

THE INFLUENCE OF LIGHT ON PATERNAL PLANTS IN *CAMPANULA AMERICANA* (CAMPANULACEAE): POLLEN CHARACTERISTICS AND OFFSPRING TRAITS¹

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Offspring trait expression is determined by the combination of parental genes and parental environments. Although maternal environmental effects have been widely characterized, few studies have focused on paternal environmental effects. To determine whether light availability influences pollen and offspring traits in the woodland herb *Campanula americana*, we reared clones of 12 genotypes in two light levels. In the parental generation we measured pollen number and size. Plants grown under high light produced more pollen grains per flower than those grown under low light. However, the response was genotype specific; some individuals responded little to changes in light availability while others substantially reduced pollen production. As a consequence, paternity ratios may vary between light environments if more pollen is associated with greater siring success. We crossed a subset of these plants to produce the offspring generation. The paternal and maternal light environments influenced offspring seed mass, percentage germination, and days to germination, while only maternal light levels influenced later life traits, such as leaf number and size. Maternal and paternal environmental effects had opposite influences on seed mass, percentage germination and days to germination. Finally, there was no direct relationship between light effects on pollen production and offspring trait expression.

Key words: *Campanula americana*; Campanulaceae; germination; maternal effects; maternal environmental effects; paternal environmental effects; plasticity to light; pollen number.

In addition to the genetic contribution that plants make to their offspring, the parental environment may also affect phenotypic expression of traits in subsequent generations. Parental environmental effects can be transmitted to offspring either through the maternal or paternal plant (Rossiter, 1996). Because seeds mature on the maternal plant, the maternal environment can have both prezygotic and postzygotic influences on the offspring phenotype (Lacey, 1996). The ubiquity of maternal environmental effects in plants (Rowe, 1964; Schaal, 1984; Roach and Wulff, 1987; Rossiter, 1996; Mousseau and Fox, 1998) may be because all stages of ovule and seed development occur within the maternal plant. In addition, the maternal plant may influence seeds once they are mature by directing seed dispersal (Donohue, 1999). In contrast, the paternal environment can only influence offspring prezygotically (Lacey, 1996). For this reason paternal environmental effects have typically been assumed to be negligible (Roach and Wulff, 1987). However, recent results suggest that this assumption may not be valid; a few studies have demonstrated that paternal environments affect offspring trait expression (Schmid and Dolt, 1994; Lacey, 1996; Galloway, 2001a, b).

Both maternal and paternal environmental effects may influence trait evolution. Theoretical studies have shown that maternal and paternal inheritance can facilitate or retard response to selection relative to strict Mendelian inheritance (Kirkpatrick and Lande, 1989; Lande and Kirkpatrick, 1990). Furthermore, parental environmental effects may alter the pattern of selection response. For example, maternal or paternal effects on traits can result in lags in selection response or con-

tinued evolution after selection has ceased (Riska, Rutledge, and Atchley, 1985; Kirkpatrick and Lande, 1989; Lande and Kirkpatrick, 1990). To understand potential evolutionary response to selection, it is important to identify traits that are influenced by parental effects in the range of environments that natural populations experience.

Paternal environmental effects are of particular relevance to quantitative genetic studies where sire effects are assumed to reflect additive genetic variation (Falconer and Mackay, 1996). Paternal environmental effects can alter estimates of genetic variance and, therefore, potential response to selection. This is important for experiments in natural populations where the environment is likely to vary among sires. Variation in trait expression among offspring of these sires would reflect both genetic variation and variation in the paternal environment. The magnitude of paternal environmental effects on offspring trait expression may also vary among sires. Such genotype-by-environment interaction in the expression of paternal effects will contribute to the estimate of additive genetic variance. Through either mechanism, paternal environmental effects are likely to influence heritability estimates.

Environmental factors may also influence pollen number and quality. Stressful environmental conditions such as reduced nutrient levels (Young and Stanton, 1990; Lau and Stephenson, 1993) and herbivory (reviewed in Delph, Johannsson, and Stephenson, 1997) result in fewer pollen grains per flower. Plants from these resource-limited conditions also sire fewer seeds when they compete for ovules with pollen from plants with ample resources (e.g., Young and Stanton, 1990; Quesada, Bollman, and Stephenson, 1995; Aizen and Raffaele, 1998). However, it is not known whether there is a link between environmental effects on pollen characters and offspring trait expression. To determine whether environmental effects on pollen production and quality are also expressed as paternal environmental effects requires an association between envi-

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ronmental influences on pollen traits and on the offspring. To date, studies have focused on either pollen traits and their association with siring ability or the offspring generation.

In this study we examine the influence of both the maternal and paternal genotype and environment on the offspring phenotype in the forest herb *Campanula americana*. Natural populations typically span dramatically different light conditions, including deep shade under a closed canopy and full sun (forest gaps or road cuts). Seeds disperse passively and, therefore, offspring are likely to experience similar light levels to maternal plants. However, pollen dispersal may exceed seed dispersal in this bumble bee-pollinated plant; therefore, the paternal light environment may differ from the maternal light environment.

In this study we use clonal replicates of genotypes to quantify parental environmental effects in *C. americana* in response to light intensity, one component of the different light environments that populations experience in nature. We measured response of pollen number and size under full sun and 73% reduction in full spectrum light and evaluated whether these traits were associated with paternal environmental effects in the offspring. Use of clonal replicates of maternal and paternal genotypes permitted separating genetic and environmental effects on offspring traits. Specifically, we addressed the following questions: (1) Does light intensity affect paternal fitness in terms of pollen number and size? (2) Do maternal and/or paternal light environments influence offspring trait expression? (3) If so, do these parental environmental effects vary with either the maternal or paternal genotype? We report patterns of intergenerational phenotypic plasticity that can be traced to the parental genotypes and environments.

MATERIALS AND METHODS

Campanula americana L. (Campanulaceae) is a monocarpic insect-pollinated herb. It has a polymorphic life history schedule with plants growing as either annuals or biennials (Baskin and Baskin, 1984). Plants flower from mid-July through late August. The primary pollinators are bumble bees foraging for nectar and to a lesser extent, halictids collecting pollen (Johnson, Delph, and Elderkin, 1995; Galloway, Cirigliano, and Gremski, 2002). The flowers are protandrous, and most fruits contain 20–40 seeds.

The objective of this experiment was to determine the influence of paternal genotype and light environment on pollen and offspring traits. Because previous work found that the expression of paternal environmental effects depended on the maternal environment (Galloway 2001a), we also included maternal light as a factor. To examine these parental effects, we reared clones of 12 genotypes in two treatments that simulate the divergent light intensity conditions that plants experience in nature. In late August 1999, rosettes at least 0.5 m apart were collected along transects through a natural population located on Bean Field Mountain, Giles County, Virginia, USA (elevation 1143 m). After 3-mo growth in the greenhouse, plants were clonally propagated from leaf cuttings. One or two leaf sections of the rosette with associated axillary buds were dipped in rooting hormone and placed in vermiculite. Cuttings were kept moist and fertilized weekly until rooted (about 2 wk). Clones were then transplanted into pots (Promix HP potting medium; Premier Horticulture, Red Hill, Pennsylvania, USA), moved into a growth chamber, and vernalized to induce bolting (5°C, 12-h days, 6 wk). In April 2000, six clones per genotype were individually transplanted into 1-L pots (1 : 1 native soil : Promix HP) and transported to an open field at the Mountain Lake Biological Station (3.5 km from the natural population). Three clones of each genotype were grown in a high-light treatment (full sun) and three in a low-light treatment (73% reduction in full spectrum light). Plants were randomly grouped into blocks of 4–5 individuals (9 full-sun blocks, 8 shade blocks). Blocks were fenced to prevent herbivory from large mammals, and fences were surrounded with shade cloth as needed. On 17 June plants were damaged by

| | | MATERNAL GENOTYPE | | | | |
|------------------|------------|-------------------|-----|------------|-----|---|
| | | Low Light | | High Light | | |
| | | 52 | 182 | 52 | 182 | |
| PATERAL GENOTYPE | Low Light | 44 | X | X | X | X |
| | | 52 | X | X | X | X |
| | | 55 | X | X | X | X |
| | | 141 | X | X | X | X |
| | | 148 | X | X | X | X |
| | | 179 | X | X | X | X |
| | 182 | X | | X | | |
| | High Light | 44 | X | X | X | X |
| | | 52 | | X | | X |
| | | 55 | X | X | X | X |
| | | 141 | X | X | X | X |
| | | 148 | X | X | X | X |
| 179 | | X | X | X | X | |
| 182 | X | | X | | | |

Fig. 1. Schematic of the crossing design employed to produce the offspring generation. A clone of each genotype was reared in high and low light. Crosses were done in a factorial design (excluding self-fertilization) with each paternal genotype in each light treatment serving as a pollen donor for each of the two maternal genotypes in each light treatment.

voles and, depending upon the extent of the damage, were either eliminated from the experiment or branch, instead of the main-stem buds, were used as the source of pollen samples. Plants were fertilized twice a month (Peters 20 : 20 : 20 NPK) and watered as needed.

To evaluate pollen traits, we collected anthers from clones in the high- and low-light treatments. Full-size but undehiscent anthers were harvested from four early flowers per plant the day prior to anthesis (typically the first flowers). The anthers were dried at room temperature in open microcentrifuge tubes for approximately 2 wk and then stored until analysis. Modal pollen diameter and the number of pollen grains per flower were measured by rehydrating the pollen in a 2% NaCl solution and then analyzing the solution with an electronic particle counter (Elzone 280PC) the following day. In some cases, the distribution of pollen diameter was bimodal, indicating that a fraction of the pollen was aborted. We quantified this by calculating the percentage of pollen that was aborted.

A single high- and low-light clone of each of seven genotypes was selected without respect to pollen traits to serve as parents for the next generation. These plants were moved into a single block in each light level. Two genotypes were designated as females (MG). All genotypes were used as pollen donors (PG) and crossed to both MGs. However, self-crosses were not conducted, the two MGs, therefore, served as pollen donors to only one MG. As a result, each MG was crossed to six PG (Fig. 1). Because our primary research objective was to characterize paternal effects, more plants served as males than females in the crossing design. Crosses included all possible combinations of paternal light (PL) and maternal light (ML) environments for a total of 48 different cross types (6 PG × 2 PL × 2 MG × 2 ML = 48). Flowers were emasculated during the male phase and protected from pollinators with plastic straws until the initiation of the female phase the following day. Female-phase flowers were hand-pollinated and protected from pollinators for one more day, at which time they had wilted, indicating successful fertilization. Three replicate pollinations of each cross type were successfully completed.

The influence of parental light environment on offspring characters was evaluated in the greenhouse. Seeds from different fruits of the same cross type were pooled. Ten seeds were randomly selected from each cross type and individually weighed. A total of 472 seeds were used because only two seeds were produced for one of the cross types (full design, 48 cross types × 10 seeds/cross type). The seeds were germinated in a fully randomized design in a growth chamber under near-optimal conditions (21°C /14°C, 12-h days; Baskin and Baskin, 1984). Seeds were individually placed on the surface of 1.5-mL microcentrifuge tubes filled with potting mix (Promix HP). Seeds were kept moist and germination was monitored daily for 40 d (only one seed

TABLE 1. Analysis of variance to determine the effect of genotype and light level on pollen traits in *Campanula americana*. * $P < 0.05$; *** $P < 0.001$.

| Source | Pollen number | | Pollen diameter | |
|-------------------------|---------------|----------|-----------------|-------|
| | df | F | df | F |
| Genotype | 11, 11 | 8.85*** | 11, 11 | 4.06* |
| Light | 1, 12 | 34.68*** | 1, 12 | 0.92 |
| Genotype \times light | 11, 177 | 2.19* | 11, 142 | 1.27 |
| Block | 2, 177 | 8.09*** | 2, 142 | 1.13 |

germinated after day 24). Thirty days after the start of the experiment, seedlings were transplanted into 15-cm (6-inch) Cone-tainers (Stuewe and Sons, Corvallis, Oregon, USA) filled with potting mix (Metromix 200; Scotts-Sierra Horticultural Products Company, Marysville, Ohio, USA). The seedlings were vernalized for 8 wk to induce flowering (5°C, 12-h days). Following vernalization, plants were transferred to the greenhouse where leaf number and the length of the longest leaf were recorded. Adult traits were not measured because of disease damage incurred in the greenhouse.

Statistical analysis—Parental traits, pollen number and diameter, were analyzed using mixed-model analysis of variance (ANOVA). Groups of three adjacent blocks were combined in each light level to create three new blocks. The new blocks reflected a spatial gradient in daily light levels in the field, with the blocks of each treatment closer to the field edge in shadow for longer in the morning than those in the field center. Block and light treatment were considered fixed effects and paternal genotype a random effect. We included two blocking factors that reflected the position of the flower on the plant in the initial analyses (branch vs. mainstem and node position). Neither of these factors explained a significant fraction of the variation and therefore were not included in the final analysis. The following factors were included in the final model: genotype, light treatment, genotype \times light treatment, and block.

Progeny traits were analyzed with a factorial mixed-model ANOVA (PROC GLM, SAS Institute, 2000) with maternal genotype and maternal and paternal light treatment considered fixed effects and paternal genotype a random effect. There was not sufficient replication to evaluate the four-way interaction, therefore only three-way interactions are reported. Parental effects may act directly on later-life traits or through changes in seed size. If seed mass is included as a covariate, the analysis tests for parental effects that act both through seed size and independent of seed size. Seed mass was initially included as a covariate in the analyses of days to germination, leaf number, and leaf size, but was only retained for leaf size, where it accounted for a significant fraction of the variance. All variables satisfied the ANOVA assumptions of homoscedasticity and normality of the residuals and therefore were not transformed. Variance among progeny attributable to maternal light (ML) and paternal light (PL) is interpreted as direct parental environmental effects. The maternal and paternal genotype factors (MG or PG) indicate direct genetic inheritance in the broad sense. The interaction between the parental genotypes and their environments (MG \times ML and PG \times PL) indicates that parental environmental effects varied among genotypes. Likewise, interactions between maternal (paternal) genotype and paternal (maternal) environment (MG \times PL and PG \times ML) suggest that the offspring phenotype is determined jointly by one parent's genotype and the other parent's environment. The interaction between the maternal and paternal genotype (MG \times PG) indicates that phenotypic expression of the progeny sired by each genotype differed when the paternal genes were expressed in the alternate maternal genetic backgrounds. A significant maternal \times paternal light interaction (ML \times PL) suggests that the parental environments interact to jointly influence the progeny phenotype. Higher order interactions indicate that the expression of the above interactions varies with the parental environment or genotype.

Presence or absence of germination was analyzed using a loglinear analysis assuming a binomial distribution and a logit link (PROC GENMOD, SAS Institute, 2000). Maternal and paternal genotype and maternal and paternal light levels were included as factors and likelihood-ratio tests were used to

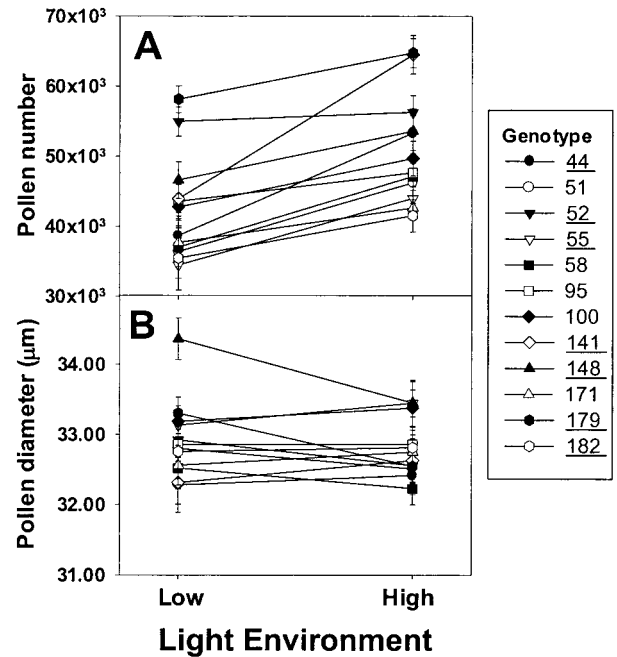


Fig. 2. Least-square means (± 1 SE) of pollen number and pollen diameter for each of the 12 genotypes in the parental generation. (A) Number of pollen grains per flower for clones of each genotype reared in high- and low-light treatments. (B) Modal pollen diameter for each genotype reared in high- and low-light treatments. Genotypes chosen to serve as parents in the crossing design are underlined.

determine the appropriate model (Agresti, 1996). The fully saturated model did not converge due to insufficient replication; however, models converged when the four-way interaction was not included.

To determine whether there was a relationship between plasticity in response to light for paternal traits and plasticity in response to paternal light in the offspring generation, we calculated the correlation between the coefficient of variation for pollen traits (e.g., pollen number) and the coefficient of variation for each offspring trait. However, the sample size of seven paternal genotypes did not permit a robust analysis. Therefore, we report a qualitative comparison of paternal and offspring response to the paternal environment using the ANOVAs on progeny traits (above).

RESULTS

Pollen traits—The light environment influenced pollen number. Pollen number ranged from 26 066 to 74 569 pollen grains per flower, and modal pollen diameter from 30.53 to 36.50 μ m in diameter. Genotypes differed significantly with respect to both of these traits (Table 1, Fig. 2). The light environment significantly influenced pollen number, with plants reared in the low-light treatment producing an average of 17% less pollen than plants reared in the high-light treatment (Table 1, Fig. 2). Genotypes varied in the degree to which pollen number differed between the light environments (Table 1). For example, pollen number did not differ between the high- and low-light clones of genotype 52. In contrast, the low-light clones of genotype 141 produced 32% less pollen than the high-light clones (Fig. 2). Pollen diameter was not influenced by the light treatment, nor was there evidence of a genotype \times environment interaction for this trait.

The pollen diameter distributions for two genotypes were consistently bimodal, indicating pollen abortion. Genotypes 55 and 148 had an average pollen abortion fraction of 25 and

TABLE 2. Analysis of variance to determine the effect of parental genotype and light environment on seed juvenile traits in *Campanula americana*. †*P* < 0.10; **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

| Source | Seed mass | | Days of germination | | Leaf number | | Leaf size | |
|------------------------|-----------|-----------|---------------------|----------|-------------|----------|-----------|----------|
| | df | <i>F</i> | df | <i>F</i> | df | <i>F</i> | df | <i>F</i> |
| Paternal genotype (PG) | 6, <1 | 2.50 | 6, <1 | 2.49 | 6, <1 | 3.21 | 6, <1 | -9.13 |
| Paternal light (PL) | 1, 6 | 14.41** | 1, 7 | 0.02 | 1, 9 | 0.14 | 1, 11 | 0.03 |
| Maternal genotype (MG) | 1, 4 | 22.01** | 1, 5 | 9.10* | 1, 6 | 2.47 | 1, 12 | 8.71* |
| Maternal light (ML) | 1, 6 | 6.76* | 1, 7 | 3.81† | 1, 10 | 55.13*** | 1, 12 | 20.69*** |
| PG × MG | 4, 5 | 0.47 | 4, 4 | 0.62 | 4, 1 | 1.93 | 4, 4 | 0.23 |
| PG × PL | 6, 9 | 0.17 | 6, 4 | 0.53 | 6, 2 | 1.20 | 6, 2 | 0.88 |
| PG × ML | 6, 8 | 1.66 | 6, 3 | 1.38 | 6, 3 | 0.40 | 6, 3 | 0.69 |
| PL × MG | 1, 4 | 3.89 | 1, 5 | 0.24 | 1, 10 | 2.93 | 1, 5 | 0.54 |
| PL × ML | 1, 6 | 8.26* | 1, 9 | 3.62† | 1, 8 | 1.86 | 1, 10 | 1.61 |
| MG × ML | 1, 4 | 148.04*** | 1, 5 | 2.27 | 1, 6 | 7.00* | 1, 10 | 0.64 |
| PG × PL × MG | 4, 427 | 10.39*** | 4, 283 | 3.90** | 4, 239 | 0.48 | 4, 238 | 1.39 |
| PG × PL × ML | 6, 427 | 9.88*** | 6, 283 | 1.10 | 6, 239 | 1.31 | 6, 238 | 0.80 |
| PL × MG × ML | 1, 427 | 18.21*** | 1, 283 | 1.14 | 1, 239 | 0.21 | 1, 238 | 0.74 |
| PG × MG × ML | 4, 427 | 3.21* | 4, 283 | 1.43 | 4, 239 | 0.98 | 4, 238 | 1.76 |
| Seed mass | — | — | — | — | — | — | 1, 238 | 14.19*** |

70%, respectively. Percentage abortion was associated with light level for genotype 148, which produced 52% aborted pollen in the high-light treatment but 88% in the low-light treatment. The other genotypes had little aborted pollen; over 97% of samples were unimodal.

Offspring traits—Seed mass was influenced by the paternal light environment (Table 2, Fig. 3A and B). Seeds sired by males reared in high light were significantly heavier than those sired by males reared in low light (mean mass H = 0.261 and L = 0.241 mg). However, the maternal environmental effect on seed mass was in the opposite direction; seeds that developed on maternal plants in low light were on average heavier

than those from high-light plants (mean mass H = 0.258 and L = 0.284 mg). The maternal genotypes differed with regard to the light environment that promoted the development of heavier seeds (MG 52, low light; MG 182, high light, MG × ML; Fig. 3A and B). Ultimately, seed mass was determined by complex interactions between the maternal and paternal genotypes and light environments; all three-way interactions between these factors were significant (Table 2).

Germination percentage of seeds was influenced by the combination of paternal genotype and the paternal and maternal light environments. Overall, 69.4% of seeds from the controlled crosses germinated. Germination was significantly influenced by paternal genotype (Table 3, Fig. 4); percentage

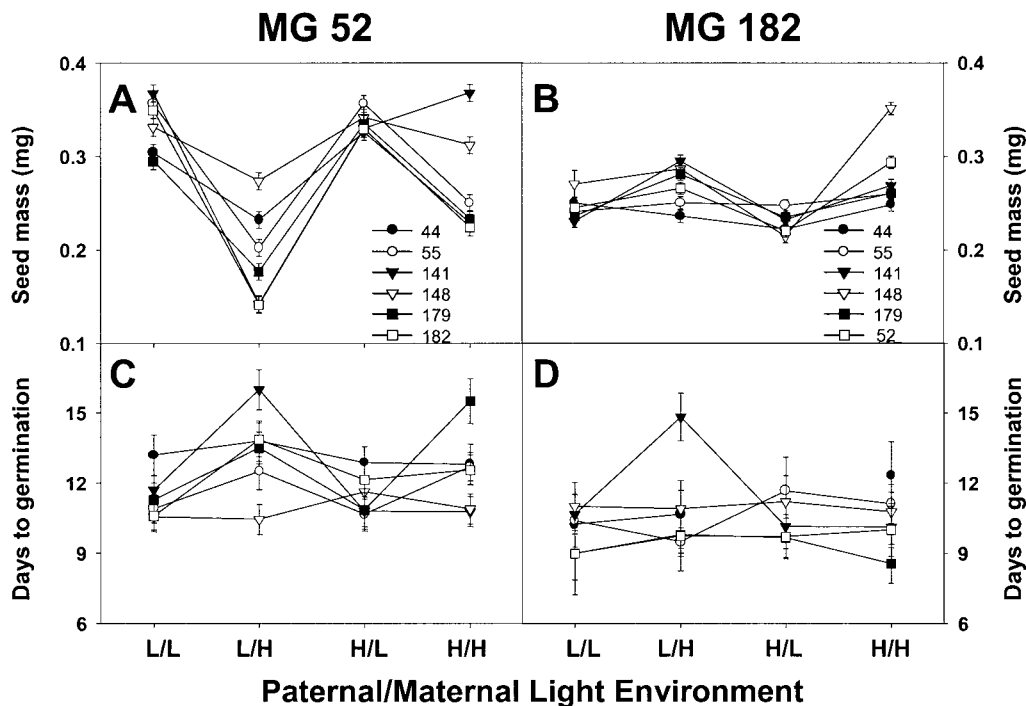


Fig. 3. Least-square means (±1 SE) of seed mass and number of days to germination for each combination of parental genotypes in each light treatment. Seed mass of progeny of (A) MG 52 and (B) MG 182. Number of days to germination of progeny of (C) MG 52 and (D) MG 182.

TABLE 3. Loglinear analysis to determine the association between germination success and parental genotype and light environment.

| Source | df | χ^2 | P |
|----------------------------|----|----------|--------|
| Paternal genotype (PG) | 6 | 61.54 | <0.001 |
| Paternal light (PL) | 1 | 2.23 | <0.14 |
| Maternal genotype (MG) | 1 | 1.16 | <0.28 |
| Maternal light (ML) | 1 | 0.46 | <0.50 |
| PG \times MG | 4 | 9.52 | <0.05 |
| PL \times ML | 1 | 12.41 | <0.001 |
| MG \times ML | 1 | 6.09 | <0.02 |
| PG \times PL \times ML | 18 | 34.95 | <0.01 |

germination per male genotype ranged from 41% (PG 44) to 92% (PG 148). There were no significant main effects of maternal genotype on seed germination. However, specific combinations of maternal and paternal genotypes differed with regard to percentage germination (PG \times MG, Table 3). For example, 57% more seed germinated from crosses between PG 44 and MG 52 than between PG 44 and MG 182. The two maternal genotypes differed with regard to the light treatment that resulted in high rates of seed germination (MG \times ML, Table 3). Twenty-one percent more seed germinated from the low-light clone of MG 52 compared to the high-light clone. In contrast, 10% more seed germinated from the high-light clone of MG 182 as compared to the low-light clone. Maternal and paternal light treatments jointly influenced germination, such that seeds whose parents experienced the same light environment had higher percentage germination ($74 \pm 5\%$) than those whose parents experienced different light environments ($69 \pm 5\%$, PL \times ML). However, the exact pattern differed among paternal genotypes (Table 3, Fig. 4).

The number of days to germination was governed by the interaction between parental genotypes and the paternal light treatment (PG \times PL \times MG, Table 2). The influence of paternal light on number of days to germination varied among different combinations of parental genotypes (Fig. 3C and D). Maternal genotype was the only factor that directly influenced the number of days to germination; seeds from MG 52 germinated an average of 1.5 d slower than those of MG 182 (Table 2). The maternal light environment did not influence the days to germination.

Neither paternal genotype nor the paternal light environment influenced leaf number or size in the progeny generation. However, the maternal environment influenced offspring vegetative traits. Offspring from maternal plants reared in low light produced 27% more leaves than those from maternal plants reared in high light (Table 2, Fig. 5). The strength of this pattern differed among the maternal genotypes and was more evident for MG 52 (MG \times ML). When the effects of seed mass were statistically removed by including this trait as a covariate, there were significant maternal genotype and light effects; progeny from maternal plants reared in low light produced leaves that were on average 23% larger than progeny from maternal plants reared in high light (Table 2, Fig. 5). When seed mass was excluded from the model, and therefore the parental effects of seed mass may also influence leaf size, more parental effects were found including MG \times ML ($F_{1,5} = 10.96, P < 0.02$) as well as PG \times PL \times MG ($F_{4,239} = 3.08, P < 0.02$) in addition to the maternal genotype and light effects reported in Table 2.

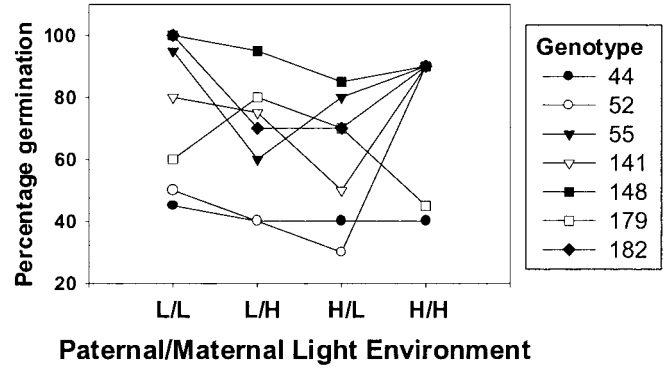


Fig. 4. Percentage germination of seeds sired by each paternal genotype when the maternal and paternal plants were grown under low-light and high-light levels. Data are averaged over two maternal genotypes.

Comparison of pollen traits to offspring traits—Paternal genotypes differed in their response to light for pollen number. If this variation in response is associated with variation in offspring trait expression, i.e., genotypes that differ between light environments for pollen traits have offspring that also differ across the paternal light levels, it would be expressed as a paternal genotype \times paternal light interaction (PG \times PL). The combination of paternal light and genotype influenced offspring expression of seed mass, percentage germination, and number of days to germination (Tables 2, 3). In all cases expression of the interaction varied with either maternal genotype or maternal light. For example, for percentage germination the expression of the PG \times PL differed with maternal light environment (Table 3). The PG \times PL interaction did not influence percentage germination when maternal plants were reared in low light ($\chi^2 = 8.92, df = 6, P < 0.18$), whereas it did when they were grown in high light ($\chi^2 = 18.15, df = 6,$

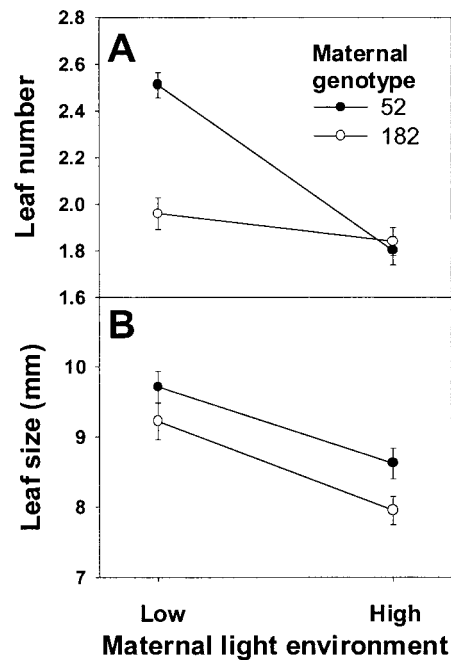


Fig. 5. Least-square means (± 1 SE) of (A) leaf number and (B) leaf size for offspring of two maternal genotypes that were each grown under low- and high-light conditions.

$P < 0.006$). For seeds of high-light maternal plants, there was not a relationship between changes in paternal pollen number across the light environments and paternal environmental effects on percentage germination. For example, genotype 52 had similar pollen number in both light environments (low light : 54 965 pollen grains; high light : 56 316 pollen grains) but percentage germination was greater for offspring of high-light paternal plants than low-light plants ($\chi^2 = 5.94$, $df = 1$, $P < 0.015$; cf. Figs. 2, 4). In contrast, pollen number for genotype 141 differed dramatically across the light environments (low light : 43 954 pollen grains; high light : 64 505 pollen grains), but paternal light level did not influence percentage germination ($\chi^2 = 1.80$, $df = 1$, $P < 0.18$). Similarly for seed mass and days to germination, the magnitude of a genotype's response to light for pollen traits did not predict the expression of paternal environmental effects.

DISCUSSION

Campanula americana is a highly outcrossing (Galloway, Etterson, and Hamrick, unpublished manuscript), bumble bee-pollinated woodland herb. Natural populations often span divergent light environments, from deep shade to full sun. Plants have no special dispersal mechanisms; seeds fall passively from pores on erect capsules when jostled by wind or animals. Thus the offspring light environment is likely to be similar to the maternal light environment. However, pollen is likely to disperse more widely than seed (Levin and Kerster, 1974; McCauley, 1994), providing the opportunity for plants from different light conditions to parent offspring. The primary goal of this study was to examine the consequences of differences in light intensity on paternal traits and the offspring that they sire. A further goal was to relate plasticity of these paternal traits to plasticity in the offspring generation.

Our findings indicate that the paternal light environment may create differences in paternity among genotypes. Plants reared under low light produced substantially less pollen than those grown in high light. However, the degree of the response was genotype specific. As a consequence, the rank order of pollen production among genotypes differed between the two light treatments. Thus, if the number of offspring sired is correlated with pollen number, a different collection of genotypes may sire more seeds in each light environment. In this manner, genotype \times environment interactions for pollen number could alter paternity in different light regimes.

The paternal environment may also directly influence the expression of traits in the offspring. Paternal environmental effects were found for all seed and germination characters but not for juvenile traits, whereas maternal environmental effects were detected for all traits. Maternal environmental effects are typically strong in the juvenile stage of the life cycle (reviewed in Roach and Wulff, 1987; Donohue and Schmitt, 1998). Similarly, paternal environments are likely to have the greatest effect early in the life cycle due to the proximity of these traits to the prezygotic stage. While our results support this generalization, there is limited support for the attenuation of paternal environmental effects over an individual's life span in other studies. The paternal environment only influenced adult vegetative traits of greenhouse-grown offspring in *Solidago* (Schmid and Dolt, 1994) and percentage germination and flowering in *Plantago* (Lacey, 1996; Lacey and Herr, 2000). In *C. americana*, the paternal environment may also affect later life-cycle characters indirectly. For example, when seed

mass was included as a covariate in the analysis of leaf size, there was no evidence for paternal environmental effects. However, when the covariate was removed, the paternal environment significantly influenced leaf size. This indicates that the paternal environment indirectly influences leaf size, a later life-cycle character, through its effects on seed size, an early life-cycle character. Ultimately, this paternal environmental influence on phenotypic expression may affect fitness. In a natural population, rosette leaf length explains 50% of the variation in fruit number (L. F. Galloway, unpublished data).

A previous study in *C. americana* found that maternal and paternal light levels influenced seed and germination traits (Galloway, 2001a, b). The experimental design simulated conditions of natural populations where flowers receive mixed pollen loads from several donors. However because of this design, it was not possible to unambiguously ascribe paternal effects to environmental factors. Pollination with mixed pollen loads provided the opportunity for genotypes to compete. If families differed in siring success in different light levels, paternity may have been biased in the treatments resulting in a genetic source to the "environmental" paternal effects. Genotype \times environment interactions for pollen number, viability, or performance could produce such an effect. In the current study, we were able to distinguish genetic and environmental sources of paternal effects because we reared clones of each genotype in each light environment and conducted crosses using single pollen donors. We found both genotype \times environment interactions for pollen number and paternal environmental effects on seed mass, percentage germination, and days to germination. This suggests that paternal environments act both through changes in paternity as well as by direct influence on offspring trait expression.

Offspring traits were influenced by interactions between parental genotypes and environments and between parental genotypes. This is an important finding because genetic variation is necessary for adaptive parental environmental effects to evolve. For all traits except leaf size, the expression of parental environmental effects varied with the maternal or paternal genotype. Similarly, the influence of maternal light on offspring trait expression consistently varies among genotypes in other studies (e.g., Schmitt, Niles, and Wulff, 1992; Wulff, Caceres, and Schmitt, 1994; Sultan, 1996), as does the expression of paternal environmental effects (Schmid and Dolt, 1994; Lacey, 1996). Parental genotypes may also interact with each other to influence trait expression. We found that specific combinations of maternal and paternal genotypes influenced offspring seed mass, the probability of germination, and germination timing. This supports a previous study on different populations of *C. americana* that found that parents contributed jointly to days to germination and initial leaf production (Richardson and Stephenson, 1992).

The combination of the maternal and paternal light environments often influenced offspring trait expression. For all traits where both parental environments influenced the offspring, the maternal and paternal environments had opposing effects on the phenotype. Seeds borne on maternal plants reared in full sun weighed less, germinated at a greater rate, and germinated slower. In contrast, seeds sired by plants reared in full sun weighed more, germinated at a lower rate, and germinated faster. Differences in fertilization success could have influenced seed number per fruit and thus seed mass. However, previous work found no trade-off between seed size and seed number for this population under a similar series of

light conditions (Galloway, 2001a). The two other species in which paternal environmental effects have been documented also found opposing contributions of the maternal and paternal environments to trait expression (Schmid and Dolt, 1994; Lacey, 1996). For example, in *Plantago* there was a greater germination percentage when paternal plants were grown under cool temperatures and when maternal plants were grown in warm conditions (Lacey, 1996). Examples of other types of inheritance where several factors influence trait expression show similar patterns of opposing influences. For example, nuclear and cytoplasmic genes have been shown to have opposing effects on trait expression (Galloway and Fenster, 2001). Similarly, negative genetic correlations are typically found between maternal and offspring contributions to trait expression (reviewed in Cheverud and Moore, 1994; Thiede, 1998). These opposing contributions to phenotypic expression may develop when different genes, e.g., parent and offspring, jointly influence the expression of a trait that is under stabilizing selection (Wolf and Brodie, 1998). While maternal and paternal environmental effects follow a different inheritance pattern, similar adaptive processes may underlie the opposing effects.

This experiment demonstrated that the paternal light environment influences both pollen and offspring traits in *C. americana*. However, there was no association between the magnitude of the environmental effects on a genotype's pollen number and the expression of paternal environmental effects in the offspring sired by that genotype. The lack of correspondence between environmental effects on parents and offspring suggests there are no "responsive" genotypes and that environments influence traits independently within and between generations. While the number of pollen grains per flower does not directly affect the progeny, it can be interpreted as an index of a genotype's response to the light environment. For example, some genotypes apparently compensate for lost resources at the level of the flower as indicated by limited change in pollen number with a 73% decrease in available light. In contrast, other genotypes showed sensitivity to light for paternal traits as indicated by changes in pollen number across the light environments. However, the notion of a "responsive" genotype that expresses phenotypic change across environments in the parental and offspring generations is not supported. Perhaps this result is not surprising given that offspring traits typically differ in the extent to which they are influenced by parental effects (e.g., Miao, Bazzaz, and Primack, 1991; Wulff and Bazzaz, 1992; Schmid and Dolt, 1994; Case, Lacey, and Hopkins, 1996; Lacey and Herr, 2000).

We found genotype \times environment interactions for both paternal and offspring traits when plants were exposed to divergent light intensities comparable to field conditions. In the parental generation, genotype \times environment interactions would maintain genetic diversity among pollen donors in natural populations (Gillespie and Turelli, 1989; Delph, Johannsson, and Stephenson, 1997). In the offspring generation, genotype \times parental environment interactions were found for almost all traits. This result could influence predictions of evolutionary trajectories in natural populations in several ways. First, genotype \times paternal environmental effects could bias estimates of heritability in quantitative genetic studies where differences among sires are assumed to reflect additive genetic variance. If paternal environmental effects are not explicitly taken into account, estimates of heritability and additive genetic variance from studies conducted in nature may be in-

flated. It is not clear how commonly paternal effects introduce this bias since there are few studies of paternal environmental effects. Secondly, parental environmental effects may facilitate or retard response to selection and this may vary among traits and genotypes (Kirkpatrick and Lande, 1989). Many traits were governed by complex interactions between the maternal and paternal genotypes and light environments indicating the context-dependent nature of evolutionary response. Thirdly, genotype \times environment interactions for parental effects represent broad-sense genetic variation. With genetic variation, parental environmental effects that enhance fitness under common light levels are expected to increase in frequency and contribute to adaptive evolution (Mousseau and Fox, 1998).

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