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# Contribution of direct and maternal genetic effects to life-history evolution

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## Summary

• Maternal effects are ubiquitous in nature. In plants, most work has focused on the effects of maternal environments on offspring trait expression. Less is known about the prevalence of genetic maternal effects and how they influence adaptive evolution. Here, we used multivariate genetic models to estimate the contributions of maternal and direct genetic (co)variance, the cross-generation direct-maternal covariance, and **M**, the matrix of maternal effect coefficients, for life-history traits in *Campanulastrum americanum*, a monocarpic herb.

• Following a three-generation breeding design, we grew paternal half-sib families with full-sib relatives of each parent and measured juvenile and adult traits.

• Seed size was influenced exclusively by maternal environmental effects, whereas maternal genetic effects influenced traits throughout the life cycle, including strong direct and maternal additive genetic correlations within and between generations for phenological and size traits. Examination of **M** suggested that both juvenile and adult traits in maternal plants influenced the expression of offspring traits.

• This study reveals substantial potential for genetic maternal effects to contribute to adaptive evolution including cross-generation direct-maternal correlations that may slow selection response, maternal effects on phenology that reinforce genetic correlations, and within- and between-generation genetic correlations that may influence life-history polymorphism.

## Introduction

Both mothers and fathers contribute genes to their offspring, but the influence of mothers often extends beyond simple genetic transmission. For example, the quality of the environment that a maternal plant experiences or its inherent ability to provision seeds may have a strong impact on the phenotype and fitness of its offspring (reviewed in Roach & Wulff, 1987; Donohue & Schmitt, 1998; Galloway, 2005). The importance of such maternal effects on the process of adaptive evolution has become increasingly recognized (Mousseau & Fox, 1998; Räsänen & Kruuk, 2007). When maternal effects have a genetic basis, a population's response to selection depends not only upon the genetic variation expressed in the current generation, but also upon sources of variation in previous generations. This time lag between the source of phenotypic variation and its effect on evolutionary change may alter patterns of evolution in unexpected and counterintuitive ways, including enhanced or reduced responses to selection, reversals in the direction of the response, and oscillatory dynamics (Kirkpatrick & Lande, 1989; Lande & Kirkpatrick, 1990; Cheverud & Moore, 1994). Although models of trait expression and evolution have been developed that include genetic maternal effects (Willham, 1963, 1972; Falconer, 1965; Kirkpatrick & Lande, 1989), few empiricists working in natural plant populations have used these tools to evaluate their importance to the process of adaptive evolution (but see Byers *et al.*, 1997; Thiede, 1998).

Measurements of quantitative genetic variation are useful because they allow predictions of a population's potential for evolutionary change (Falconer & MacKay, 1996; Lynch & Walsh, 1998). For example, the rate of adaptive evolution in flowering time in response to climate change can be predicted using estimates of additive genetic variance (Franks *et al.*, 2007). However, flowering time in offspring may also be influenced by genetically based attributes of the maternal plant. In this

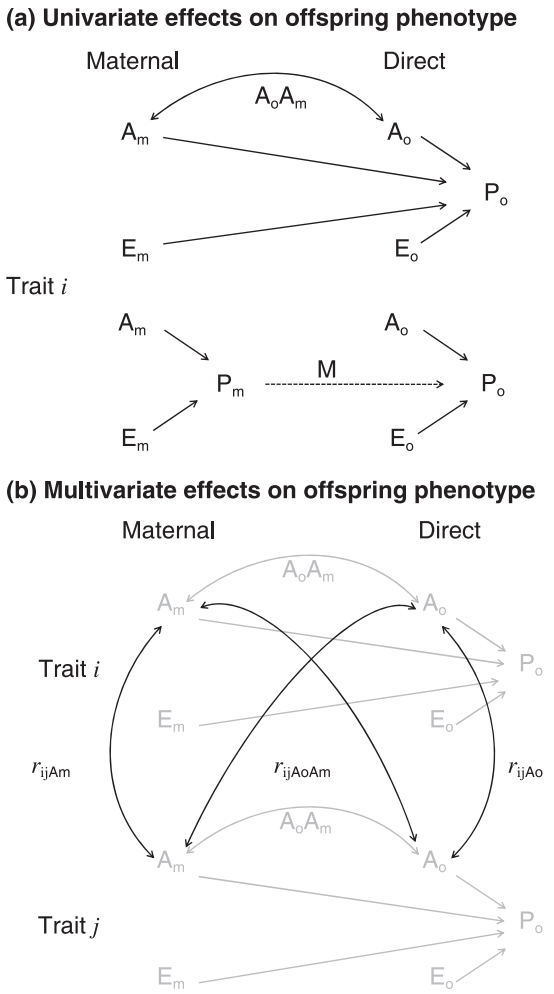


**Fig. 1** Schematic diagrams of a trait with maternal inheritance. (a) Direct genetic (A) and environmental (E) effects on trait expression (P) are indicated with the subscript 'o'. Maternal genetic and environmental effects on trait expression are indicated with the subscript 'm'. The upper diagram depicts the analysis using the variance components approach and the double-headed arrow indicates the cross-generation direct-maternal genetic covariance ($A_oA_m$). The lower diagram depicts the trait-based model, and the maternal effect on trait expression is indicated by the dotted arrow (M). (b) Multivariate extrapolation of the variance components approach. The between-trait relationships are indicated by black lines and the within-trait associations are indicated by grey lines. Between-trait relationships include the between-trait maternal ($r_{ijAm}$) and direct ($r_{ijAo}$) additive genetic correlations as well as direct-maternal correlations ($r_{ijAoAm}$).

case, a variance-components approach may be used to partition additive genetic variance into the direct effects of an individual's own genes as well as the indirect effects of genes expressed in the maternal generation. Trait evolution (e.g. timing of flowering) may result from changes in either an individual's own genes or in maternally acting genes. Direct and indirect genetic effects may also covary across the generations (Dickerson, 1947; Willham, 1963; Fig. 1a). Previous studies have demonstrated that this direct-maternal covariance is a common feature of the genetic architecture of traits with maternal inheritance (reviewed in Räsänen & Kruuk, 2007). Frequently the covariance is negative, reducing the total genetic variation across generations, and therefore may retard the selection response. However it may also be positive, accelerating potential evolutionary change relative to simpler predictions based on single-generation estimates of additive genetic variance.

The contribution of the maternal generation to trait expression may be extended to incorporate a multivariate phenotype. Many traits under selection, in particular life-history traits such as timing of flowering and timing of germination, are genetically correlated and therefore will not evolve independently in response to selection. A multivariate analysis of quantitative genetic variation allows this lack of independence to be quantified with the estimation of within-generation genetic correlations between traits ($r_{ijAo}$, $r_{ijAm}$) as well as across-generation genetic correlations between traits ($r_{ijAoAm}$ and $r_{jiAoAm}$; Fig. 1b). Because univariate analyses have been more commonly employed than multivariate analyses, it is unclear whether cross-generation effects, such as a correlation between maternal genetic effects on the timing of flowering with direct genetic effects on the timing of germination, are common. Like direct-maternal correlations within traits, such cross-generation correlations between traits may act to enhance or reduce the response to natural selection.

Further insight into cross-generation effects can be obtained using an alternative trait-based model of maternal effects (Falconer, 1965). This model recognizes that the phenotypic expression of a specific trait in the maternal generation (e.g. timing of flowering), may influence the expression of the same trait, or of a different trait, in the offspring (e.g. timing of germination). This approach has been used to evaluate environmentally mediated maternal effects in plants, such as the effect of maternal branch architecture on seed-dispersal distance and hence offspring branch architecture (Donohue, 1999). Similarly, estimates of the maternal-effect coefficient $M$ may be derived for the genetic component of maternal trait expression (Fig. 1a). $M$ is subtly different from the cross-generation covariance ($\sigma_{AoAm}$) because it describes a causal relationship between the traits, assuming that offspring phenotype is directly affected by a specific maternal trait.

A multivariate analogue of this model, described by the matrix **M**, allows estimation of the effects of multiple maternal traits on multiple offspring traits (Kirkpatrick & Lande, 1989). The components of **M**, $M_{ij}$, can be thought of as partial regression coefficients that measure the effect of maternal trait $j$ (e.g. flowering time) on offspring trait $i$ (e.g. timing of germination) after controlling for Mendelian transmission. Until recently, there has been no generalized method available for measuring **M**. However, recent theoretical work has demonstrated that trait-based models of maternal effects are equivalent to variance-components models and that **M** can be estimated using the variance-components approach (McGlothlin & Brodie, 2009). We make use of this new methodology in the research presented

here and use estimates of direct maternal effects ($A_o$) and the maternal–offspring genetic covariance ($A_{m,o}$) to calculate **M**. Much like multivariate estimates of response to selection ($\Delta \mathbf{z} = \mathbf{G}\boldsymbol{\beta}$; Lande & Arnold, 1983), which combine estimates derived from empirical data to make quantitative predictions (e.g. Grant & Grant, 1995), estimates of **M** may be used to generate hypotheses of how maternal traits influence the expression of traits in the offspring generation.

Our goal in the current study was to provide a comprehensive assessment of genetic maternal effects in *Campanulastrum americanum*, a forest-edge herb in which previous work has demonstrated environmental and phenotypic maternal effects (reviewed in Galloway, 2005). *C. americanum* populations typically straddle understory and light-gap habitats. We have found that maternal effects elicited by the alternate light environments enhance offspring performance such that offspring have three times greater fitness when they are appropriately cued for their growth environment by their mothers (Galloway & Etterson, 2007). This response is mediated, in part, by effects of maternal light on season of germination because fall-germinating seeds become annuals while spring-germinating seeds become biennials. Season of germination is also determined by maternal flowering time (Galloway & Burgess, 2009). Interactions between these maternally acting mechanisms create a web of potential transgenerational effects in *C. americanum*. Explicit genetic analysis of traits that cause maternal effects (e.g. flowering time) and those that demonstrate maternal inheritance (i.e. whose expression is altered by maternal effects, e.g. germination time) will inform our understanding of whether genetic maternal effects, together with these environmental and phenotypic maternal effects, contribute to adaptive evolution of life-history schedule. Juvenile and adult-size traits are also included in our analysis because changes in phenology often result in changes in size. We conducted this analysis using a three-generation breeding design and determined univariate and multivariate estimates of direct genetic effects, maternal genetic effects and the maternal–offspring covariance. These estimates were then used to calculate **M** to develop hypotheses concerning causal influences of maternal traits on the offspring phenotype.

## Materials and Methods

### Study system

*Campanulastrum americanum* (Campanulaceae) Small (= *Campanula americana* L.) is a predominately outcrossing autotetraploid herb (Galloway *et al.*, 2003; Galloway & Etterson, 2005). Individuals are monocarpic, growing either as annuals or biennials. Life-history schedule is determined by season of germination, which is, in part, genetically determined, varying among families (Galloway & Burgess, 2009), and in part influenced by the local light environment. Fall-germinating annuals predominate in light gaps, and spring-germinating biennials predominate under the forest canopy (Galloway & Etterson, 2007). Populations typically grow at wood margins near road cuts, streams and tree falls. The study population is located near the Mountain Lake Biological Station on Rt 613, Giles Co, Virginia, USA (see also Galloway, 2001, 2002, 2005).

### Breeding design

To estimate direct and maternal genetic effects, we conducted a three-generation breeding design. Plants grown in the first generation ($G_0$) were crossed to form full-sib families ($G_1$). Seeds from each family were grown and crossed in a paternal half-sib design ($G_2$). We then grew seeds of the half-sib families ($G_2$), together with siblings of their parents ($G_1$), to provide the two generations of known relatedness required for estimating direct and maternal genetic effects, their covariance and the maternal effect matrix **M** (Willham, 1963; Eisen, 1967; Kirkpatrick & Lande, 1989; McGlothlin & Brodie, 2009). Parental and offspring generations ($G_1$ and $G_2$) were grown together at the same time in a common glasshouse environment.

Seeds to produce the first generation, $G_0$, were collected from 236 individuals scattered throughout the natural population. They were germinated in plug trays in a growth chamber (12 h day (21°C): 12 h night (15°C)) for 4 wk. Some lines were replanted because of poor germination. Seedlings were vernalized at 5°C for 6 wk to induce flowering and were then transplanted into 0.5 l pots and placed in an outdoor enclosure 3.5 km from their home population. The enclosure is a clearing surrounded by trees and has a light environment similar to light gaps in the home population.

Full-sib $G_1$ families were produced by randomly pairing 194 plants and assigning each to serve as a male or a female. Although highly outcrossing, *C. americanum* is self-compatible; therefore, the protandrous flowers were emasculated before the onset of the female phase. Pollen is presented on the outer surface of the style, and in the wild, insects typically remove all the pollen within 2 h of anthesis (Evanhoe & Galloway, 2002). We selected male-phase flowers that had no pollen remaining, wiped styles with a wet paintbrush to remove any remaining grains and individually protected them from insect visits by covering them with short lengths of a drinking straw. The next day, flowers entered the female phase and we removed the straws, pollinated flowers with the appropriate donor and replaced straws until the flower wilted. Up to 25 pollinations were conducted on each plant, typically representing *c.* one-sixth of the flowers produced. Plants were moved to a glasshouse midway through fruit ripening; additional pollinations were conducted on late-flowering plants at this time (20% of the total).

Individuals from each full-sib family were grown and used to create $G_2$ paternal half-sib families. Seeds from 97 full-sib $G_1$ families were germinated, vernalized and transplanted following the procedures of the previous generation. The pots were placed in a light gap in their home population and

surrounded with fencing to protect plants from mammalian herbivores. To create paternal half-sib families, 26 individuals were randomly selected to serve as sires and each was crossed to three unique randomly assigned plants (dams). Up to 25 hand-pollinations were conducted on each dam following the same procedure as the previous generation. Because a few plants failed to flower, seven sires were crossed to only two dams, resulting in total of 71 maternal full-sib families in $G_2$.

## Evaluation of genetic variation

Full-sibs of the sires and dams used in the paternal half-sib crosses ($G_1$) and paternal half-sib families ($G_2$) were grown in a glasshouse under controlled conditions. Fifteen seeds were selected from each of the 71 $G_2$ families, 71 $G_1$ full-sibs of dams used to produce $G_2$ (maternal relatives) and 26 $G_1$ full-sibs of sires used to produce $G_2$ (paternal relatives). Four families had insufficient seed ($n = 4, 10, 10, 12$), resulting in a total of 2496 seeds. Seeds were individually weighed on a microbalance and divided into 15 blocks with one seed/family/block. Seeds were then surface-sown into plug trays filled with a soil-less potting mix (210 plugs per tray) and located randomly within each block. A total of 12 trays was used, and some blocks were divided among trays. The trays were placed in three growth chambers set to: 12 h day (21°C) : 12 h night (15°C) and were kept moist. Because environmental variation that influenced germination and early growth varied among plug trays, and plants were located in the same order in the glasshouse as during germination, 'Tray' was included as a blocking factor in all analyses.

Juvenile and adult phenological traits and size were recorded for each individual. Germination was scored daily for 47 d and then plants were vernalized at 5°C for 7 wk. Following vernalization, plants were moved to a glasshouse, transplanted into $4 \times 14$ cm tubular pots containing potting mix : fritted clay (3:1), and grown under conditions of extended days (16 h). Plants were watered as necessary and fertilized every 2 wk until flowering and then fertilized weekly thereafter. At transplant, we counted leaf number and measured the longest leaf (mm). These variables were multiplied together to give an index of rosette size. After bolting, plants were checked daily for initiation of flowering, recorded as the number of days postvernalization. Above-ground biomass was harvested *c.* 8 wk after an individual had initiated flowering; this harvest time was chosen to approximate the ripening date of a fruit from a flower open in the second week of blooming and therefore represents the reproductive life span of natural individuals. Biomass was dried and weighed. In total, we estimated direct and maternal genetic parameters for three juvenile traits (seed mass, days to germination and rosette size) and for two adult traits (days to flower and final biomass). Days to germination, days to flower and final biomass were $\log_e$-transformed and rosette area was square-root transformed before analysis to improve normality.

## Statistical analysis

We used an 'animal model' (Kruuk, 2004; Kruuk & Hadfield, 2007), and the program ASREML 2.0 (Gilmour *et al.*, 2006) to analyze data from the three-generation breeding design. The animal model fits genetic parameters using a pedigree, allowing all relationships to be used as information, and is appropriate for estimating additive genetic effects in autotetraploids such as *C. americanum* (Wricke & Weber, 1986). Fitting this model required that we assume that epistasis was negligible and, more importantly, that direct and maternal dominance variances and their covariance were negligible. This later assumption is commonly made because it is difficult to obtain all types of relatives necessary to estimate these additional variance components (Lynch & Walsh, 1998). Trait means were similar to those found in other glasshouse studies of *C. americanum* (Supporting Information Table S1, e.g. Burgess *et al.*, 2007).

We initially fitted univariate models to the five traits. First, we fitted a fully specified model, including Tray as a fixed effect, direct ($\sigma^2_{Ao}$) and maternal ($\sigma^2_{Am}$) genetic effects and the covariance between them ($\sigma_{AoAm}$), a maternal environmental effect ($\sigma^2_{Em}$) and a residual component ($\sigma^2_{Eo}$) as random effects (Model 1). The (co)variance matrix of genetic effects was constrained to be positive definite (positive variances and correlations between −1 and 1). The maternal environmental effect was unconstrained because in some cases, such as when direct and maternal residuals are negatively correlated, the expected maternal environmental variance is negative (Bijma, 2006; McGlothlin & Brodie, 2009). In one case (seed mass), Model 1 could not converge on a final solution and therefore we dropped the direct-maternal covariance term from the model (Model 2).

We used likelihood ratio tests to evaluate the statistical significance of the genetic and environmental parameters using a hierarchical approach (Gilmour *et al.*, 2006). The test statistic for a model parameter, $D$, is equal to twice the difference in the log likelihood between the full model and the reduced model without that parameter. The expected distribution of $D$ for a single parameter is a composite of two distributions, $\chi^2_0$ and $\chi^2_1$, and for this reason, we report $P = 0.5[1 - \Pr(\chi^2_1 \leq D)]$, as recommended by Gilmour *et al.* (2006; see also Visscher, 2006). The maternal environmental effect was tested first. In three cases, the maternal environmental effect was nonsignificant ($P \geq 0.27$) and we used a reduced model with all parameters, except $\sigma^2_{Em}$ (Model 3), to test the remaining parameters. This was carried out because the maternal environmental effect can absorb variance previously attributed to the maternal genetic effect after the latter is removed, invalidating the likelihood ratio test. The direct-maternal covariance was removed next and tested, and direct and maternal genetic variances were tested against this further reduced model.

For univariate models, we calculated direct, maternal and total heritabilities. Direct heritability was calculated as $h^2_{Ao} = \sigma^2_{Ao}/\sigma^2_p$, where $\sigma^2_p = \sigma^2_{Ao} + \sigma^2_{Am} + \sigma_{AoAm} + \sigma^2_{Eo} + \sigma^2_{Em}$. Maternal

heritability was $h^2_{Am} = \sigma^2_{Am}/\sigma^2_p$, and total heritability was $h^2_{total} = (\sigma^2_{Ao} + 1.5\ \sigma_{AoAm} + 0.5\ \sigma^2_{Am})/\sigma^2_p$ (Willham, 1972). Approximate standard errors for these variance ratios were calculated using the delta method in ASReml (Gilmour *et al.*, 2006).

All traits, except seed mass, showed evidence of significant or nearly significant genetic variance (see the Results) and were retained for a four-trait multivariate model. We report estimates of (co)variance components from a fully specified multivariate model that included the same effects as univariate Model 1, specifically matrices of direct ($\mathbf{E}_o$) and maternal ($\mathbf{E}_m$) environmental effects and a composite genetic (co)variance matrix composed of $\mathbf{A}_o$ (in the upper left corner), $\mathbf{A}_m$ (in the lower right corner), $\mathbf{A}_{o,m}$ (in the upper right corner) and its transpose $\mathbf{A}_{m,o}$ (in the lower left corner). For ease of interpretation, this composite matrix was constrained to be positive definite.

Significance testing of the multivariate model followed a procedure similar to that used for univariate models. Initially, we removed the environmental maternal-effects matrix ($\mathbf{E}_m$). To test components of the composite matrix of $\mathbf{A}_o$, $\mathbf{A}_m$ and $\mathbf{A}_{o,m}$, we removed the positive-definite constraint because many constrained models would not converge if individual components were removed or fixed at zero. The significance of the individual genetic components of the composite matrix was tested by removing $\mathbf{A}_{o,m}$, $\mathbf{A}_o$ and $\mathbf{A}_m$, each in turn, and performing the appropriate likelihood ratio tests. We report $P = 0.5[1 - \Pr(\chi^2_k \leq D)]$, where $k$ is the number of parameters removed from the model (Gilmour *et al.*, 2006). We also performed likelihood-ratio tests for individual parameters in the genetic (co)variance matrix by constraining each of them to zero (or a very small positive number, in the case of diagonal components), and testing them against the model including all genetic (co)variance components.

ASReml generates approximate standard errors for (co)variance components by inverting the average information matrix (Gilmour *et al.*, 2006). However, in some cases, as with our constrained model, this method cannot generate standard errors. Therefore, we determined approximate standard errors generated by ASReml for the unconstrained model. To calculate approximate standard errors for our constrained model, we used the formula: $SE_{const} = SE_{unconst}(G_{const}/G_{unconst})$, where $G$ is a component of the composite genetic (co)variance matrix. These approximate standard errors can be considered to be upper bounds of the true standard error (Gilmour *et al.*, 2006); significance testing using likelihood-ratio tests, however, is not affected by this approximation.

To quantify cross-generation effects in more detail, we calculated the matrix of maternal-effect coefficients (**M**) from the trait-based model of maternal effects of Kirkpatrick & Lande (1989). Measurements of **M** suggest how specific offspring traits are affected by specific maternal traits, after taking other genetic correlations into account, and are useful for generating mechanistic hypotheses. Recently, it has been shown that **M** can be estimated as follows:

$$\mathbf{M} = \mathbf{A}_{m,o}\mathbf{A}_o^{-1}$$

where **M** is a square matrix of maternal effect coefficients $M_{ij}$, which measure the effect of maternal trait $j$ on offspring trait $i$ (McGlothlin & Brodie, 2009). Before we performed this calculation, we transformed all genetic (co)variance components to unit variance with the formula: $A_{ij}/\sqrt{(P_{ii}P_{jj})}$, where $P_{ii}$ and $P_{jj}$ are phenotypic variances. This places all traits on the same scale, allowing comparison among components of **M**. A method for estimating the confidence intervals around these estimates has not yet been derived. We therefore based our interpretation on the magnitude of each element of **M**, and limited our conclusions to the development of hypotheses. We note that like measurements of selection gradients for correlated traits, our measurement of **M** is dependent upon the set of traits included in our study. The inclusion of other correlated maternal or maternally influenced traits may alter the magnitude and/or direction of the elements of **M**.

## Results

Trait expression was significantly influenced by both maternal environmental and genetic components of variance. Environmental maternal effects ($\sigma^2_{Em}$) were significant and substantial for both the juvenile trait seed mass and the adult trait days to flower (Table 1). Significant additive genetic variation was found for all traits, except seed mass (Tables 1–3, Fig. 2). Direct and maternal additive genetic variance ($\sigma^2_{Ao}$ and $\sigma^2_{Am}$), and the covariance between direct and maternal additive genetic effects ($\sigma_{AoAm}$), contributed to the expression of phenology and size (Table 1). Days to germination was strongly influenced by all forms of genetic variation. However, for days to germination and rosette size (both juvenile traits), the maternal additive genetic effects, including both variance and covariance components, had stronger statistical support than direct genetic effects. By contrast, for the adult traits, days to flower and biomass, there was stronger evidence for direct additive genetic effects than for maternal genetic effects.

For almost all traits, the heritabilities calculated using the direct ($h^2_{Ao}$) and maternal ($h^2_{Am}$) additive genetic variances were larger than the total heritability ($h^2_{total}$), calculated using all types of additive genetic variance (Table 2). This was particularly true for the juvenile traits, days to germination and rosette size, where the direct and maternal heritabilities were approximately three times larger than the total heritability. The discrepancy between the different heritability estimates was exclusively the result of large negative direct-maternal genetic correlations (Tables 1, 2). For example, the cross-generation genetic correlation, $r_{AoAm}$, was nearly −0.8 for both days to germination and rosette size (Table 2). Large negative correlations indicate that direct and maternal effects share an underlying genetic basis but have antagonistic effects on offspring trait expression. The $r_{AoAm}$ for adult traits, although still large, was substantially less than that of the juvenile traits. As a result,

**Table 1** Causal components of variance for the univariate model of maternal inheritance that best describes each trait in *Campanulastrum americanum*

| | Model | $\sigma^2_{Ao}$ | $\sigma_{AoAm}$ | $\sigma^2_{Am}$ | $\sigma^2_{Eo}$ | $\sigma^2_{Em}$ |
|---|---|---|---|---|---|---|
| Seed mass | 3 | 0.0004 | | 0.0000 | 0.0015 | 0.0043* |
| | | (0.0015) | | | (0.0008) | (0.0009) |
| Days to germination | 2 | 0.0166* | –0.0121** | 0.0139** | 0.0275 | |
| | | (0.0073) | (0.0056) | (0.0052) | (0.0039) | |
| Rosette size | 2 | 0.8332+ | –0.6812* | 0.8922** | 2.3875 | |
| | | (0.4578) | (0.3892) | (0.3583) | (0.2516) | |
| Days to flower | 1 | 0.0127*** | –0.0070+ | 0.0129+ | 0.0062 | –0.0050* |
| | | (0.0040) | (0.0054) | (0.0077) | (0.0021) | (0.0028) |
| Biomass | 2 | 0.0315* | –0.0105+ | 0.0113+ | 0.0532 | |
| | | (0.0139) | (0.0087) | (0.0075) | (0.0074) | |

Model 1 fits all components, including the direct additive genetic variance ($\sigma^2_{Ao}$), the direct-maternal additive genetic covariance ($\sigma_{AoAm}$), the maternal additive genetic variance ($\sigma^2_{Am}$), the residual environmental component ($\sigma^2_{Eo}$) and the maternal environmental covariance ($\sigma^2_{Em}$); model 2 fits all but $\sigma^2_{Em}$; and model 3 fits all but $\sigma_{AoAm}$. See the Materials and Methods 'Statistical analysis' section for a description of model-selection procedures. +, $P \leq 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

**Table 2** Direct ($h^2_{Ao}$), maternal ($h^2_{Am}$) and total ($h^2_{total}$) heritabilities, and the direct-maternal genetic correlations ($r_{AoAm}$) for *Campanulastrum americanum*, calculated from univariate analyses using the inheritance model indicated in Table 1; approximate SE in parentheses

| | $h^2_{Ao}$ | $h^2_{Am}$ | $h^2_{total}$ | $r_{AoAm}$ |
|---|---|---|---|---|
| Seed mass | 0.064 (0.242) | 0.000 | 0.064 (0.242) | |
| Days to germination | 0.361 (0.154) | 0.303 (0.110) | 0.119 (0.122) | –0.793 (0.181) |
| Rosette size | 0.243 (0.132) | 0.260 (0.103) | 0.075 (0.108) | –0.790 (0.241) |
| Days to flower | 0.641 (0.187) | 0.649 (0.382) | 0.439 (0.198) | –0.544 (0.244) |
| Biomass | 0.368 (0.157) | 0.133 (0.088) | 0.249 (0.112) | –0.558 (0.267) |

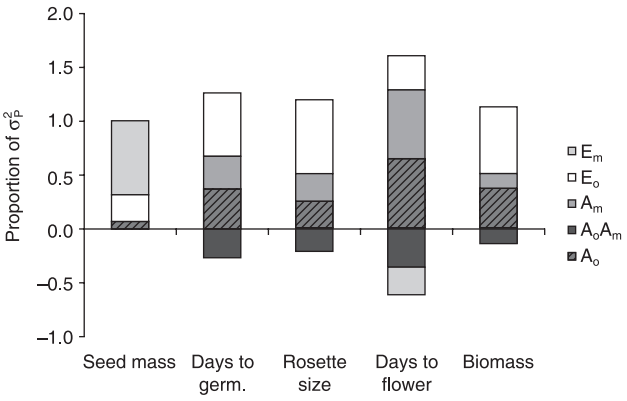


**Fig. 2** The relative contribution of each variance component to the total phenotypic variance ($\sigma^2_P$) in the univariate analysis. Variance components are given for the best-fit model and include the direct additive ($\sigma^2_{Ao}$), maternal additive ($\sigma^2_{Am}$), direct environmental (residual, $\sigma^2_{Eo}$) and maternal environmental ($\sigma^2_{Em}$) variance, and the direct-maternal additive covariance ($\sigma_{AoAm}$). See Table 1 for models and significance of each component of variance. germ, germination.

total heritability for days to flower and biomass was greater than for the juvenile traits. Days to flower had the greatest direct, maternal and total heritabilities (Table 2), suggesting that direct selection on this trait will yield the strongest evolutionary response.

The multivariate analysis revealed the importance of direct additive, maternal additive and the direct-maternal additive genetic covariance between traits (Table 3). Likelihood ratio tests indicated that each of the component matrices of the four-trait model contributed to the model fit, although the $\mathbf{A}_{o,m}$ matrix was marginally nonsignificant ($\mathbf{A}_o$: $D = 28.42$, degrees of freedom (df) = 10, $P < 0.001$; $\mathbf{A}_m$: $D = 22.20$, df = 10, $P < 0.007$; $\mathbf{A}_{o,m}$: $D = 21.18$, df = 10, $P < 0.086$; Table 3, see Table S2 for environmental and phenotypic matrices).

A comparison of $r_{ijAo}$ and $r_{ijAm}$ revealed fundamental similarities between the two matrices (Table 4). For example, the sign of the between-trait direct additive genetic correlations ($r_{ijAo}$) and the between-trait maternal additive genetic correlations ($r_{ijAm}$) was consistent (Table 4). This common pattern of relationship between traits for both forms of additive genetic variance suggests a similar underlying genetic basis that influences trait expression in the same direction. However, the between-trait maternal additive genetic correlations were larger than the direct additive genetic correlations for four of the six pairs of traits. Maternal additive genetic correlations were also more frequently significant (five of six) than direct additive genetic correlations (two of six with an additional genetic correlation being marginally nonsignificant). Regardless of the source, many of the genetic correlations among the four traits

**Table 3** Direct genetic ($\mathbf{A}_o$, a), maternal genetic ($\mathbf{A}_m$, b) and direct-maternal ($\mathbf{A}_{o,m}$, c) (co)variance matrices for *Campanulastrum americanum* estimated from the multivariate model

(a) $\mathbf{A}_o$

| | Days to germination | Rosette size | Days to flower | Biomass |
|---|---|---|---|---|
| Days to germination | 0.01226* | | | |
| | (0.00674) | | | |
| Rosette size | –0.09339* | 0.91809+ | | |
| | (0.04623) | (0.55642) | | |
| Days to flower | 0.00448+ | –0.03354 | 0.00951** | |
| | (0.00291) | (0.03105) | (0.00366) | |
| Biomass | 0.00257 | –0.05122 | 0.01226** | 0.02856*** |
| | (0.00334) | (0.04164) | (0.00494) | (0.00965) |

(b) $\mathbf{A}_m$

| | Days to germination | Rosette size | Days to flower | Biomass |
|---|---|---|---|---|
| Days to germination | 0.00642*** | | | |
| | (0.00237) | | | |
| Rosette size | –0.05826** | 0.87460** | | |
| | (0.02801) | (0.38700) | | |
| Days to flower | 0.00505** | –0.04815* | 0.00567 | |
| | (0.00222) | (0.02360) | (0.00727) | |
| Biomass | 0.00415* | –0.05688* | 0.00379+ | 0.00551+ |
| | (0.00210) | (0.03366) | (0.00316) | (0.00441) |

(c) $\mathbf{A}_{o,m}$

| | | Maternal | | | |
|---|---|---|---|---|---|
| | | Days to germination | Rosette size | Days to flower | Biomass |
| Offspring | Days to germination | –0.00483* | 0.05618** | –0.00605** | –0.00466+ |
| | | (0.00268) | (0.03070) | (0.00253) | (0.00295) |
| | Rosette size | 0.03902 | –0.58207+ | 0.04321+ | 0.04460 |
| | | (0.03364) | (0.46566) | (0.02900) | (0.05868) |
| | Days to flower | –0.00424+ | 0.03187 | –0.00164 | –0.00354+ |
| | | (0.00310) | (0.02924) | (0.04095) | (0.00233) |
| | Biomass | –0.00317 | 0.05648+ | 0.00167 | –0.00650+ |
| | | (0.00288) | (0.03552) | (0.00490) | (0.00439) |

Each matrix gives parameter estimates with the significance level and approximate SE (in parentheses). +, $P \leq 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

were very strong; eight of the 12 correlations exceeded 0.6, and three were > 0.8.

Phenological traits had positive direct and maternal additive genetic correlations across life-cycle stages (Table 4a). This indicates that there were families with faster life cycles, germinating early and flowering early, and those with slower life cycles, being later to both germinate and flower. By contrast, plant-size traits were negatively correlated across the life cycle. Rosette size had negative direct and maternal genetic correlations with final biomass, although this relationship was only significant for the latter. Strong negative direct and maternal genetic correlations were also evident between the juvenile traits timing of germination and rosette size, indicating that earlier germinating seeds become large rosettes, whereas later germinating seeds become small rosettes. For adult traits there were strong positive direct and maternal genetic correlations between days to flower and final biomass, such that early flowering plants were typically smaller than late flowering plants (although it was only significant for $r_{ijAo}$, Table 4a).

As found in the univariate analysis, $r_{AoAm}$ was consistently negative in within-trait comparisons (diagonal elements, Table 4b). This negative $r_{AoAm}$ was > 0.5 for three traits, but smaller and not significant for days to flower. Regardless of significance, negative within-trait $r_{AoAm}$ is expected to reduce the total genetic variance because it is opposite in sign to both the direct and maternal variances for each trait (Table 3), as seen in the univariate analysis. The same pattern is also apparent in the correlation structure between traits within generations ($r_{ijAo}$, $r_{ijAm}$) compared with the correlation structure between traits and across generations ($r_{ijAoAm}$). For example, days to

**Table 4** Direct ($r_{ijAo}$) and maternal ($r_{ijAm}$) additive genetic correlations between traits (a), and the between-trait direct-maternal genetic correlations ($r_{ijAoAm}$) (b) estimated from the multivariate model

(a) $r_{ijAo}$ are below the diagonal and $r_{ijAm}$ are above the diagonal

| | Days to germination | Rosette size | Days to flower | Biomass |
|---|---|---|---|---|
| Days to germination | | –0.778** | 0.837** | 0.698* |
| | | (0.146) | (0.436) | (0.317) |
| Rosette size | –0.880* | | –0.684* | –0.819* |
| | (0.106) | | (0.356) | (0.413) |
| Days to flower | 0.414+ | –0.359 | | 0.678 |
| | (0.206) | (0.273) | | (0.196) |
| Biomass | 0.137 | –0.316 | 0.744** | |
| | (0.162) | (0.216) | (0.081) | |

(b) $r_{ijAoAm}$

| | | Maternal ($A_m$) | | | |
|---|---|---|---|---|---|
| | | Days to germination | Rosette size | Days to flower | Biomass |
| Offspring ($A_o$) | Days to germination | –0.544* | 0.542** | –0.725** | –0.567+ |
| | | (0.172) | (0.209) | (0.439) | (0.371) |
| | Rosette size | 0.508 | –0.650+ | 0.599+ | 0.627 |
| | | (0.364) | (0.327) | (0.436) | (0.794) |
| | Days to flower | –0.543+ | 0.349 | –0.223 | –0.490+ |
| | | (0.359) | (0.292) | (–6.251) | (0.237) |
| | Biomass | –0.234 | 0.357+ | 0.131 | –0.518+ |
| | | (0.205) | (0.204) | (0.437) | (0.167) |

Approximate standard errors are given in parentheses as well as the significance associated with the covariance estimate. +, $P \leq 0.1$; *, $P < 0.05$; **, $P < 0.01$.

**Table 5** The maternal-effects matrix (**M**, Kirkpatrick & Lande, 1989)

| | | Maternal | | | |
|---|---|---|---|---|---|
| | | Days to germination | Rosette size | Days to flower | Biomass |
| Offspring | Days to germination | 0.216 | 0.469 | –0.460 | 0.364 |
| | Rosette size | –0.086 | –0.654 | 0.046 | 0.095 |
| | Days to flower | –0.379 | 0.339 | –0.289 | 0.520 |
| | Biomass | –0.206 | 0.070 | 0.035 | –0.214 |

Elements reveal the maternal effect of specific traits on offspring trait expression.

germination and days to flower had large, positive direct additive and maternal additive genetic correlations (Table 4a). By contrast, the genetic correlations across generations between days to germination and days to flower were large and negative (Table 4b). These antagonistic transgenerational relationships are expected to have a constraining effect on the evolution of combinations of maternal and offspring traits, similar to the expected effect for single traits.

The magnitude of the cross-generation genetic correlations ($r_{ijAoAm}$) differed among life-cycle stages for the direct genetic effects but not for the maternal genetic effects. Cross-generation correlations between traits were greater for direct-acting genes underlying juvenile traits than adult traits. In keeping with this observation, all the highly significant $r_{ijAoAm}$ were for the combination of direct genetic effects on days to germination with maternally acting genes for other traits (Table 4b). In particular, there was a very strong negative cross-generation correlation between direct genetic effects on days to germination and maternally acting genes for days to flower (Table 4b). By contrast, $r_{ijAoAm}$ was similar for maternally acting genes throughout the life cycle.

Days to germination and days to flower were influenced most by genetic maternal effects. After taking genetic correlations between traits into account, the average magnitude of the maternal effect coefficients in **M** on offspring phenological traits was twice that found on offspring size traits (Table 5). The

effects of maternal phenological traits on offspring phenology contributed to this pattern (Table 5). Earlier maternal flowering delayed offspring germination and flowering; earlier maternal germination delayed offspring flowering. By contrast, the maternal timing of germination reinforced a similar timing of germination in offspring. This positive maternal effect contrasts with the negative cross-generation genetic correlation ($r_{iiAoAm}$) for days to germination. The difference may be related to the strong genetic correlations between timing of germination and rosette size ($r_{ijAo} = -0.88$, $r_{ijAm} = -0.78$) as genetic correlations with other traits are accounted for in **M** but not in $\mathbf{A}_{o,m}$. Finally, size, regardless of whether measured in juveniles or adults, had a positive maternal effect on both offspring phenological traits. Large maternal plants had later-germinating and later-flowering offspring, holding all else constant, whereas smaller maternal plants had earlier-germinating and earlier-flowering offspring. By contrast, there was little effect of maternal size on offspring size measured at other stages of the life cycle, although effects of maternal size traits on offspring size traits within a life cycle stage were larger and negative (Table 5).

## Discussion

We found that maternal inheritance contributed to trait expression throughout the life cycle in the monocarpic herb *C. americanum*. With the exception of seed mass, the expression of all traits was influenced by direct genetic effects, maternal genetic effects and the correlation between direct and maternal genetic effects. Maternal genetic effects influenced patterns of genetic correlation between traits in addition to the expression of traits themselves. Indeed, maternal genetic correlations between traits tended to be stronger than direct genetic correlations. The strong evidence for genetic maternal effects on inheritance suggests that the evolutionary potential of *C. americanum* cannot be appropriately evaluated using the breeders' equation, $R = h^2S$, or its multivariate analogue, $\Delta\mathbf{z} = \mathbf{G}\boldsymbol{\beta}$, but requires predictive formulas that include cross-generational effects (e.g. Kirkpatrick & Lande, 1989). Our results support the longstanding evidence for genetic maternal effects and direct-maternal genetic correlations in agricultural plants and animals (reviewed in Meyer, 1992; Shaw & Byers, 1998; Wilson & Réale, 2006), as well as similar findings in a small, but growing number of investigations of wild plant and animal populations (e.g. Byers *et al.*, 1997; Thiede, 1998; Perry *et al.*, 2004; Räsänen & Kruuk, 2007).

A common theme throughout these studies is the prevalence of maternal effects early in the life cycle, followed by an increase in the relative magnitude of direct genetic effects and a decrease in maternal genetic effects for postjuvenile traits (Meyer, 1992; Lindholm *et al.*, 2006; Wilson & Réale, 2006). Many studies of plants and animals have focused on traits very early in the life cycle when it is likely that maternally acting genes will have important effects. For example, strong maternal effects have been detected for traits such as cotyledon diameter in plants (Thiede, 1998), yolk volume and juvenile body size in fish hatchlings (Perry *et al.*, 2004; Lindholm *et al.*, 2006), and birth date, litter size and birth weight in mammals (Meyer, 1992; McAdam *et al.*, 2002; Wilson *et al.*, 2005; Wilson & Réale, 2006; Kruuk & Hadfield, 2007). For such traits, it is not always clear whether a trait is controlled by the mother or the offspring, and it is perhaps expected that maternal genetic and/or environmental effects will have a greater influence on expression than direct effects.

Supporting this pattern, we found that seed mass in *C. americanum* was determined almost exclusively by the maternal environment. Maternal plants were grown under field conditions and therefore the maternal environmental effects may be a result of the microenvironmental variation common to natural habitats, which may have affected seeds directly or altered maternal resource conditions, indirectly influencing seed provisioning. An earlier study in *C. americanum*, using a classic paternal half-sib crossing design to estimate $\sigma^2_{Ao}$, also found that genetic variation for seed mass was not statistically distinguishable from zero (J. R. Etterson & L. F. Galloway, unpublished). Similarly, seed mass in *Collinsia verna* was only explained by the maternal environment when the data were analyzed using the model employed here; $\sigma^2_{Am}$ and $\sigma^2_{AoAm}$ contributed to seed mass when a more complex model that also included $\sigma^2_{EoEm}$ was used (Thiede, 1998). Direct heritability ($h^2_{Ao}$) of seed mass is very small in many plant species (reviewed in Platenkamp & Shaw, 1993; also Montalvo & Shaw, 1994; Byers *et al.*, 1997; Thiede, 1998). However, maternal heritability ($h^2_{Am}$) of seed mass in the few wild taxa evaluated is larger (Byers *et al.*, 1997; Thiede, 1998). Our results, combined with those from other studies, suggest that it may be more meaningful to consider seed mass as a trait of the mother rather than of the offspring, much as egg size is considered in animals (e.g. Fox *et al.*, 1999).

We also found that the contribution of maternal genetic inheritance ($\sigma^2_{Am}$, $\sigma_{AoAm}$) was statistically stronger for the juvenile traits, timing of germination and rosette size, whereas direct genetic inheritance, $\sigma^2_{Ao}$, was statistically stronger for the adult traits of flowering date and biomass. Surprisingly, expression of adult traits was also influenced by maternal genetic effects. There is little other evidence for maternal genetic effects on adult traits in natural plant populations; examples from animals include adult mass in burying beetles (Rauter & Moore, 2002), mass, gender ratio and caste ratio in ants (Linksvayer, 2006), and sexual behaviour in zebra finches (Forstmeier *et al.*, 2004). Also, in contrast to expectations, we did not find larger $M_{ij}$ values for offspring juvenile traits than for offspring adult traits. Our finding, of maternal genetic effects on traits expressed throughout the life cycle, suggests that further exploration of later-life traits is warranted to fully understand the evolutionary contribution of maternal inheritance.

## Effect of genetic correlations across generations on trait evolution

The correlation between direct genetic effects and maternal genetic effects was substantial and negative for all traits, a pattern that has been demonstrated in a wide range of agricultural and natural plants and animals (reviewed in Räsänen & Kruuk, 2007). Owing to these negative direct-maternal correlations, total heritability in *C. americanum* was smaller than either the direct or maternal heritability in almost all cases. Because the direct-maternal correlation was so large for juvenile traits, total heritability was only about one-third as large as the heritabilities estimated from the direct or maternal genetic effects. Consequently, the presence of maternal inheritance is expected to slow the response to selection and therefore the rate of evolutionary change in *C. americanum*. Previous work on this species supports these results. Direct heritability for days to flower, calculated using a paternal half-sib design, was $h_o^2 = 0.73$ (J. R. Etterson & L. F. Galloway, unpublished), whereas realized heritability calculated from artificial selection for earlier flowering, expected to be comparable to total heritability, was $h_R^2 = 0.31$ (Burgess *et al.*, 2007).

Cross-generation correlations expected to constrain selection response were also found between traits. We estimated $\mathbf{A}_o$, $\mathbf{A}_m$ and $\mathbf{A}_{o,m}$, and consistently found that within-generation correlations between traits ($r_{ijAm}$, $r_{ijAo}$) were of the same sign, and this sign was opposite to that found for the cross-generation correlations between the traits ($r_{ijAoAm}$). Although it is a novel observation that cross-generation contributions to multivariate evolution are simply an extension of the univariate case, it is perhaps not surprising. Traits under stabilizing selection are predicted to have cross-generation effects that oppose those found within a generation because negative direct-maternal correlations effectively reduce the total genetic variance (Wolf & Brodie, 1998). Stabilizing selection has been found in *C. americanum* for both timing of germination and flowering when their phenotypic distributions have been expanded by experimental manipulation (L. F. Galloway & K. S. Burgess, unpublished), but not in natural populations (Kilkenny & Galloway, 2008). In addition, combinations of these central life-history traits may also be under multivariate stabilizing selection.

## Effect of genetic correlations on life-history evolution

Positive correlations between phenological traits expressed at different times in the life cycle could influence life-history evolution in *C. americanum*. Timing of germination determines life-history schedule because fall-germinating individuals grow as annuals while spring-germinating seeds are biennial. Maternal flowering time also has a phenotypic effect on the timing of offspring germination; seeds from early flowering plants ripen and are dispersed earlier than seeds of late-flowering plants (Galloway & Burgess, 2009). These early dispersed seeds from early flowering plants are more likely to germinate as annuals, whereas late-dispersed seeds from later-flowering plants are more likely to germinate as biennials (Galloway, 2002; Galloway & Burgess, 2009). We detected positive direct and maternal genetic correlations between timing of germination and flowering, suggesting that the relationship between these phenological traits also has a genetic basis. Indeed, artificial selection for early flowering resulted in an increase in the frequency of annuals in nature, confirming that the evolution of these traits is nonindependent (K. S. Burgess & L. F. Galloway, unpublished). As a result, response to selection favouring early germinating annuals is expected to be greater than would be predicted based on the genetic variation in germination time alone because change in the timing of germination will alter the timing of flowering in the same direction, which in turn will influence germination time.

In contrast to the positive within-generation genetic correlations ($r_{ijAo}$, $r_{ijAm}$), the cross-generation correlation between timing of germination and timing of flowering ($r_{ijAoAm}$) is negative. These negative cross-generation effects should counteract the positive within-generation effects. Such conflicting interactions among genetic (co)variance components can be explored using the multivariate equivalent of total heritability (Willham, 1972). The composite genetic (co)variance matrix, calculated with the equation $\mathbf{A}_{total} = \mathbf{A}_o + 0.5\mathbf{A}_{o,m} + \mathbf{A}_{m,o} + 0.5\mathbf{A}_m$, predicts a population's response to natural selection when genetic maternal effects are present (J. W. McGlothlin & E. D. Brodie III, unpublished). Total additive genetic correlations between traits can then be explored by scaling genetic covariances by their respective genetic variances, $r_{total} = A_{total,\,ij} / \sqrt{(A_{total,\,ii}\, A_{total,\,jj})}$. Applying these calculations to the current data results in very low total genetic correlations between adult and juvenile traits (mean $|r_{tot}| = 0.06$), but large total correlations for traits within a life-cycle stage (mean $|r_{tot}| = 0.84$). This effect arises primarily from cross-generational genetic correlations ($r_{ijAoAm}$) that are opposite in sign to within-generation correlations, resulting in a decoupling of juvenile traits from adult traits. This is of interest from an evolutionary perspective because genetic correlations within and across life-cycle stages are a fundamental component of life-history theory (Roff, 2002).

We also found evidence for genetic correlations between phenological and size traits, which may influence life-history evolution, although these correlations were less consistently significant than correlations between phenological traits. Patterns of correlation within a developmental stage followed expectations based on life-history trade-offs (Stearns, 1992; Roff, 2002). For example, in the juvenile stage we would expect earlier-germinating seeds to become large rosettes and later-germinating seeds to become small rosettes. This expectation is upheld by a negative genetic correlation between these traits. Similarly, given that growth in monocarpic adults typically stops with the initiation of reproduction (Geber, 1990), we would expect earlier-reproducing plants to be smaller than later-reproducing plants. Again, the expectation is supported

at the adult stage by a positive genetic correlation between timing of flowering and final biomass. However, the patterns are more complex when correlations across juvenile and adult stages of the life cycle are considered. For example, the negative correlation between juvenile size and timing of reproduction means that small rosettes bloom late while large rosettes bloom early. This, in turn, creates a positive relationship between the timing of germination and final plant size; later-germinating seeds become small juveniles that bolt late and therefore are larger. These inter-relationships between size and phenology, as well as associated cross-generation effects, must be considered for a comprehensive understanding of potential response to selection on life-history schedule.

### Maternal effects on life-history evolution estimated by **M**

The maternal-effect coefficients in **M** can be seen as hypotheses for how specific maternal traits affect offspring phenotypes. We found that maternal effects on phenology were substantially stronger than those on size. In most cases, phenological traits expressed in the maternal generation had a negative effect on the phenological traits expressed in the offspring generation, paralleling the pattern found in $r_{ijAoAm}$. In other words, the maternal effect of mothers with earlier phenology tended to result in offspring with later phenology. By contrast, the maternal effect of size traits on offspring phenological traits was exclusively positive. Larger maternal plants, whether at the juvenile stage or at the adult stage, caused later germination and later flowering in their offspring.

Unlike the relationship between phenological traits, maternal effects of size on offspring phenology may create a positive-feedback cycle for life-history evolution. For example, because of maternal effects, larger plants have later-germinating seed. These seeds are therefore likely to germinate in the spring and grow as biennials. Within-generation correlations indicate that late-germinating plants (i.e. biennials) are larger as a result of genetic correlations in addition to the longer growth period before flowering. In total, the maternal effect of size reinforces the positive within-generation correlation such that large biennials are likely to produce large biennials. Theory predicts that this maternal effect would be adaptive because biennials, having double the life span of annuals, must have substantially larger reproductive success for comparable levels of fitness (Metcalf *et al.*, 2003; e.g. Galloway & Etterson, 2007). The association of larger size with later germination enables greater population growth for biennials and supports the contribution of maternal effects in maintaining the polymorphic life-history schedule.

In summary, **M** has produced a causal scenario of how maternally expressed traits may influence offspring trait expression, leading to the prediction that these genetic maternal effects contribute to the life-history variation and evolution. Because the concept of maternal effects posits a causal relationship between maternal and offspring phenotypes (Wolf & Wade, 2009), measuring **M** in addition to genetic (co)variances provides further insight into the potential influence of maternal effects on the evolutionary process.

### Conclusion

Previous work on *C. americanum* has demonstrated that maternal environmental effects have a substantial influence on offspring performance. First, offspring appropriately cued by their maternal light environment have three times greater fitness than those grown in an alternate environment (Galloway & Etterson, 2007). Second, maternal flowering time influences offspring germination time, which determines whether plants develop into annuals or biennials (Galloway & Burgess, 2009). Here, we have demonstrated a third way by which cross-generation maternal effects may influence trait evolution. Trait inheritance is determined by maternal genetic effects and the covariance between maternal and offspring genetic effects. In total, cross-generation effects influence phenotypic expression, genetic variation, and the potential for response to selection. Together, these studies suggest that we need to include across-generation contributions to trait expression in addition to within-generation estimates of additive genetic variance to understand patterns of adaptive evolution.

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## Supporting Information

Additional supporting information may be found in the online version of this article.

**Table S1** Mean, standard deviation, coefficient of variation and sample sizes for traits measured on *Campanulastrum americanum* in the $G_1$ and $G_2$ generations

**Table S2** Direct environmental, maternal environmental, and phenotypic (co)variance matrices for *Campanulastrum americanum* estimated from the multivariate model

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