

Plasticity to canopy shade in a monocarpic herb: within- and between-generation effects

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Summary

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- Plants exhibit plasticity in response to their current environment and, in some cases, to that of the previous generation (i.e. maternal effects). However, few studies have evaluated both within- and between-generation plasticities and the extent to which they interact to influence fitness, especially in natural environments.
- The plasticity of adult traits to two generations of natural differences in light was determined for *Campanulastrum americanum*, a forest-edge herb that expresses annual and biennial life histories.
- Plasticity was found to an individual's light environment (within generation) and the maternal light environment (between generations). Responses to ambient light for size traits and timing of flowering were probably passive, whereas apparently adaptive responses were found for light acquisition traits. Maternal light influenced the expression of most adult traits but had the strongest effect when plants were germinated in natural environments.
- The transgenerational effects of light were consistent with adaptive plasticity for several traits. Plastic within-generation changes in flowering time may also result in adaptive between-generation effects by altering the offspring life history schedule. Finally, the results underscore the importance of conducting studies of within- and between-generation plasticity in natural populations, where the environmental context is relevant to that in which the traits evolved.

Introduction

Changes in phenotype in response to environmental conditions are ubiquitous in plants and animals. These changes may be a passive consequence of resource limitation, for example smaller size or slower growth when resources are limited (Coleman *et al.*, 1994; Van Kleunen & Fischer, 2005; Bell & Galloway, 2007). Alternatively, they may be an adaptive response that enhances individual performance under the prevailing conditions (Kingsolver, 1995; Dudley & Schmitt, 1996; Van Buskirk *et al.*, 1997). One of the clearest examples of adaptive plasticity in plants is the shade-avoidance syndrome (reviewed in Schmitt *et al.*, 2003; Franklin & Whitelam, 2005). When sunlight filters through a vegetative canopy and passes through leaves, red wavelengths are disproportionately absorbed by chlorophyll. This loss of red light closer to the ground is detected by phytochromes as a reduction in the ratio of red to far-red (R:FR) wavelengths. For plants of open habitats, a reduced R:FR ratio indicates the presence of neighbors and

typically elicits a shade-avoidance response characterized in part by enhanced stem and petiole elongation, reduced branching, and proportionately larger and thinner leaves (e.g. Leeflang *et al.*, 1998; Stuefer & Huber, 1998; Causin & Wulff, 2003; Weijschede *et al.*, 2006). This response is adaptive, permitting plants to overtop their neighbors (Dudley & Schmitt, 1996; Weinig, 2000). However, for plants from understory habitats, increased elongation is less likely to result in increased light interception. As a consequence, these plants typically display a more modest response to vegetative shade (Donohue *et al.*, 2001; von Wettberg & Schmitt, 2005; Bell & Galloway, 2008). In contrast to these distinct light habitats, we have little information about responses to light for taxa whose populations inhabit more heterogeneous environments, such as forest edges, where individuals routinely experience neighbor shading in open areas and tree shade under the canopy.

Plasticity may also occur between generations if the environment individuals experience influences the expression of traits in their offspring. Transgenerational plasticity in plants

is typically dominated by the maternal environment because it has both pre- and postzygotic effects on the offspring, in contrast to the paternal environment's strictly prezygotic effect (Lacey, 1996). Transgenerational plasticity may also be adaptive, enhancing offspring performance (Fox *et al.*, 1997; Agrawal *et al.*, 1999; Galloway & Etterson, 2007; Allen *et al.*, 2008). However, adaptive between-generation plasticity is only expected to evolve if the maternal environment predicts the offspring environment and if maternal effects are genetically variable and enhance fitness (Donohue & Schmitt, 1998). Results to date indicate that a number of environments are predictable between generations (Donohue & Schmitt, 1998; Galloway, 2005), and that there is genetic variation for maternal environmental effects (e.g. Schmitt *et al.*, 1992; Wulff *et al.*, 1994; Lacey, 1996; Sultan, 1996; Munir *et al.*, 2001; Galloway & Etterson, 2007). Studies have also found that maternal effects are often strong for juvenile traits (reviewed in Donohue & Schmitt, 1998). This is not surprising because seeds develop on the maternal plant and therefore directly experience the maternal environment. As a consequence of their proximity to the maternal generation, most previous work has focused on juvenile traits and we know much less about the influence of maternal environments on the expression of adult characters, especially in natural conditions (but see Donohue & Schmitt, 1998; Lacey & Herr, 2000; Donohue *et al.*, 2005a). Indeed, it has been suggested that maternal effects contribute little to later life cycle stages (Weiner *et al.*, 1997). More information on the expression of maternal effects in adult traits in natural environments is needed to understand the importance of these effects for adaptive evolution.

In many plant species, mothers and offspring share the same light environment, whether it is unshaded, thickly vegetated, or beneath a forest canopy (Galloway, 2005). This suggests that a plastic shade-avoidance response may be as relevant between generations as within a generation. Indeed, transgenerational plasticity to light has been demonstrated to enhance both maternal (Donohue & Schmitt, 1998) and offspring fitness (Galloway & Etterson, 2007). These effects may be mediated by the influence of phytochromes on germination (Donohue *et al.*, 2007, 2008). Alternatively, they may be attributable to direct modification of the offspring phenotype, for example seed mass, by the maternal environment. Seed mass often determines the probability of germination, timing of germination, and offspring survival (e.g. Stanton, 1984; Houssard & Escarre, 1991; Simons & Johnston, 2000; Benard & Toft, 2008). These juvenile traits in turn will influence fitness directly or through their effect on adult characters. Finally, the maternal environment influences the timing of seed dispersal and the dispersal environment. Because dispersal determines the probability of germination, establishment, and fitness (Donohue, 2003), it provides a powerful link between maternal and offspring trait expression that may reflect adaptive transgenerational evolution.

The expression of the shade-avoidance syndrome, like any plastic response, will depend on the environmental context. Many studies, especially those conducted under controlled conditions, have focused on relatively simple environmental settings. While this has enabled a clear understanding of the phenotypic changes associated with shade-avoidance, plants in nature are not likely to experience environmental changes in isolation of other factors. Combinations of environmental factors may lead to unanticipated responses. For example, the fitness benefit of the shade-avoidance phenotype in *Impatiens capensis* depends on local moisture levels (Huber *et al.*, 2004); this is perhaps not surprising, as plants with elongated stems have reduced roots (Maliakal *et al.*, 1999). Therefore, to understand functional, potentially adaptive, within- and between-generation plasticity, it is necessary to evaluate trait expression in the complex environmental conditions under which those responses evolved.

We studied plasticity to light in *Campanulastrum americanum*, an outcrossing herb with annual and biennial life histories that inhabits forest-edge habitats. In *C. americanum*, a transgenerational response to maternal light enhances offspring fitness (Galloway & Etterson, 2007). Here we explored the within- and between-generation components of the shade-avoidance response by evaluating an individual's response to its own light environment as well as to its maternal light environment to gain insight into how maternal effects influence fitness in this species. Specifically, we addressed the following questions.

- What is the within-generation effect of canopy shade on traits important for light capture?
- Do these plastic responses to light differ between annuals and biennials?
- Does the maternal light environment influence the expression of traits throughout the life cycle, including those important for light acquisition?
- How does canopy shade affect traits that may influence the offspring phenotype?

Materials and Methods

Study system

Campanulastrum americanum Small (= *Campanula americana* L.; Campanulaceae) is an outcrossing woodland herb with a polymorphic life history. Individuals grow as annuals or biennials depending on the timing of germination. Seeds that germinate in the fall grow as annuals and flower the following summer, whereas seeds that delay germination until the spring grow as rosettes for their first year and flower in their second summer as biennials. Seeds are nondormant (Baskin & Baskin, 1984). The timing of germination, and therefore the life history schedule, is determined by multiple factors including a seed's dispersal environment, its maternal environment, the timing of seed dispersal, and individual and maternal genetic effects (Galloway, 2002; Galloway & Etterson, 2007; L. F.

Galloway & J. R. Etterson, unpublished). As a consequence, annuals and biennials are expected to be similar except for size-related differences associated with life span.

Campanulastrum americanum populations are typically located at forest edges. In our study population, most individuals growing under the deciduous forest canopy are biennials, whereas most plants growing in tree-fall light gaps are annuals (Galloway & Etterson, 2007). On average, light gaps receive 10 times more radiation than understory habitats (Galloway & Etterson, 2007).

Following the winter, both annuals and biennials bolt and then initiate flowering in midsummer. *Campanulastrum americanum* is highly outcrossing (Galloway *et al.*, 2003); the primary pollinators are bumblebees foraging for nectar and halictids collecting pollen (Galloway *et al.*, 2002; Kilkenny & Galloway, 2008). Flowers are located in compact inflorescences at reproductive nodes on the main stem and lateral branches. When fruits mature, four pores at the top open and seeds are shed passively. The timing of fruit maturation and seed dispersal influence the offspring life history schedule (Galloway, 2002; Galloway & Burgess, in press).

Creation of experimental seed

Experimental seeds that were genetically similar but differed in their maternal light environment were created using two generations of hand-pollinations. For the first generation of crosses, seeds from 236 plants collected throughout the focal population were germinated and vernalized under controlled conditions (cf. Galloway *et al.*, 2003). Rosettes were then transplanted into a light-gap habitat and, when flowering, plants were paired randomly. One individual in each pair was assigned to serve as the pollen donor and the other as the pollen recipient. Pairs were hand-pollinated to produce 111 full-sib families. For the second generation of crosses, several seeds from each full-sib family were germinated and vernalized under controlled conditions. Siblings from 71 families were split across light environments in the natural population, with one transplanted into the light gap and a second into the forest understory. These siblings served as the maternal plants and their planting location determined the maternal light environment. One individual from each of the remaining 40 families was planted in the light gap and these plants were used as sires. Each sire pollinated light-gap and understory siblings of three families to form the experimental generation. This design resulted in seeds for 71 full-sib experimental families that were replicated in both light environments. We refer to these families as 'lines' because of their replication across light environments.

Experimental design

Experimental seeds were used to produce annuals and biennials that were planted into light-gap and understory regions of the

natural population in a partial reciprocal transplant design. We manipulated the life history schedule so that annuals and biennials bloomed in the same year by planting seeds in the spring, where immediate germination would produce biennials, and again in the fall for germination of annuals. Seeds from light-gap and understory maternal habitats were planted in understory and light-gap regions of the natural population. Spring-planted seeds failed to germinate in the natural time window. Therefore, in late spring, we germinated experimental seeds from light-gap and understory habitats under controlled conditions to produce biennial plants (in plug trays with day:night temperatures of 22 : 14 °C and 12-h days). Seeds germinated and grew rapidly; when they were the size of native seedlings, they were transplanted into light-gap (eight blocks) and understory (eight blocks) areas of the natural population ('individual' light environments). Approximately 440 biennial seedlings were transplanted for each combination of maternal and individual light environments (except 100 for seedlings from shade-grown mothers planted in light gaps). Experimental seeds from light-gap and understory habitats were planted into both environments at the end of the summer, and annuals germinated in the fall (see Galloway & Etterson, 2007 for details). The next spring, annual seedlings were transplanted within each light habitat from germination plots into light-gap (four) and understory (one) blocks. Limited germination resulted in 14–22 annual plants for each combination of maternal and individual light environments with little replication of lines except for seedlings from sun-grown mothers planted in light gaps (375 plants). Including both annuals and biennials and both maternal environments, nine replicates per line were planted on average in each light environment.

All blocks were surrounded by fencing to prevent herbivory by large mammals; however, burrowing mammals entered the enclosures several times, eating leaves (rosettes) or apical meristems (bolting plants). Herbivory was scored from 0 to 4, reflecting the severity of the damage.

Three categories of traits were measured on the experimental generation to quantify response to light: size traits, shade-avoidance traits, and traits anticipated to have direct intergenerational effects. Plant size was determined at two life stages. Rosette size was estimated for biennials at the end of their first summer as the product of leaf number and length of the longest leaf. Each plant was harvested when its first fruit ripened and above-ground biomass was determined when dry. The shade-avoidance response was quantified by measuring leaf area, specific leaf area, and internode length. On the date of first flower for each plant, the leaf subtending that flower was harvested and pressed, and the length of the internode above that flower was measured. The area of the harvested leaf was divided by its weight to estimate specific leaf area. Possible effects of light on the next generation (i.e. offspring of the experimental generation) were measured by date of first flower and seed mass. When plants were harvested, a nearly ripe fruit

Table 1 Analysis of (co)variance for size (rosette size and harvest biomass), traits important for light capture (leaf area, specific leaf area (SLA) and internode length), and timing of flowering for genetic lines of *Campanulastrum americanum* grown in understory and light-gap regions of their natural population

Source	Rosette size	Biomass	Leaf area (biomass) ^c	SLA	Internode length	Flower day	Flower day (biomass) ^c
Light ^a	301.60***	144.19***	4.41*	65.46***	29.85***	105.27***	33.22***
Maternal light (ML)	0.01	0.98	0.00	0.15	9.75**	3.94*	7.10**
Light × ML	0.51	0.83	4.19*	1.53	0.03	0.81	0.16
Life history (LH) ^a	–	246.11***	0.00	2.20	0.82	51.97***	1.22
Light × LH ^a	–	2.75	0.13	0.97	0.31	6.41*	4.26+
ML × LH	–	1.63	0.05	0.23	5.72*	2.21	4.96*
Light × ML × LH	–	0.93	5.81*	1.78	0.09	1.07	0.28
Line	0.41	0	2.25*	0.36	1.64*	2.78**	3.49***
Line × light	0.63	1.09	0	1.70*	0.94	0.82	0.04
Block	2.51**	2.33**	2.61**	2.79**	2.40**	1.54+	1.54+
Herbivory	–	2.37	8.61**	1.71	0.19	–	–
Biomass			284.05***				122.48***
Error df ^b	1300	937	912	1191	1004	609	608

Annuals and biennials (life history) were grown in either their maternal light environment or the alternate light environment. *F*-statistics are given for fixed effects and *Z*-values for random effects. +, $P < 0.10$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

^aDenominator df = 17.

^bNumerator df = 1 for all fixed effects and interactions.

^cANCOVA with biomass as the covariate.

that had not yet dehisced was collected. Seed mass was only determined for biennials because there were few seeds for understory annuals. Seed mass was estimated for a subset of lines present in all light combinations by weighing a group of 10 seeds from the harvested fruit. Some seeds were dull colored and slightly flat suggesting, they that would be less likely to germinate (possibly because of premature harvest). This variation was visually assessed and seeds were assigned to one of three 'quality' categories (high, moderate and poor).

Statistical analysis

Mixed model analysis of covariance was used to determine the influence of individual (within-generation) and maternal (between-generation) light environments on trait expression. Individual light environment, maternal light, and life history (whether plants were annual or biennial) were fixed effects in the model. Line, the interaction between line and individual light environment, and block (nested in individual light environment and life history) were included as random factors. Other possible interactions with line (e.g. maternal light and life history) were not included because there was not sufficient replication at these levels. Plant size is expected to differ between light environments. To account for potential size-associated differences, additional analyses were conducted for leaf area and date of first flower with biomass included as a covariate. Herbivory was included as a covariate for all factors except rosette size (little herbivory on young plants) and days to flower (analysis limited to individuals that did not experience herbivory). Only biennials form rosettes, and therefore life

history was not included as a factor in the analysis of that trait. For the analysis of seed mass, maternal genotype (nested within maternal light environment; random) and quality (fixed) were added to the model; high-quality seeds were also analyzed separately. Rosette size, biomass, internode length, and seed mass were log-transformed and leaf area was square-root-transformed to meet the assumptions of ANOVA.

Results

Plant size was influenced by both individual environment and life history. Rosettes and mature plants were at least fourfold larger in light-gap than understory regions of the population (Table 1, Fig. 1a,b). The final biomass of biennials was also fourfold larger than that of annuals (Fig. 1b).

Most traits important for light capture were influenced by individual and maternal light environments. The leaf area of gap-grown plants was 37% larger than that of understory-grown plants ($F_{1,17} = 11.05$, $P = 0.004$). After accounting for differences in leaf area associated with plant size, understory individuals had larger leaves than those growing in light gaps (Table 1, Fig. 2a). However, annuals from maternal plants grown in the sun were an exception. Their leaf size, adjusted for differences in plant size, was relatively constant across growth environments; smaller than average in the shade and larger than average in the sun (Fig. 2a). Individual light environment, maternal light and life history also jointly influenced leaf area independently of plant size (light × maternal light (ML) × life history (LH): $F_{1,1192} = 3.90$, $P < 0.048$). Understory plants had larger specific leaf area than those

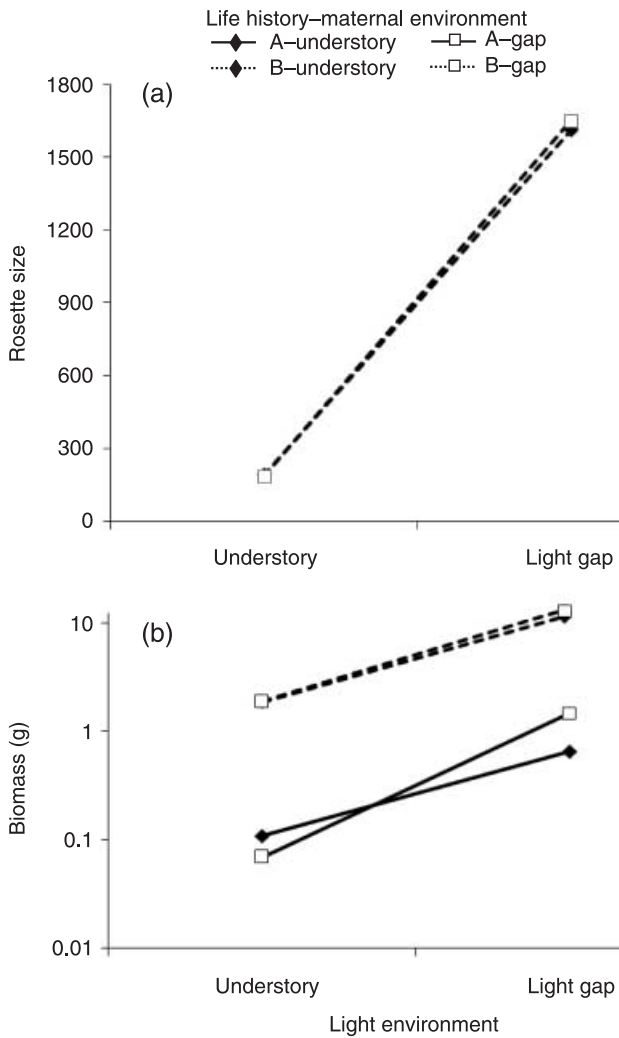


Fig. 1 Mean index of rosette size (a) and aboveground biomass (b) for annual (A) and biennial (B) *Campanulastrum americanum* grown in understory and light-gap regions of a natural population. Rosettes are only present in biennials. Plants were grown in either their maternal light environment or the alternate light environment.

growing in light gaps (Fig. 2b). This pattern of thinner understory leaves was found across maternal light environments and life histories. Internode length was greater for plants growing in light gaps and for those with gap-grown mothers (Table 1, Fig. 2c). However, the effect of maternal light environment on internode length was greater for annuals than for biennials (Fig. 2c).

Light environment may influence an individual's offspring through flowering time and seed mass. Plants in the understory flowered 10 d after those growing in light gaps (Table 1, Fig. 3a). This difference was greater for the annuals (13 d) than the biennials (8 d). Maternal light also influenced flowering time, with individuals with gap-grown mothers flowering significantly later than those with shade-grown mothers. Finally, biennials flowered on average 7 d earlier than annuals (Fig. 3a). This difference in flowering time was largely attributable to differences

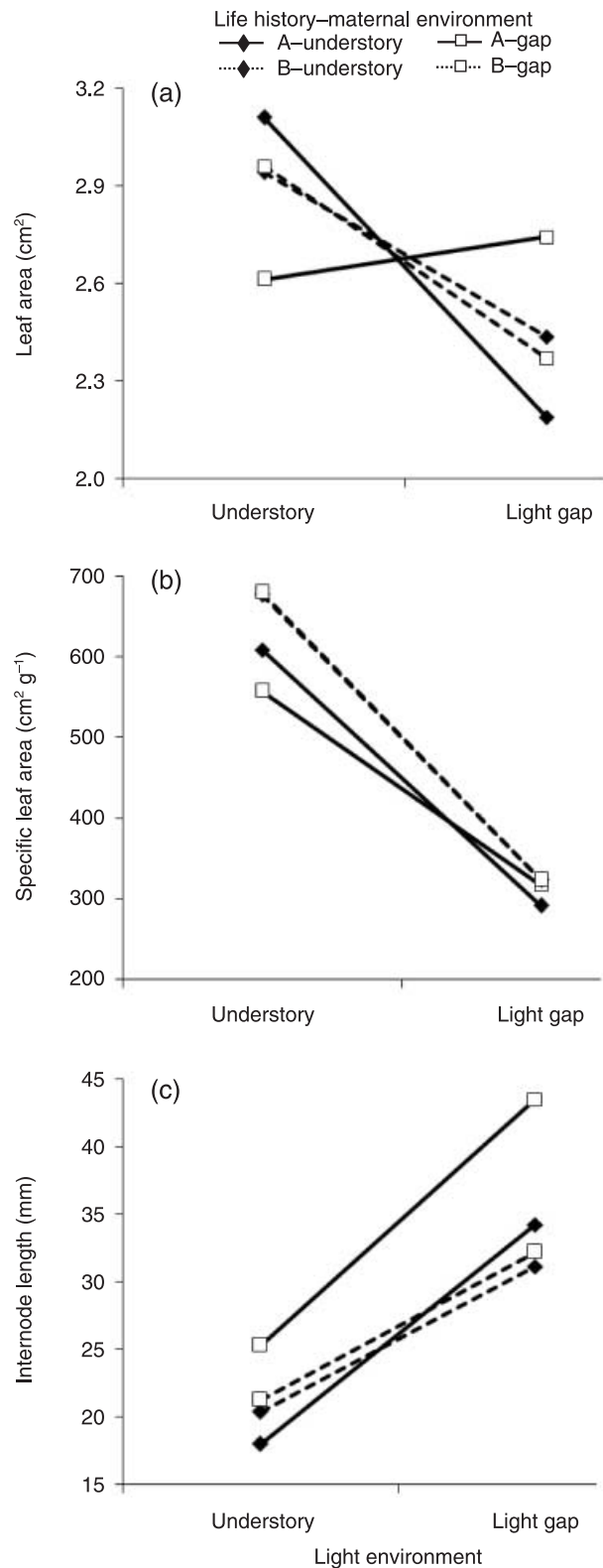


Fig. 2 Least-square means for leaf area (a), specific leaf area (b), and internode length (c) for annual (A) and biennial (B) *Campanulastrum americanum* grown in understory and light-gap regions of a natural population. Plants were grown in either their maternal light environment or the alternate light environment.

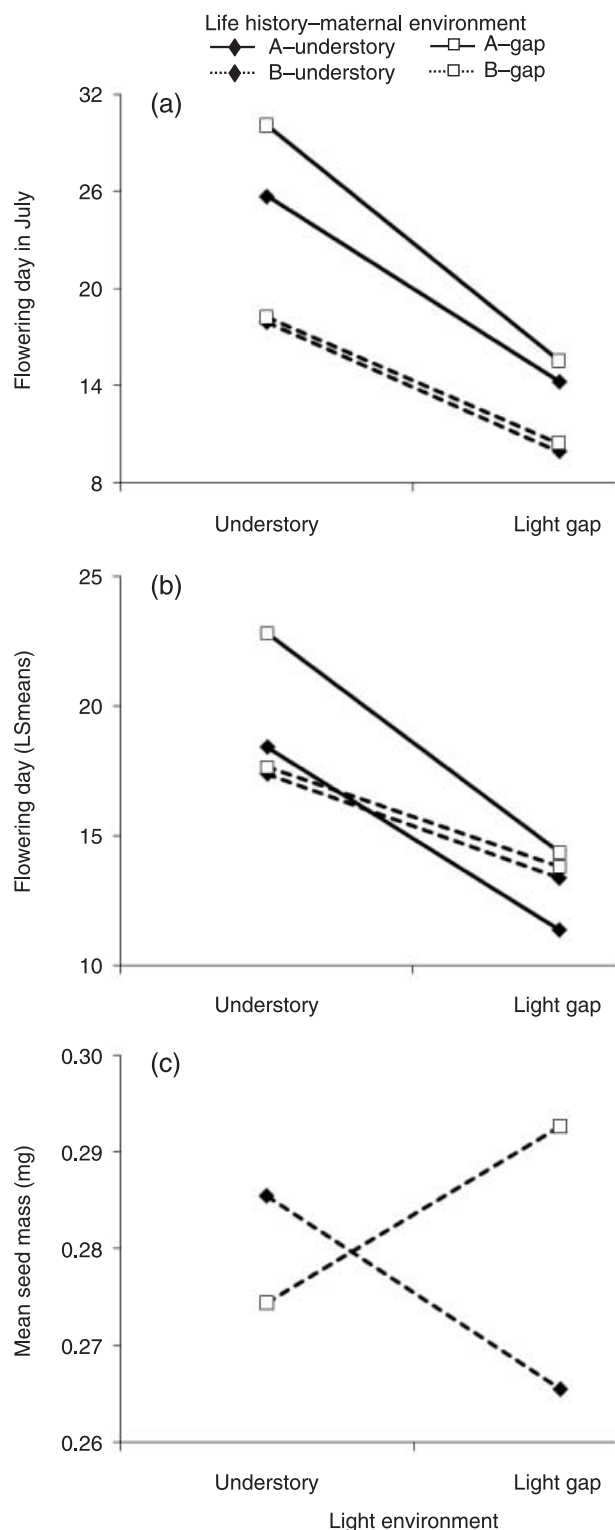


Fig. 3 Date of first flower (a), least-square (LS) means for date of first flower adjusted for plant size (b) and mean mass of high-quality seeds (c) for annual (A) and biennial (B) *Campanulastrum americanum* grown in understory and light-gap regions of a natural population. Seed mass is shown only for biennials. Plants were grown in either their maternal light environment or the alternate light environment.

Table 2 Analysis of covariance of seed mass for genetic lines of biennial *Campanulastrum americanum* grown in understory and light-gap regions of their natural population

Source	Seed mass	
	df	F/Z
Light	1,11	1.35
Maternal light (ML)	1,19	1.23
Light × ML	1,50	4.90*
Line	–	0
Line × light	–	0
Mother (ML)	–	1.39+
Block (light)	–	0.64
Quality	2,50	172.33***

Plants were grown in either their maternal light environment or the alternate light environment. Seed quality is included as an additional blocking factor. *F*-statistics are given for fixed effects and *Z*-values for random effects. +, $P < 0.10$; *, $P < 0.05$; ***, $P < 0.001$.

in size. Once size was included as a covariate, flowering time no longer differed between annuals and biennials (Table 1, Fig. 3b). In addition, maternal light resulted in greater differences in flowering time for annuals than for biennials after controlling for size (Table 1, Fig. 3b). The seed mass of biennials was determined by the combination of individual and maternal environments (Table 2). High-quality seeds from gap-grown plants were larger if their mothers had grown in light gaps, and high-quality seeds from shade-grown plants were larger if their mothers had grown in the understory (light × ML: $F_{1,19} = 3.21$, $P = 0.089$; Fig. 3c). The pattern was the same when all seeds were considered; seeds on understory plants were 3% larger and seeds on gap-grown plants were 12% larger if individuals were grown in the same environment as their mother.

There was variation among lines for leaf area, internode length, and timing of flowering, indicating genetic variation for these traits across light environments. For specific leaf area there was variation in response to the individual light environment among lines, indicating genetic variation in plasticity (Table 1).

Discussion

Campanulastrum americanum has both passive and active plastic responses to the ambient light environment. To understand active, potentially adaptive, shade-avoidance responses, it is important to identify passive responses to light habitat. In this study, individuals growing under the forest canopy were smaller with reduced rosette size, leaf area, internode length, and final biomass relative to those growing in light gaps. In a previous study, fruit and seed production on understory individuals was also found to be lower than that on light-gap plants (Galloway & Etterson, 2007). This smaller size is probably a passive response to the reduced photosynthetic resources of

the forest understory (i.e. light quantity). Understory plants tend to be smaller because of slower growth rates in the low-light habitat (Coleman *et al.*, 1994). Consistent with this, a separate study found that *C. americanum* exhibited slower regrowth after deer herbivory in understory habitats (S. Lin & L. F. Galloway, unpublished). Shade-grown plants also flowered later than light-gap individuals. Later flowering of understory *C. americanum* may also be a passive response to the limited-resource conditions under the forest canopy. In contrast to these plastic responses to the poor growth environment which are probably passive, specific leaf area and relative size of individual leaves were both larger under the forest canopy than in light gaps. Relatively larger, thinner leaves under the forest canopy allow for greater light capture per unit of resource allocation. These shifts in leaf morphology are consistent with adaptive expectations of enhanced allocation to light capture in shaded environments. However, in understory habitats light quality (R:FR ratio) is altered in conjunction with light quantity; these distinct aspects of light must be experimentally decoupled to clearly distinguish passive from adaptive plastic responses (e.g. Griffith & Sultan, 2005).

Forest-edge taxa, such as *C. americanum*, whose populations span open and understory habitats, may express different patterns of plasticity to light than species from habitats where light availability is more uniform. Plants from high-light habitats typically have substantial shade-avoidance responses including elongated internodes (or petioles which serve as vertical spacers for taxa with prostrate growth patterns), increased specific leaf area, reduced branching, and earlier flowering (Franklin & Whitelam, 2005). Studies have demonstrated that selection favors shade-induced elongation in open habitats where such a shade-avoidance response allows plants to overtop neighbors (Dudley & Schmitt, 1996; Donohue *et al.*, 2000; Weinig, 2000; Weijschede *et al.*, 2006; Bell & Galloway, 2008). By contrast, both the plastic response to reduced R:FR light and selection for elongation are reduced under closed canopies (Donohue *et al.*, 2001; von Wettberg & Schmitt, 2005; Bell & Galloway, 2008), where carbon invested in increased elongation does not yield substantially greater light capture and may result in structurally weaker individuals (Schmitt *et al.*, 1995). In *C. americanum* populations that span natural light environments and therefore experience both selective regimes, apparently an elongation response to shade has not been favored by selection as internodes were longer on light-gap-grown plants. However, the greater relative leaf area and specific leaf area found in understory *C. americanum* could be considered shade 'tolerance' responses (e.g. Griffith & Sultan, 2005), enhancing light acquisition but not neighbor avoidance, and are expected to result in greater light interception regardless of the source of shade. Phenotypes that enhance light acquisition in the understory but not neighbor overtopping represent functionally adaptive responses to the mixed shade cues found in forest-edge populations. Later flowering under shade conditions of forest-

edge populations may also represent tolerance if it permits greater resource accumulation before flowering but little neighbor avoidance. By contrast, earlier flowering under reduced R:FR conditions of open habitat taxa may represent adaptive plasticity that enhances reproductive output under competitive conditions (Smith & Whitelam, 1997; Donohue *et al.*, 2000). Future studies of forest-edge species that combine overhead canopy with response to neighbor density would permit evaluation of these hypotheses.

Trait expression and plasticity differed between annual and biennial *C. americanum*. Not surprisingly, biennials were substantially larger than annuals; this difference in biomass is associated with greater fruit and seed production (Galloway & Etterson, 2007), and earlier flowering. Changes in plant size in response to light were similar for both life histories. Annuals were also more plastic than biennials for timing of reproduction. Differences in trait expression between individuals that experience common environmental conditions but differ in life history may result in differential fitness and patterns of selection between the life histories (Donohue *et al.*, 2005b). However, it should be noted that annuals and biennials experienced different early life environments as a consequence of the experimental design. These differences in early growth conditions may have contributed to the differences observed between the two life histories (see also later).

Maternal light environment influenced the expression of all adult traits in *C. americanum* except size and specific leaf area. Generally, these maternal effects depended on life history, with maternal light influencing trait expression in annuals but having very little effect in biennials (except on seed mass). Annuals were germinated in understory and light-gap habitats and transplanted as seedlings within those habitats. Therefore, annuals experienced germination cues and seedling light regimes that were similar to the maternal environments and correlated with their growth environment. The matching light environments between generations, where the maternal habitat provided appropriate transgenerational cues for the juvenile habitat, appear to have elicited maternal effects that persisted into later life stages. By contrast, biennials were germinated in a uniform controlled environment and then seedlings were transplanted into understory and light-gap habitats. The shared germination and early life environmental conditions of light-gap and shade-grown mothers may account for the lack of maternal light effects, suggesting that maternal light may also affect biennials that germinate under natural conditions. Alternatively, the reduced effect of maternal light on trait expression in biennials may reflect their longer life span, as maternal effects are often strongest early in the life cycle (Donohue & Schmitt, 1998). The difference in response between annuals and biennials reveals the importance of conducting plasticity studies in the relevant environmental context.

Previous work in *C. americanum* has demonstrated that the maternal light environment strongly influences fitness

(Galloway & Etterson, 2007). In particular, population projection models show that plants have over three times higher fitness when grown in their maternal light environment than in an alternate light environment, despite the lack of a measurable direct effect of maternal light on survival and fecundity. The maternal light effects found here on relative leaf area and seed mass suggest that they may contribute to this adaptive transgenerational plasticity. Leaf area and seed mass were larger when plants were grown in their maternal light environment, especially in light gaps. These maternal effects are likely to be adaptive because higher values of these traits are typically associated with higher performance. There are few other studies for comparison where consecutive generations have been grown in relevant natural environments. In *Plantago lanceolata*, maternal plants had higher fitness when their seeds were planted into the maternal habitat (Donohue & Schmitt, 1998), indicating adaptive maternal effects (Marshall & Uller, 2007). However, specific traits that contribute to this adaptive response are not known.

The local light environment may also influence the expression of traits that themselves cause maternal effects. Plants growing under the forest canopy initiated flowering on average a week and a half after those in light-gap habitats. The initiation of flowering can be used as an index for average time of fruit maturation because flowering phenology and the time from flower production to fruit maturation are largely independent of date of first flower (Galloway & Burgess, in press). Therefore, the earlier light-gap flowers will become earlier ripening fruits that disperse their seeds before the later flowers of understory individuals. The timing of seed dispersal influences the offspring life history schedule (Galloway, 2002; Galloway & Burgess, in press), with seeds that are dispersed earlier in light gaps expected to yield a higher frequency of fall-germinating annuals and seeds that are dispersed later in the shade producing a larger fraction of spring-germinating biennials. Fitness measured using the population growth rate is higher for annuals in light gaps and biennials under the forest canopy (Galloway & Etterson, 2007). Therefore, although changes in flowering time in response to light habitat appear to be passive (see above), the changes that they are expected to induce in offspring life history are adaptive. A more detailed study of fruit maturation in the two habitats, combined with a projection matrix analysis, would allow a quantitative assessment of the expected differences in fitness. In summary, plastic responses to the ambient light environment in flowering time will result in phenotypic maternal effects that enhance offspring fitness in *C. americanum*.

Specific maternal traits also influence offspring trait expression in other taxa. For example, maternal density influences offspring growth form (Donohue, 1999), solar tracking of maternal flowers influences seed size (Stanton & Galen, 1989), maternal seed dispersal time influences offspring germination patterns (Lacey & Pace, 1983, Donohue *et al.*, 2005a), maternal inflorescence color alters seed development temperature (Lacey

& Herr, 2000, 2005), and maternal tuber size determines offspring performance (Hangelbroek *et al.*, 2003). This substantial and varied list of examples speaks to widespread occurrence of intergenerational phenotypic effects. However, more information is needed to determine the contribution of these effects to adaptive evolution.

This study of phenotypic change in response to natural variation in light in *C. americanum* has revealed a number of interacting mechanisms through which light-gap and understory habitats may influence performance. Combining these results with previous work (Galloway & Etterson, 2007), we find that while maternal environmental effects are strongest early in the life cycle, influences on the later life cycle act in the same direction and together result in substantial cumulative effects of transgenerational plasticity on fitness. The within-generation plastic effect of light environment on reproductive phenology had between-generation effects on germination season. Because germination season determines the expression of fitness associated adult traits (e.g. life history schedule), plastic changes in reproductive timing will also yield intergenerational fitness effects. In total, the light environment of *C. americanum* elicits apparently adaptive shade-avoidance (or shade-tolerance) responses within generations as well as adaptive between-generation changes in phenotype. Further studies are required to understand the generality of such effects. However, future work should be conducted in natural populations because the expression of adaptive plasticity is environment dependent and therefore best studied in the complex environmental context in which it evolved.

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