the level of maternal plant and were conducted using PROC GLM of the SAS statistical package (SAS Institute, 1990). However, the three-way interaction among maternal environment, paternal environment, and family was not included for seed number/fruit since there was not sufficient replication at this level. In addition, for this variable interactions were combined with the error if not significant. A significant interaction between the maternal and the paternal environment indicated that the parental environments jointly contributed to expression of offspring characters. To understand the pattern of these joint parental effects, ANOVA was used to compare paternal environments within each level of the maternal environment (analyses not shown, but results are indicated on the figures as differences in paternal environment means). When there were significant parental environmental effects, Tukey multiple comparison tests were used to compare treatment levels within a factor.

Because percentage germination was calculated for each family in each maternal and paternal treatment combination, interactions between parental environments and family were not evaluated for this character. In addition, populations were analyzed separately because percentage germination was calculated at the peak germination time, and this time differed between the populations. Parental biomass was square-root transformed, days to germination natural log transformed, and percentage germination arcsine transformed to meet assumptions of normality.

Statistical interactions between the parental environment and population demonstrate that the populations respond differently to the parental environmental treatments. To understand these differences, further analyses were conducted for each population following a significant interaction. Differences in parental effects between populations expressed in a common environment are likely to be genetic. However, because the parental generation was raised from field-collected seed, environmental differences between the sites may have contributed to population differentiation through second-generation "grandparental" environmental effects (cf. Alexander and Wulff, 1985; Miao, Bazaz, and Primack, 1991; Case, Lacey, and Hopkins, 1996). The contribution of the grandparental field environment is likely to have had a much smaller effect on offspring seed characters than the disparate parental conditions in the greenhouse (e.g., Wulff et al., 1999). The interaction between the parental environments and family reveals that the expression of parental environmental effects varies across families. Variation among field-collected families in a common environment represents broadsense genetic variation (although it may include grandparental environmental effects, but see above; Kalisz and Wardle, 1994).

Last, parental effects may act directly on germination characters or may alter patterns of germination through changes in seed size. If seed mass is included in an analysis as a covariate, then the analysis tests for the presence of parental effects that act independently of seed size. If seed mass is not included as a covariate, the analysis tests for parental effects that act both through seed size and independent of seed size. By exploring the consequences of including seed mass as a covariate, the contribution of parental effects on seed size to later characters can be determined.

## RESULTS

**Parental generation**—Aboveground biomass increased from  $18.2 \pm 1.3$  g in low light, to  $37.5 \pm 1.5$  g in medium light, and to  $46.9 \pm 2.4$  g for plants growing in full sun ( $F_{2,108} = 79.23$ ,  $P < 0.001$ ). There was also a dramatic response to the nutrient treatment: plant biomass increased fivefold across the nutrient gradient from  $11.2 \pm 0.5$  to  $56.6 \pm 1.8$  g ( $F_{2,107} = 424.37$ ,  $P < 0.0001$ ). Medium-nutrient plants differed from both the high- and the low-nutrient individuals ( $16.8 \pm 0.8$  g). For both gradients, the response was similar across populations

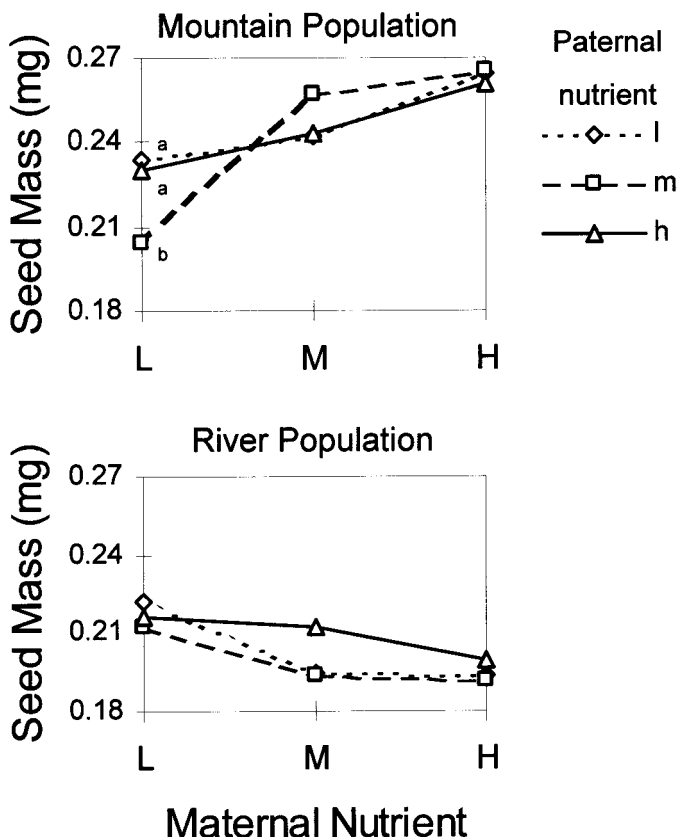


Fig. 1. Mass of individual *Campanula americana* seeds from two populations with three maternal and three paternal nutrient environments. Letters that differ within a maternal nutrient environment indicate differences in the effect of the paternal environment on seed mass at  $\alpha < 0.05$ .

(Pop  $\times$  Light,  $F_{2,97} = 0.94$ ,  $P = 0.39$ ; Pop  $\times$  Nutrient,  $F_{2,107} = 0.51$ ,  $P = 0.60$ ). In summary, for both light and nutrients there were dramatic differences between the treatment levels for the plants that served as the female and male parents to evaluate parental environmental effects.

**Offspring response to parental nutrients**—Both maternal and paternal nutrient levels influenced seed mass, but the pattern differed between populations (Mat nut  $\times$  Pop,  $F_{2,34} = 3.93$ ,  $P < 0.03$ ). Seed mass increased in response to increasing maternal nutrient levels in the Mountain population (nearly significant  $P < 0.055$ ), but decreased slightly in the River population (Fig. 1). However, the pattern of increase in the Mountain population depended on the paternal nutrient level (Table 1). Seeds produced under a low maternal nutrient environment were smaller if they were fathered by plants growing under medium-nutrient conditions; when maternal nutrients were not as limited the paternal environment did not influence seed mass (Fig. 1). In the River population, the paternal nutrient level had a nearly significant effect on seed mass (Table 1,  $P < 0.06$ ); seeds with high-nutrient fathers tended to be heavier (Fig. 1). In both populations there was a significant maternal nutrient by paternal nutrient by family interaction, revealing genetic variation in the response of seed mass to specific maternal and paternal nutrient combinations (Table 1). Therefore, the patterns of parental effects in Fig. 1 simply represent the average across families for response to the parental nutrient environment.

TABLE 1. Analysis of variance to determine the effect of maternal and paternal nutrient levels on individual seed mass in *Campanula americana*. Separate analyses were conducted for the Mountain and River populations. † 0.1 > P > 0.05, \* P < 0.05, \*\*\* P < 0.001.

Source	Mountain			River		
	df	MS	F	df	MS	F
Maternal nutrient	2,14	0.0317	3.59†	2,18	0.0252	1.31
Paternal nutrient	2,14	0.0007	0.22	2,18	0.0064	3.29†
Mat nut × Pat nut	4,24	0.0051	3.10*	4,32	0.0022	1.32
Family	7,15	0.0314	3.01*	9,16	0.0135	0.64
Mat nut × Fam	14,15	0.0098	0.34	17,25	0.0209	1.23
Pat nut × Fam	14,24	0.0031	1.82†	18,31	0.0020	1.17
Mat nut × Pat nut × Fam	23,519	0.0017	2.36***	31,758	0.0017	3.91***
Plant (Fam, Mat nut)	14,519	0.0296	40.67***	22,758	0.0166	37.43***

Variation in seed mass was not simply due to changes in the number of seeds per fruit. Seed number per fruit increased with increasing maternal nutrients from  $50.7 \pm 1.7$  in low-nutrient plants to  $61.4 \pm 1.7$  in high nutrient individuals ( $F_{2,85} = 3.73, P < 0.03$ ). Seed number per fruit and individual seed mass were not correlated on average ( $r = -0.005 \pm 0.25$ , average of correlations for each maternal environment in each population), although there was a significant positive association ( $r = 0.44$ ) between seed number and seed size under low maternal nutrient conditions in the Mountain population.

The effect of parental nutrient level on the number of days to germination differed among families in both populations, indicating genetic variation in parental nutrient effects (Table 2). There was also trend toward different patterns of parental effects in the two populations (Mat nut × Pop,  $P < 0.08$ , Mat nut × Pat nut × Pop,  $P < 0.07$ ; Table 2). However, there was no clear effect of the maternal or paternal nutrient environment on offspring days to germination in either population. Seeds from the River population germinated earlier ( $11.1 \pm 0.4$  d) than those from the Mountain population ( $14.1 \pm 0.7$  d). Seed size influenced the number of days to germination (Table 2); large seeds germinated earlier. If parental effects were allowed to influence days to germination through seed size (i.e., no covariate), there was no qualitative change in the results (analysis not shown).

Maternal nutrient level influenced percentage germination in both populations (Table 3). Although tested independently because percentage germination was calculated at the peak germination of each population, high maternal nutrient con-

ditions reduced percentage germination, while medium-nutrient conditions resulted in greater rates of germination in both populations (Fig. 2). Low maternal nutrient conditions resulted in greater germination in the River population but were statistically indistinguishable from the other maternal environments in the Mountain population. Percentage germination differed among families in the River population (Table 3). Genetic variation in maternal effects was not evaluated because family means were used to estimate percentage germination. Seed size influenced germination in both populations (Table 3): larger seeds had greater rates of germination. The pattern of parental environmental effects was the same if they were allowed to act through seed size (i.e., no covariate), instead of independently (analysis not shown). However, the pattern was not as strong and maternal nutrient in the Mountain population was no longer significant ( $P < 0.056$ ), suggesting that maternal effects due to seed size may oppose those that act independently for percentage germination.

**Offspring response to parental light**—Patterns of response of seed mass to the parental light environment were similar between populations and depended on both maternal and paternal light levels (Table 4). Under low maternal light conditions, seeds were relatively small, and the paternal environment did not affect seed size (Fig. 3). Under intermediate maternal light conditions, seeds fathered by plants in the same light environment were largest while those with high-light fathers were smallest. Seeds produced on plants grown in full sun were largest when fathered by plants grown under medium- or high-light conditions (Fig. 3). The difference in paternal effects between medium and high maternal light treatments is largely because seeds with high-light fathers increased in size with increasing maternal light while seeds with fathers in

TABLE 2. Analysis of variance to determine the effect of maternal and paternal nutrient levels on days to germination in *Campanula americana*. Days to germination was natural log transformed prior to analysis, and seed mass was used as covariate. † 0.1 > P > 0.05, \* P < 0.05, \*\* P < 0.01, \*\*\* P < 0.001.

Source	df	MS	F
Maternal nutrient	2,48	0.420	1.59
Paternal nutrient	2,63	0.229	1.66
Mat nut × Pat nut	4,104	0.182	1.45
Population	1,26	2.972	9.93**
Mat nut × Pop	2,50	0.670	2.62†
Pat nut × Pop	2,64	0.246	1.79
Mat nut × Pat nut × Pop	4,108	0.274	2.19†
Family (Pop)	16,26	0.598	1.49
Mat nut × Fam (Pop)	31,45	0.395	1.14
Pat nut × Fam (Pop)	32,54	0.163	1.13
Mat nut × Pat nut × Fam	48,701	0.148	1.54*
Seed mass	1,701	0.744	7.73**
Plant (Pop, Fam, Mat nut)	35,701	0.351	3.64***

TABLE 3. Analysis of variance to determine the effect of maternal and paternal nutrient levels on percentage germination in *Campanula americana*. Separate analyses were conducted for the Mountain and River populations. Percentage germination was arcsine transformed prior to analysis, and mean seed mass was used as covariate. \* P < 0.05, \*\* P < 0.01.

Source	Mountain			River		
	df	MS	F	df	MS	F
Maternal nutrient	2	0.480	4.31*	2	0.442	5.06**
Paternal nutrient	2	0.027	0.24	2	0.093	1.06
Mat nut × Pat nut	4	0.007	0.06	4	0.029	0.34
Family	7	0.151	1.35	9	0.281	3.21**
Seed mass	1	0.528	4.74*	1	1.013	11.58**
Error	50	0.111		65	0.087	

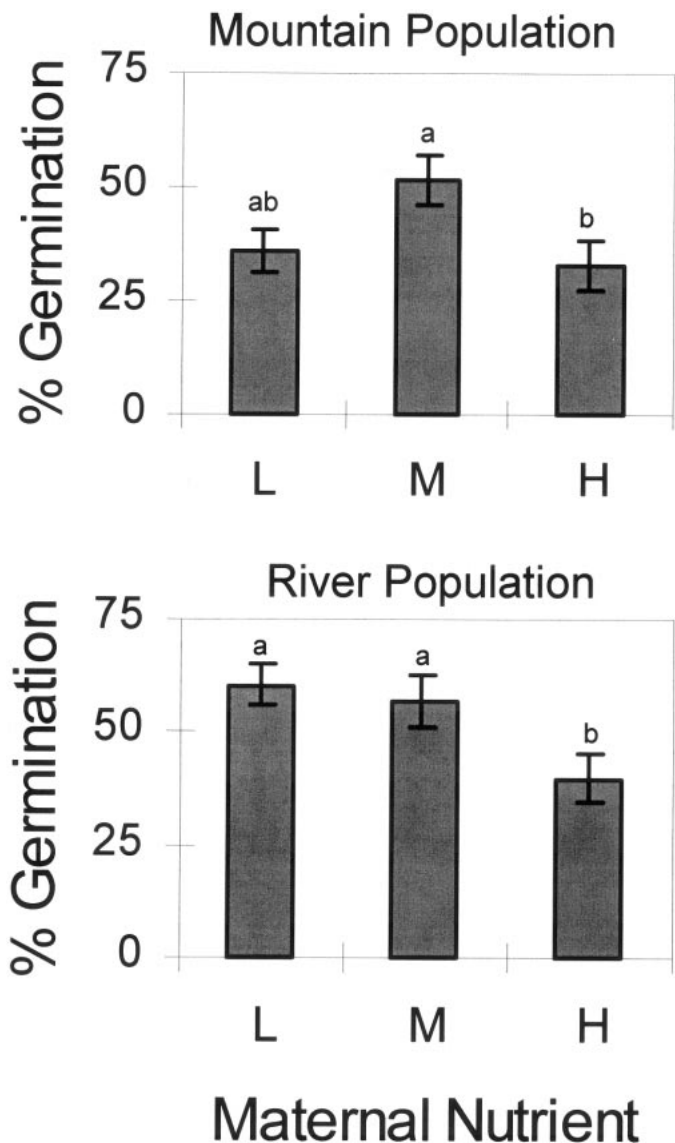


Fig. 2. Percentage germination of *Campanula americana* seeds from two populations with three maternal nutrient environments. Different letters indicate that the means within a population differ at  $\alpha < 0.05$ .

limited light conditions were largest under medium maternal light levels. There was a significant interaction among the maternal light environment, the paternal light environment, and family indicating genetic variation in parental effects in response to light for seed mass (Table 4).

Differences in seed mass across the parental light environments were not typically due to a trade-off between seed size and seed number. Seed number per fruit increased with increasing light from  $34.3 \pm 2.9$  for low light individuals to  $61.5 \pm 2.5$  for those grown under full sun ( $F_{2,52} = 9.78, P < 0.001$ ). There was a small negative correlation between the number of seeds per fruit and individual seed mass on average ( $r = -0.12 \pm 0.23$ , average of correlations for each maternal environment in each population). With the exception of a significant negative association between seed number per fruit and seed size under medium maternal light conditions in the River population ( $r = -0.61$ ), correlations were near zero and not significant.

TABLE 4. Analysis of variance to determine the effect of maternal and paternal light levels on individual seed mass in *Campanula americana*. †  $0.1 > P > 0.05$ , \*  $P < 0.05$ , \*\*\*  $P < 0.001$ .

Source	df	MS	F
Maternal light	2,28	0.0680	3.96*
Paternal light	2,29	0.0018	1.61
Mat light $\times$ Pat light	4,54	0.0089	8.65***
Population	1,14	0.1418	3.28†
Mat lt $\times$ Pop	2,28	0.0021	0.12
Pat lt $\times$ Pop	2,29	0.0003	0.27
Mat lt $\times$ Pat lt $\times$ Pop	4,54	0.0021	2.01
Family (Pop)	14,26	0.0453	2.53*
Mat lt $\times$ Fam (Pop)	27,40	0.0180	0.83
Pat lt $\times$ Fam (Pop)	28,52	0.0011	1.11
Mat lt $\times$ Pat lt $\times$ Fam	51,1285	0.0011	2.62***
Plant (Pop, Fam, Mat lt)	38,1285	0.0215	52.82***

On average, parental light environments had a greater influence on timing of germination in the Mountain population (Mat light  $\times$  Pat light  $\times$  Pop,  $F_{4,126} = 4.52, P < 0.002$ ; Fig. 4). In the Mountain population, the maternal and paternal light environments jointly affected days to germination (Table 5). While average days to germination were similar for seeds produced in all maternal light levels, those produced on maternal plants growing in low-light environments germinated earlier if their father grew in intermediate-light conditions (Fig. 4). There was also variation in days to germination in response to maternal light environment among families (Table 5), revealing genetic variation for maternal effects in days to germination in this population. Seed mass did not influence timing of germination in either population.

Both maternal and paternal light levels influenced percentage germination under controlled conditions in *C. americana*. Under low and high maternal light levels germination rates depended on the paternal environment in the Mountain population (Table 6, Fig. 5). This interaction between parental environments is largely because percentage germination increased with increasing maternal light levels for seeds with low-light fathers, while percentage germination decreased with increasing maternal light for seed with medium- or high-light fathers (Fig. 5). In the River population only the maternal light levels influenced percentage germination (Table 6, Fig. 5). Seeds from mothers grown under low light levels had a 58%

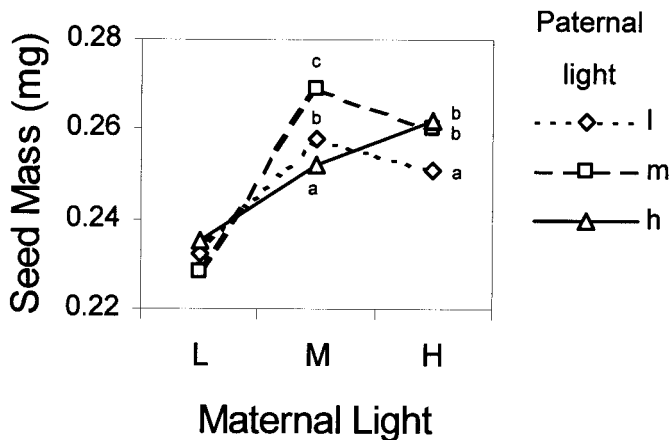


Fig. 3. Mass of individual *Campanula americana* seeds with three maternal and three paternal light environments. See Fig. 1 for details.

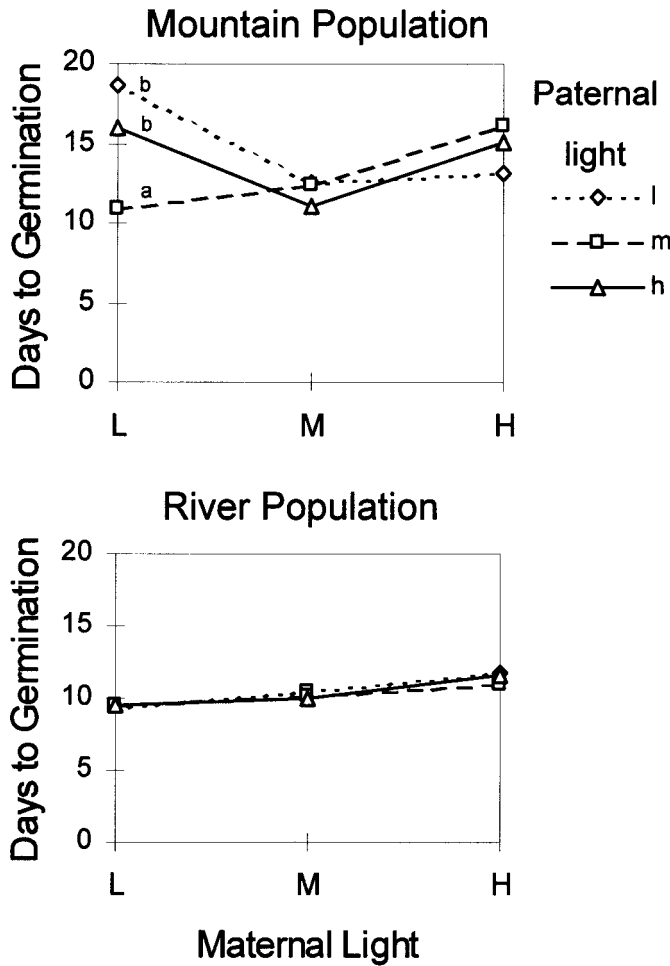


Fig. 4. Days to germination of *Campanula americana* seeds from two populations with three maternal and three paternal light environments. See Fig. 1 for details.

germination rate, while those from mothers grown under high- and medium-light levels germinated at lesser rates (46 and 43%, respectively). Percentage germination varied among families in the Mountain population, but family variation in parental effects was not evaluated because germination percentage was calculated for each family. Seed mass did not influence percentage germination in the Mountain population, but larger seeds had greater rates of germination in the River population (Table 6).

TABLE 6. Analysis of variance to determine the effect of maternal and paternal light levels on percentage germination in *Campanula americana*. Separate analyses were conducted for the Mountain and River population. Percentage germination was arcsine transformed prior to analysis, and mean seed mass was used as covariate in the River analysis. \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .

Source	Mountain			River		
	df	MS	F	df	MS	F
Maternal light	2	0.015	0.22	2	0.507	5.26**
Paternal light	2	0.088	1.28	2	0.028	0.28
Mat It × Pat It	4	0.226	3.30*	4	0.034	0.35
Family	7	0.512	7.48***	7	0.155	1.61
Seed mass	—	—	—	1	0.836	8.67**
Error	52	0.068		53	0.096	

DISCUSSION

Both parental light and nutrient environments influenced seed characters in *Campanula americana*. Perhaps the most striking finding was that the maternal and the paternal environments frequently influenced seed characters jointly, such that the combination of parental environments determined the offspring phenotype. For example, paternal nutrient status only affected seed mass under low maternal nutrient conditions in the Mountain population. Similarly, the paternal light environment only influenced seed size when maternal plants were grown under medium- and high-light levels. With the exception of percentage germination, interactions between the parental environments had a greater influence on offspring characters than either parental environment alone. These interactions typically resulted in the expression of paternal environmental effects under some but not all maternal environments. However, they were equally common under limited and ample maternal resources. Interaction between parental environments is supported by a study with *Plantago* that found high paternal temperature increased germination of seeds produced on low-temperature maternal plants, but decreased the germination of seeds produced on high-temperature maternal plants (Lacey, 1996). If the paternal environment typically acts jointly with the maternal environment, experiments that do not evaluate interactions between the parental environments may miss the contribution of the paternal environment to the offspring phenotype. Because studies have not explored possible interactions between the parental environments, it is not known whether the paternal environmental effects found in other studies (Schmid and Dolt, 1994; Lacey, 1996) may also vary across maternal environments.

The source of paternal environmental effects is difficult to

TABLE 5. Analysis of variance to determine the effect of maternal and paternal light levels on days to germination in *Campanula americana*. Days to germination was natural log transformed prior to analysis. † $0.1 > P > 0.05$ , \* $P < 0.05$ , \*\*\* $P < 0.001$ .

Source	Mountain			River		
	df	MS	F	df	MS	F
Maternal light	2,16	0.870	2.16	2,14	0.790	1.75
Paternal light	2,33	0.435	3.05†	2,18	0.001	0.02
Mat It × Pat It	4,20	0.504	3.64*	4,34	0.061	0.97
Family	7,11	0.518	1.03	7,14	0.552	1.10
Mat It × Fam	13,11	0.497	2.76*	14,19	0.496	1.48
Pat It × Fam	14,20	0.135	0.98	14,31	0.077	1.22
Mat It × Pat It × Fam	13,151	0.134	0.84	26,493	0.062	0.92
Plant (Fam, Mat It)	12,151	0.224	1.40	20,493	0.356	5.27***

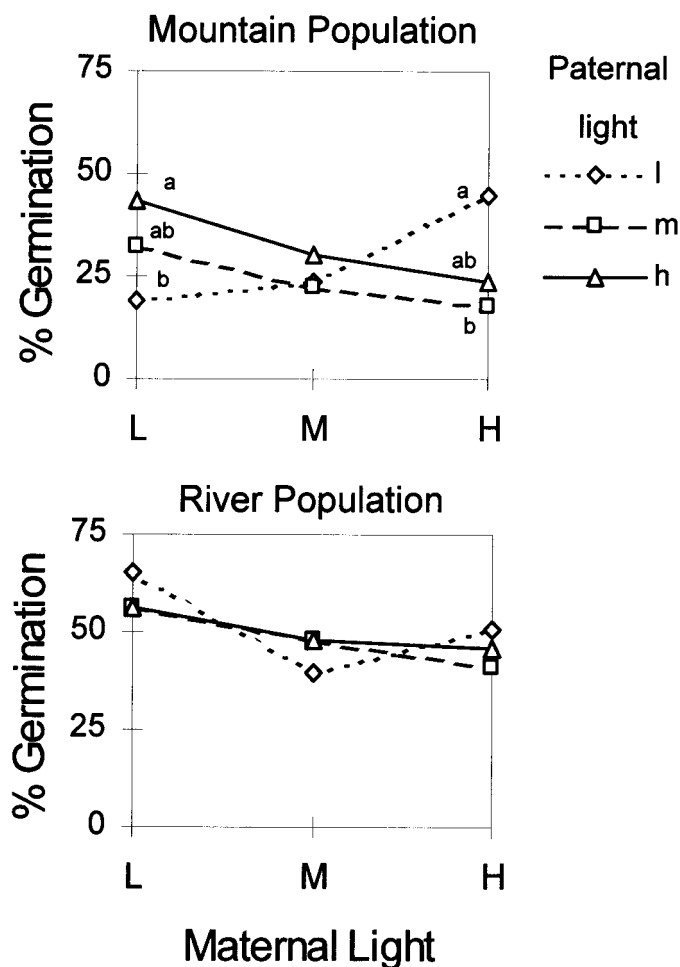


Fig. 5. Percentage germination of *Campanula americana* seeds from two populations with three maternal and three paternal light environments. See Fig. 1 for details.

determine. A number of studies have demonstrated that pollen quality and quantity varies with the environment and may affect an individual's ability to sire seeds (e.g., Young and Stanton, 1990; Delph, Johannsson, and Stephenson, 1997; Aizen and Raffaele, 1998; Lehtilä and Strauss, 1999). In natural populations, heterogeneous environments may lead to differences in siring ability among individuals due to variation in pollen quantity or quality. In the present experiment, the environment was constant across individuals within a paternal treatment. However, if the response of pollen characters to the environment varied among families (i.e., genotype-by-environment interaction), the family with the greatest amount of viable pollen may vary across environments. As a consequence, the paternal environment may bias paternity, resulting in a genetic source to the "environmental" paternal effects.

This scenario is possible in the present experiment where pollen was pooled from families in each paternal environmental treatment. In congener *C. rapunculoides*, pollen number, size, percentage germination, and pollen tube length did not vary with the environment, however, there was a genotype-by-environment interaction for pollen viability (Vogler, Peretz, and Stephenson, 1999). If true for *C. americana*, differences in pollen viability among families that also differ for offspring characters could create paternal environmental effects. How-

ever, the influence of individuals with particularly high siring ability in any given treatment should be diminished because the composition of the pollen pool differed for each recipient across days and between groups of individuals within days. In addition, paternal effects varied with the maternal environment. It seems likely that differences in pollen quality or quantity would have a consistent effect across maternal treatments (but see Marshall and Ellstrand, 1988, for a possible mechanism). The only way to fully separate the effects of the paternal environment from genotype-by-interactions for pollen characters is to compare seeds produced by clonal replicates of individuals grown in different environments (e.g., Schmid and Dolt, 1994; Lacey, 1996). We are presently using this approach to determine whether genotype-by-environment interactions contribute to the effect of paternal light levels on seed characters.

There are a number of additional potential causes of paternal environmental effects (reviewed in Mazer and Gorchov, 1996). For example, there may be selection among the developing pollen grains within each environment, such that the genetic composition of the pollen produced by a given individual depends on the environment in which the pollen matured. Alternatively, environment-specific gene expression induced in the male gametophyte may create differences between the progeny produced by the same genetic father growing in different environments. Last, paternal environmental effects may simply be due to nongenetic changes in pollen that affect the offspring phenotype, for example, resource provisioning.

In contrast to the paternal environment, the maternal environment also had an independent effect on seed characters. On average, seed mass increased with increasing maternal resources. This pattern was particularly strong between the low and medium levels for both maternal light and nutrient environments. Similarly, other studies have found seed mass to increase in response to increasing maternal light (Wolfe, 1995; Sultan, 1996) and nutrients (Aarssen and Burton, 1990; Schmitt, Niles, and Wulff, 1992; Wulff and Bazzaz, 1992), although, like the River population, there may be little response of seed mass to maternal nutrient level (Wolfe, 1995; Sultan, 1996; Weiner et al., 1997). Seed number per fruit also increased with increasing maternal resources. There was little evidence for a trade-off between seed number and seed size in *C. americana* (see also Richardson and Stephenson, 1991); on average, plants with more resources produced more larger seeds. Larger *C. americana* seeds have been found to grow faster (Richardson and Stephenson, 1992), and, therefore, favorable maternal light and nutrient environments may enhance offspring survival and fecundity via seed size effects.

Parental environments had a greater influence on percentage germination than on days to germination. While days to germination was jointly influenced by maternal and paternal light levels in the Mountain population, that interaction was largely due to paternal effects expressed when maternal plants were grown under low-light conditions. In contrast, increasing maternal resources, whether light or nutrients, decreased the fraction of seeds with early germination independent of the paternal environment for the River population and to a lesser extent the Mountain population. Similar to our findings here, seeds of high-light maternal plants had a reduced percentage germination in *Plantago* (but only under dark germination conditions; Schmitt, Niles, and Wulff, 1992). However, maternal nutrient effects on germination in other studies are not consistent. For example, high parental nutrient levels decrease (Wulff

et al., 1999) or increase (Stratton, 1989) germination percentage and slow down (Schmid and Dolt, 1994) or speed up (Stratton, 1989; Aarssen and Burton, 1990) germination. Variation in these results may be in part due to the contribution of seed size to germination. Maternal nutrient effects are likely to act through seed size in several of these studies (Stratton, 1989; Aarssen and Burton, 1990; Wulff et al., 1999).

The parental light environment influenced germination characters independent of seed size in *C. americana* (see also Richardson and Stephenson, 1992; Kalisz and Wardle, 1994). In contrast, larger seeds germinated earlier and in greater numbers than smaller seeds when parents were grown under a range of nutrient environments. If the parental nutrient environment was allowed to influence germination characters through seed mass, the magnitude of the parental effects was reduced. Therefore parental environmental effects acting through seed size appear to oppose those that act directly on juvenile characters. Other studies have shown that parental effects expressed through juvenile characters, e.g., seed size, may differ from those expressed directly on later characters (Stratton, 1989; Galloway, 1995; Sills and Nienhuis, 1995). Changes in the seed components may underlie these patterns. Seeds are composed of seed coat and the offspring tissues (endosperm and embryo). Therefore a change in seed mass may be due to either a change in offspring size or in seed coat thickness (e.g., Sultan, 1996; Lacey, Smith, and Case, 1997). Because seed mass only influenced germination characters when the parental generation was grown in a range of nutrient conditions, and the seed coat is often associated with timing of germination (cf. Sultan, 1996; Baskin and Baskin, 1998), these results suggest that the proportionate allocation to the seed coat may vary across the nutrient gradient, but change little in response to maternal light.

Parental environmental effects differed both between populations and among families within populations. Family variation in parental effects was found in almost every analysis (although it was not evaluated for percentage germination), suggesting that genetic variation for parental effects is pervasive. Genetically based parental environmental effects have been found in other studies (e.g., Schmitt, Niles, and Wulff, 1992; Plantenkamp and Shaw, 1993; Wulff, Caceres, and Schmitt, 1994; Lacey, 1996; Shaw and Byers, 1998). Genetic variation within populations provides the opportunity for parental environmental effects to contribute to adaptive evolution. Indeed, the differences between populations for parental effects found here may reflect genetic divergence in response to local selective environments.

In addition to seed characters, parental environmental effects may influence life history. Germination schedule determines life history in *C. americana*. Seeds that germinate in the fall have an annual life history, while those that germinate in the spring are biennials. The results presented here imply that both the maternal and the paternal light environment may influence life history in the Mountain population. In addition, variation among families in response to maternal light levels in the Mountain population, and in response to the joint effect of maternal and paternal nutrient environments over both populations, implies that parental environmental effects for germination time are genetically variable. Therefore, if there is an association between parental and offspring light environment (more likely for the maternal parent than the paternal parent since pollen dispersal typically exceeds seed dispersal; Levin and Kerster, 1974) and specific patterns of parental en-

vironmental effects enhance fitness, parental environmental effects may contribute to life history evolution in this species.

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