

# Manipulation of flowering time: phenological integration and maternal effects

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**Abstract.** Timing of flowering is central to reproductive success and is currently advancing in many natural populations due to a warmer climate. However, we have little understanding of how earlier initiation of flowering influences subsequent reproductive phenology or the expression of traits in the offspring. To evaluate the consequences of an altered flowering phenology we manipulated cohorts of *Campanulastrum americanum*, an herb with annual and biennial growth forms, to flower and disperse seeds up to a month earlier, at the same time, and up to a month later than a natural population in two separate years. Relative to the date of first flower, the temporal patterns of flower production and the timing of fruit maturation and seed dispersal were similar among individuals that initiated flowering over the expanded reproductive season, indicating strong phenological integration of reproductive traits. However, plants that initiated flowering substantially outside the natural window showed a change in the rate of reproduction, with a compressed reproductive schedule for early-flowering individuals and an expanded one for late-flowering plants. Changes in flowering time had more dramatic effects on the offspring generation. Initiation of flowering two weeks earlier would result in a fourfold increase in the frequency of annual offspring, and four weeks earlier would result in a tenfold increase. The frequency of annuals was less sensitive to modest delays in flowering time but decreased with greater delays in flowering time. Collectively, these results reveal a tightly integrated reproductive phenology that shifts with timing of flowering within generations but may lead to more dramatic responses to climate change between generations.

**Key words:** *Campanulastrum americanum*; flowering time; indirect genetic effects; life-history evolution; maternal effects; phenotypic integration; phenotypic manipulation; reproductive phenology.

## INTRODUCTION

The timing of flowering is critical to plant reproductive success, influencing the number of flowers produced (Picó and Retana 2000), availability of pollinators (Brody 1997), abiotic conditions during fruit maturation (Lacey et al. 2003, Giménez-Benavides et al. 2007), and fruit herbivory (Bishop and Schemske 1998, Pilsen 2000). There has been an increasing focus on this key trait because earlier flowering has been observed in many taxa in response to the warmer winters and earlier springs associated with climate change (Bradley et al. 1999, Fitter and Fitter 2002, Peñuelas et al. 2002, Parmesan and Yohe 2003). Despite this attention, we still have little understanding of how accelerating the initiation of flowering may change the timing of subsequent reproductive events, i.e., reproductive phenology, including the temporal pattern of flower deployment, the timing of fruit maturation, and the timing of seed dispersal. For example, earlier-flowering

plants have been found to mature fruit earlier (Price and Waser 1998, Peñuelas et al. 2002, Post et al. 2008), especially in species that bloom early in the growing season (Sherry et al. 2007). Are such changes in fruit maturation time due to a shift in reproductive phenology in which earlier-initiated fruits develop on the same schedule and therefore mature earlier? Alternatively, might fruits change their developmental rate when produced earlier in the season, either expanding or compressing their maturation schedule? Are changes similar for other components of reproductive phenology? A shift in reproductive phenology would suggest that the timing of reproductive events is tightly integrated due to developmental, physiological, or genetic correlations (Pigliucci 2003). In contrast, a change in the relative timing of reproductive events would suggest that these traits are externally cued, e.g., by temperature or day length, and respond plastically to the ambient environment. Ultimately, the fitness consequences associated with an altered initiation of flowering depend on whether reproductive phenology is integrated or flexible in response to changing environmental cues.

A change in the timing of plant reproduction may also influence the expression of traits in the offspring generation. A number of maternal traits have been shown to determine the offspring phenotype. For

Manuscript received 21 May 2008; revised 26 November 2008; accepted 8 December 2008. Corresponding Editor: R. J. Mitchell.

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example, maternal density influences offspring growth form (Donohue 1999), solar tracking of maternal flowers influences seed size (Stanton and Galen 1989), and maternal seed dispersal time influences offspring germination patterns (Lacey and Pace 1983, Donohue et al. 2005). Similarly, a change in the timing of reproduction may result in maternal effects that influence the expression of seed, seedling, or adult traits of the offspring. Under these conditions, the evolution of flowering time will result in altered offspring traits, not due to genetic change in the offspring, but rather to the "indirect" effects of changes in maternal trait expression (Wolf et al. 1998). To date potential cross-generation implications of altered flowering times due to warmer climates have largely been ignored.

Understanding the within- and between-generation consequences of changes in flowering time requires study of a range of phenotypes. Unfortunately, strong selection on traits closely related to fitness, such as flowering time, often reduces variation in trait expression, yielding few individuals that express extreme traits (Conner and Hartl 2004). However, phenotypic manipulation, in which the phenotype of a specific trait is altered to increase the frequency of rare forms and create phenotypes more extreme than those found in nature, provides an effective experimental approach. The resulting broad trait distribution makes it possible to evaluate mechanistic relationships among traits independent of underlying genetic relationships. Phenotypic manipulation is a powerful tool that has been used to unravel causal factors of phenotypic selection (Wade and Kalisz 1990), test adaptive hypotheses (Schmitt et al. 1999, Lenssen et al. 2005), evaluate fitness consequences of novel phenotypes (Griffith and Watson 2006), and elucidate functional attributes of traits (Arathi and Kelly 2004). In particular, this approach is well suited for determining whether changing flowering time alters subsequent reproductive phenology and results in cross-generation effects.

Here we manipulate flowering time to examine the phenological integration within and between generations in *Campanulastrum americanum*, a native wildflower with annual and biennial growth forms. Previous work on this species demonstrated that flowers that are produced earlier on a plant form early-maturing fruits whose seeds are more likely to germinate as annuals (Galloway 2002). These results suggest that environment-associated changes in flowering time may alter the timing of fruit production and the frequency of the annual and biennial growth forms in this species. To test this, we manipulated individuals from a random genetic background to flower and disperse seeds earlier, at the same time, and later than the natural population. We then measured patterns of flower production, the time to fruit maturation, and the pattern of seed dispersal, as well as the frequency of fall and spring germination. A unified shift in reproductive events with flowering time would indicate phenological integration,

whereas changes in the relative timing or duration of reproductive events among individuals that differ in flowering time would indicate that reproductive phenology is environmentally cued. Specifically, we address the following questions: (1) Does the seasonal pattern of flower production depend on when plants initiate flowering? (2) Is the time to fruit maturation constant across the flowering season? (3) What is the timing of seed dispersal from mature fruit and is it consistent across the reproductive season? (4) Does dispersal time influence the timing of germination and therefore offspring life history?

## MATERIAL AND METHODS

### *Study system*

*Campanulastrum americanum* (L.) Small (= *Campanula americana* L., Campanulaceae) is an outcrossing insect-pollinated North American herb that flowers from early July to late August (Galloway et al. 2002, 2003, Kilkenny and Galloway 2008; see Plate 1). Flowers are located in compact inflorescences at reproductive nodes on the main stem and lateral branches. Most fruits on understory plants contain 15–40 seeds (Galloway 2002, Galloway and Etterson 2007). When fruits mature, four pores open at the top and seeds are shed passively when plants are jostled. *Campanulastrum americanum* seeds have no after-ripening period (Baskin and Baskin 1984) and therefore can germinate immediately following dispersal. However, cold temperatures are required for flowering. Therefore seeds that germinate in the fall, shortly after dispersal, grow as annuals and flower the following summer. Seeds that are delayed in germination until spring grow as rosettes for their first year and flower their second summer as biennials.

We experimentally manipulated the initiation of flowering and timing of seed dispersal for two years in understory regions of a natural *C. americanum* population on Beanfield Mountain, 3.6 km from Mountain Lake Biological Station, Giles County, Virginia, USA (elevation 1143 m). Throughout, mixed-model analyses were conducted using PROC MIXED (SAS Institute 2005).

### *Manipulation of flowering time*

In two separate years, five cohorts of *C. americanum* were created to flower earlier, at the same time, and later than the natural population. Seeds collected from plants in the study population (year 1, 100 plants; year 2, 50 plants) were evenly sampled to create five cohorts of 100 individuals. Cohorts were planted at two-week intervals from 10 January through 17 March in year 1 (2004) and at three-week intervals from 6 December through 31 January in year 2 (2005). Once planted, seeds were placed in a growth chamber (21°C day, 14°C night; 12-h days), and three weeks later seedlings were moved to a cold room to stimulate flowering (5°C, 12-h days). In year 2 the transfer to the cold room was delayed four weeks to allow time for growth prior to vernalization.

Following seven weeks of vernalization, plants were moved to a greenhouse in which supplemental lighting increased day length to 16 h and plants were watered and fertilized regularly. In year 1, cohorts were moved into the field at 15-day intervals: 1 July (cohort 1), 15 July (cohort 2), 31 July (cohort 3), 15 August (cohort 4), 30 August (cohort 5). In year 2, the experiment was initiated earlier and cohorts were moved into the field at 21-day intervals: 6 June (cohort 1), 28 June (cohort 2), 18 July (cohort 3), 8 August (cohort 4), 29 August (cohort 5). Under the forest canopy *C. americanum* typically begins flowering 15 July (mean of four years; SE = 0.56), with the peak initiation of flowering 21 July (Galloway 2002; L. F. Galloway, unpublished data). Therefore in year 1, cohort 1 initiated flowering two weeks prior to the natural population, cohorts 2 and 3 within the natural window, and cohorts 4 and 5 were 3.5 and 5.5 weeks after the natural population peak. In year 2, cohort 1 initiated flowering five weeks prior to the natural population, cohort 2 two weeks prior, cohort 3 near the natural population peak, and cohorts 4 and 5 were 2.5 and 5.5 weeks later than the population peak. By holding the length of time prior to flowering constant, cohorts differed in timing of flowering but were expected to be otherwise similar.

The 25 plants in each cohort that initiated flowering closest to the designated initiation date were moved to the study population. (In a few cases plants had begun flowering within the previous week and were held at 10°C to slow their development.) Plants from each cohort were then divided among four blocks, each surrounded with plastic netting to prevent deer herbivory. In year 1 potted plants were placed in the field, but in year 2 individuals were transplanted directly into the ground. In each block, plants from each cohort were randomly located in a 1-m grid, a spacing that limited interactions among individuals. Plants were staked and watered as necessary throughout the reproductive season.

Plants were censused at five-day intervals in year 1 and seven-day intervals in year 2. The “day” of each census was recorded as the number of days since cohort 1 was placed in the field. The “standardized census day” sets the initial day that each cohort is placed in the field as zero and was calculated to facilitate the comparison of cohorts. In year 1, we counted all open flowers and marked four at each census. We checked marked flowers at subsequent censuses and noted when fruit were mature, i.e., dehisced. In year 2, three flowers were marked at alternate censuses (14-day intervals); otherwise the procedure was the same. Flowers typically remain open two to three days (Evanhoe and Galloway 2002), and thus new flowers were marked at each census. However, some late-season flowers in year 1 remained open across censuses; we report only new flowers. The sum of flowers open at each census is an index of total flower production.

Seasonal patterns of flower production for each plant were summarized with “average flower day,” the mean

census day that an individual’s flowers were produced. This was done by weighting each standardized census day by the proportion of an individual’s total flower production that was open on that day and summing over all census days (cf. Nuismer and Cunningham 2005). The number of days from the date a flower was open to fruit maturation was averaged over all flowers marked at each census on each individual; analyses were conducted on plant means. Average flower day, total flowers, and days to fruit maturation were analyzed using analysis of (co)variance with cohort (fixed) and block (random) as factors. The analysis of days to fruit maturation also included the standardized census day flowers were marked (covariate) and plant nested within cohort (random), since plants were sampled in multiple censuses. The interaction between standardized census day and cohort was tested to determine whether the seasonal pattern of days to fruit maturation varied among cohorts. Separate analyses were conducted for the two years due to variation in the timing of cohort initiation as well as the census interval between years.

#### *Pattern of seed dispersal*

The pattern of seed dispersal was determined by quantifying the number of seeds remaining in fruits at intervals after fruits dehisce. Seven young fruits near the same developmental stage were labeled on each plant in all five cohorts in year 1. Fruits were typically located at sequential nodes on the main stem and represented the first flowers on the plant. The first labeled fruit on each plant that turned straw colored, indicating it was nearly mature, was collected and used to estimate the number of seeds prior to dispersal. The remaining six fruits were checked every five days to see whether they had dehisced. At each five-day census newly dehisced fruit were assigned to be collected at sequential five-day intervals. Therefore, if three fruits were dehisced they would be collected 5, 10, and 15 days from that census (representing fruit that had been open 5–9, 10–14, and 15–19 days). If the remaining three fruits had dehisced by the following census, they would be collected 5, 10, and 15 days from that later census. This collection technique yielded estimates of seed dispersal throughout the fruit maturation period and therefore episodic environmental factors (e.g., rain, strong wind) did not disproportionately influence results. It also resulted in larger sample sizes in the earlier dispersal intervals in which seed number was more variable.

The number of seeds remaining in a fruit was analyzed using ANOVA to determine whether the pattern of seed dispersal varied over the season. Cohort and the number of days a fruit had been dehisced (in five-day intervals) were fixed effects, while block and plant (nested in cohort) were random. A significant interaction between cohort and days dehisced would indicate that the pattern of seed dispersal varied among cohorts. Seed number was square-root transformed to meet the assumptions of ANOVA.

### *Manipulation of seed dispersal*

To determine whether the timing of fruit maturation and seed dispersal influences whether seeds germinate in the fall as annuals or in the spring as biennials, we experimentally manipulated the timing of dispersal in two separate years. In year 1 (2004), field-collected seeds from 100 plants were used. To increase germination rate in year 2 (2005), we used seeds created by conducting pollinations among 12 individuals in the greenhouse. Seeds were planted into their home population earlier, at the same time, and later than natural seed dispersal. In year 1, eight cohorts of seeds were planted at two-week intervals from 27 July through 2 November. In year 2, six cohorts of seeds were planted at three-week intervals from 26 July through 8 November. Seed dispersal of native understory plants typically begins 10 September, peaks 17 September, and continues through 5 November (E. M. Yoshizuka and L. F. Galloway, *unpublished data*).

Each year seeds were planted into 30 blocks. Blocks were paired, and one member of each pair was watered regularly to test whether water availability influenced patterns of germination. Four replicates of each cohort (five in year 2) were planted in randomized locations in each block. A group of seeds was planted for each replicate. In year 1, maternal families were equally divided between 8 and 12 seeds per replicate and one (occasionally two) replicate per family was planted for each cohort. In year 2, 10 seeds were planted for each replicate and one replicate of each maternal family was planted into each pair of blocks for each cohort. In total, 960 replicates were planted in year 1 (120/cohort), and 900 in year 2 (150/cohort). Seeds were planted into  $3.8 \times 3.8 \times 15$  cm cardboard sleeves placed adjacent to one another. The sleeves were filled with local soil except the top 5 cm was a 1:1 mix of sterilized local soil and potting mix to prevent germination from the seed bank (K. L. Stuble and L. F. Galloway, *unpublished data*). Seeds were sown on the soil surface on the assigned date and covered with  $<0.5$  cm of the soil mix. After planting, the blocks were covered with a wire mesh to reduce granivory and trampling. Seeds were checked weekly for germination from planting until snowfall and from snowmelt until no new seedlings were observed for two intervals in the spring (see Plate 1). A very small percentage of the remaining seeds emerged following the first spring (Galloway 2001); therefore germination was only scored for a single fall and spring (see Plate 1). As a result there was no overlap between the studies initiated in year 1 and year 2; therefore we refer to the replicates of the experiment by the year the seeds were planted even though germination occurred across two calendar years. We report annual (fall) and biennial (spring) germination totals.

We evaluated whether timing of seed dispersal influenced the proportion that germinated as annuals or biennials. In year 2, we used an ANOVA to determine the effect of cohort, life history (annual or biennial),

family, water addition, and block (nested in water) on the proportion of seeds that germinated (number germinated/number planted). Block, family, and interactions with family were treated as random effects. Life history tests whether proportion germination differs between the fall (annual) and spring (biennial); the interaction between cohort and life history indicates whether the timing of seed dispersal influences the proportion that germinate as annuals or biennials. In year 1, only seeds in the first three cohorts germinated as annuals and fall germination rates were small, resulting in too many zeros for ANOVA. Therefore for the first four cohorts (one with no annual germination, analogous to year 2), individual replicates were assigned a value of "1" for a season if any seeds germinated and "0" if not. Use of this dichotomous variable permits analysis of low germination rates, but small values will have a greater statistical importance. A log-linear analysis was conducted on this dichotomous germination variable including cohort, life history, water addition, and block (PROC GENMOD; SAS Institute 2005). Family was not included because replication was too limited when only four cohorts were considered. In year 1, total germination proportion was analyzed as in year 2, but without "life history." In both years, water addition had little influence on germination proportion. Interactions with water addition were not significant and therefore were dropped from the statistical models.

## RESULTS

### *Manipulation of flowering time*

Flowering patterns varied among cohorts that initiated blooming at different times (Fig. 1). In both years the middle three flowering cohorts had a similar time to peak flower production. Cohort 1 peaked slightly earlier in both years, and cohort 5 peaked later in year 1. The average flower day varied among cohorts and was earlier for the early cohorts than for the later cohorts (year 1,  $F_{4,110} = 7.49$ ,  $P < 0.001$ ; year 2,  $F_{4,109} = 13.62$ ,  $P < 0.001$ ; Fig. 2), indicating that cohorts that initiated flowering later in the season deployed their flowers more slowly, despite a similar timing of peak flowering. None of the plants in year 2 flowered as long as year 1, despite regular watering in both years (see *x*-axis, Fig. 1).

The number of days to fruit maturation was similar across cohorts in year 2 (Table 1, Fig. 3). However in year 1, seasonal effects on the days to fruit maturation varied among cohorts (cohort  $\times$  standardized census day; Table 1). This interaction was due to cohort 1, which matured fruit more quickly as the season progressed (Fig. 3). An analysis of cohorts 2–5 revealed similar seasonal patterns of days to fruit maturation among cohorts (cohort,  $F_{3,75} = 2.62$ ,  $P = 0.06$ ; standardized census day,  $F_{1,160} = 1.04$ ,  $P = 0.31$ ; cohort  $\times$  standardized census day,  $F_{3,160} = 0.35$ ,  $P = 0.79$ ). In year 2, the days to fruit maturation dropped across the reproductive season within each cohort (Table 1, Fig. 3). In summary, with the exception of cohort 1 in year 1,

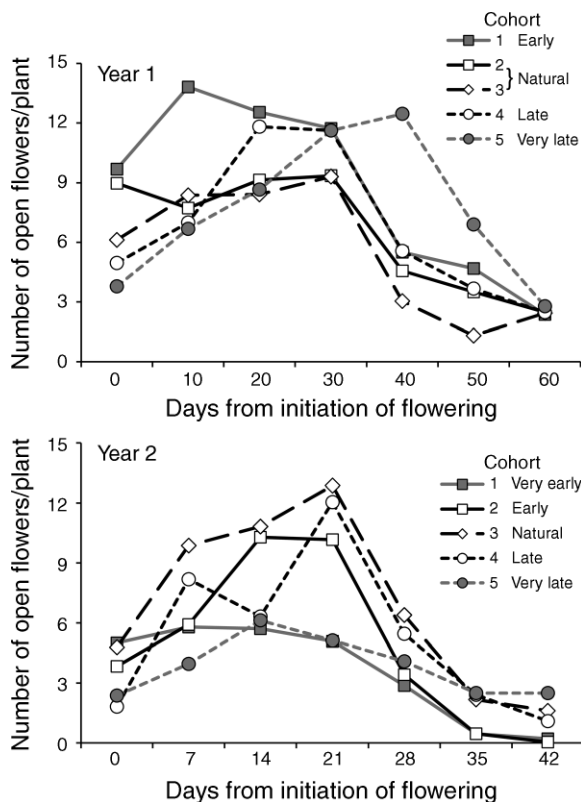


FIG. 1. Mean number of open flowers per plant at intervals from the initiation of flowering for cohorts of *Campanulastrum americanum* that initiated flowering earlier, at the same time, and later than the native population. The experiment was conducted in two years, beginning three weeks earlier in year 2 than year 1, at Mountain Lake Biological Station, Giles County, Virginia, USA. Sequential cohorts were initiated every two weeks in year 1 and every three weeks in year 2.

days to fruit maturation was tightly associated with the day the flower that produced that fruit was open.

Total flower production differed little among cohorts in year 1 ( $F_{4,111} = 2.43, P = 0.052$ ), and in year 2; cohort means were similar except that cohort 1 produced fewer flowers than cohort 3 ( $F_{4,109} = 17.05, P < 0.001$ ). In both years, later flowers were less likely to yield mature fruits in the later cohorts (Fig. 3; each successive cohort has a shorter line). Only fruit that had been initiated by census day 70 in year 1 and day 112 in year 2 had enough time to mature fruit prior to plant death with the onset of winter (Appendix).

*Pattern of seed dispersal*

On average, half of the seeds dispersed from fruit in the first five days after the pores opened (days dehiscid,  $F_{4,606} = 51.34, P < 0.001$ ; Fig. 4). Almost all seeds had dispersed within three weeks. The pattern of seed dispersal did not vary among cohorts of plants that initiated flowering at different times (cohort  $\times$  days dehiscid,  $F_{19,606} = 0.55, P = 0.94$ ), indicating a similar pattern of seed dispersal regardless of when in the season fruits mature. Therefore, if fruits mature over a long time window, seeds will also disperse over an extended period of time.

*Manipulation of seed dispersal*

The timing of seed planting strongly influenced whether seeds germinated in the fall as annuals or the spring as biennials (cohort  $\times$  life history; Table 2). Although overall germination was less in year 1, the influence of dispersal time on seasonal patterns of germination was similar in both years: the later seeds were dispersed, the fewer germinated as annuals (Table 2, Fig. 5). Limited fall germination in later-dispersed

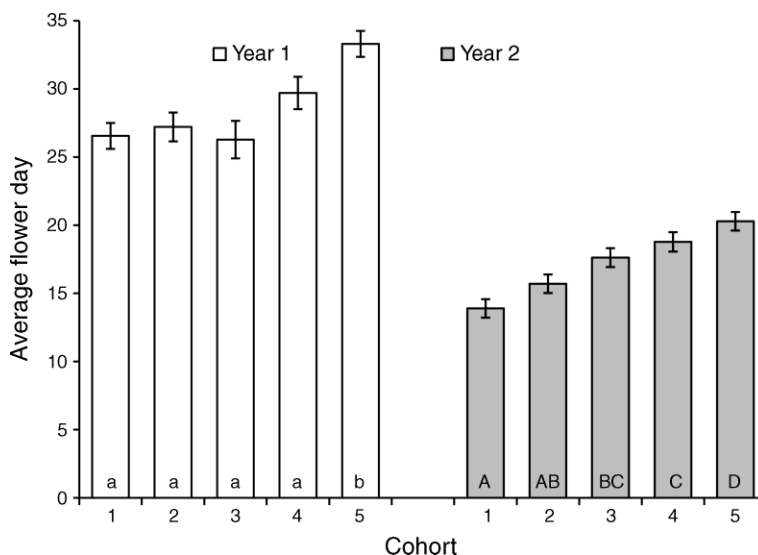


FIG. 2. Average flower day (mean  $\pm$  SE) for five cohorts of *Campanulastrum americanum* manipulated to initiate flowering earlier, at the same time, and later than the native population in two separate years. Means within each year that are different at  $\alpha \leq 0.05$  with a Tukey multiple comparison are indicated with different letters. See Fig. 1 for timing of cohort initiation.

TABLE 1. ANCOVA of the number of days to fruit maturation for flowers open at different times in the season for five cohorts of *Campanulastrum americanum* that initiated flowering earlier, at the same time, and later than the native population, studied in two years.

Source	df†	Year 1		Year 2	
		F/Z	P	F/Z	P
Cohort	4	2.86	<0.0274	1.21	<0.3125
Standardized census day	1	2.54	<0.1126	61.05	<0.0001
Cohort × Std. census day	4	4.80	<0.0010	1.55	<0.1926
Plant		2.79	<0.0026	4.82	<0.0001
Block		1.04	<0.1486	0‡	...

Notes: F statistics and degrees of freedom are given for fixed effects, and Z values for random effects. The study was conducted at Mountain Lake Biological Station, Giles County, Virginia, USA.

† Denominator df: year 1 = 246 (except cohort, for which df = 96); year 2 = 123 (except cohort, for which df = 106). Degrees of freedom are given only for fixed effects.

‡ Covariance estimates of random effects have a lower bound of zero.

cohorts resulted in a majority of biennial seedlings. Families varied in the proportion of annual and biennial offspring among cohorts (year 2; Table 2), indicating genetic variation for the effect of dispersal time on the proportion of annuals and biennials.

The total germination proportion depended upon when seeds were dispersed (year 1,  $F_{7,800} = 34.27$ ,  $P < 0.0001$ ; year 2, Table 2). Tukey multiple comparisons of germination among cohorts found the later cohorts (after 1 October) had the greatest germination in both years, significantly more than the middle cohorts (August and early September; Fig. 5). The earliest cohort and the last cohort in September had intermediate germination rates in both years.

There was little evidence that limited germination of annuals was due to lack of rain in the fall. In year 1, watered seeds had slightly greater germination rates in the first four cohorts (Table 2), but the effect was modest and not found in the analysis of all cohorts ( $F_{1,28} = 0.89$ ,  $P = 0.354$ ). In year 2, water addition did not influence germination rates (Table 2).

DISCUSSION

Reproductive phenology was similar for cohorts of *Campanulastrum americanum* manipulated to initiate flowering earlier, at the same time, and later than the natural population. Although early-flowering plants experienced a longer growing season, they shifted their entire reproductive phenology earlier and did not extend reproduction. Initiation of flowering several weeks earlier than native plants, almost twice the change typically observed in response to warming climates (e.g., Fitter and Fitter 2002, Peñuelas et al. 2002), resulted in near-constant patterns of flower production, average flower day, and days to fruit maturation. Similar patterns were found when flowering was delayed by several weeks. As a result, early flowers matured fruit early and late flowers gave rise to late-season fruit. The temporal pattern of seed dispersal was constant across the fruit maturation period. Therefore the timing of fruit maturation predicts when seeds disperse and are available for germination. There was a strong associa-

tion between the time of seed dispersal and offspring life-history schedule, with earlier-dispersed seeds more likely to germinate as annuals than later-dispersed seeds. Similar patterns of reproduction, regardless of when flowering was initiated, indicate reproductive phenology was integrated within individuals. Moreover, phenology was also integrated between generations, as indicated by the link between reproductive timing and germination timing. A consequence of this transgenerational associ-

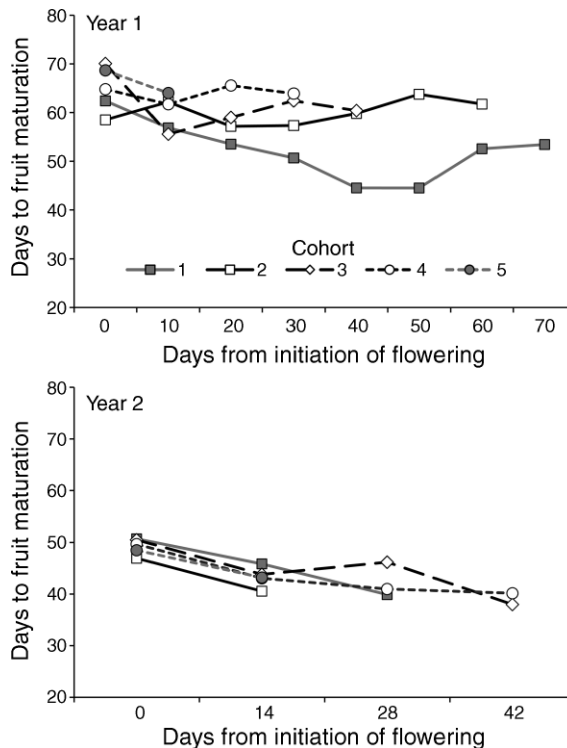


FIG. 3. Mean number of days to fruit maturation plotted against the number of days from the initiation of flowering. Fruit were produced on five cohorts of *Campanulastrum americanum* that were manipulated to initiate flowering earlier, at the same time, and later than the native population in two years. See Fig. 1 for timing of cohort initiation.

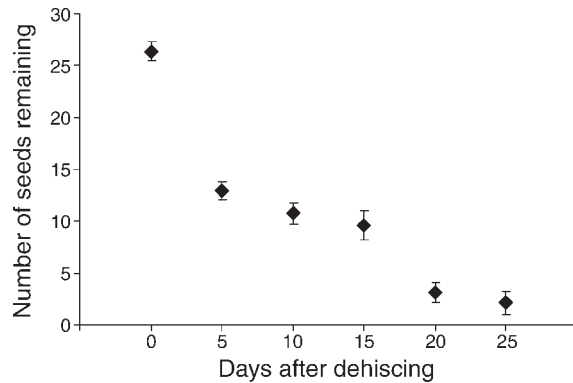


FIG. 4. Temporal pattern of seed dispersal in *Campanulastrum americanum*: number of seeds (mean ± SE) present in fruit prior to dehiscent (day 0) and at five-day intervals after fruits dehisce.

ation is that offspring life-history schedule is determined in part by maternal flowering time.

Although the traits associated with reproductive phenology were integrated within years, the duration of reproductive events differed between years. In year 2, plants reached their flowering peak a week faster, the average flower day was 11 days earlier, and individuals bloomed for only two-thirds as long as those in year 1. Similarly, fruit maturation was two weeks faster in year 2 than in year 1. As a result, plants had a compressed reproductive schedule in year 2. Annual differences in temperature may contribute to the pattern, as year 2 was warmer on average than year 1 (data not shown). However, it is also likely that the potted plants used in year 1 experienced a more benign growth environment and therefore a slower reproductive phenology than the transplanted individuals in year 2. Regardless of source, these annual differences demonstrate that reproductive phenology is plastic and can be either compressed or

expanded. They also reinforce the conclusion that reproductive phenology is integrated within individuals because plants in year 2 shifted to a more rapid phenology for all reproductive traits measured.

Reproductive phenology was somewhat different in cohorts manipulated to bloom substantially outside the natural window. Specifically, individuals that initiated flowering a month later than the natural population deployed their flowers more slowly, resulting in a later average flower day. As a consequence, late-flowering plants will have additional delays in fruit production than would be predicted by the initiation of flowering alone. In contrast, individuals that flowered a month earlier than the natural population showed the opposite pattern; they produced more flowers in the early part of their reproductive period, giving rise to an earlier average flower day and fewer total flowers. Furthermore, the cohort that flowered prior to the natural population in year 1 also matured fruit more rapidly as the season progressed. As a result, plants that flowered substantially earlier than the natural population had a reproductive phenology that was compressed as well as advanced. These changes in reproductive phenology for cohorts manipulated to bloom substantially outside the natural window are likely due to environmental factors. In particular, with the exception of cohort 1 in year 2, all cohorts were initiated after the vernal solstice, and therefore photoperiod decreased with each sequential cohort. In addition, the final cohort experienced cooler growing conditions in both years, measured as growing degree-days (data not shown), which may have resulted in the slower deployment of flowers.

Broadly analogous results are found in some experimental warming studies that report that earlier initiation of flowering is followed by a shorter duration of reproduction (Sherry et al. 2007, Post et al. 2008). However, unchanged or longer duration of reproduction is found in other species (Price and Waser 1998, Dunne

TABLE 2. Analysis of germination of *Campanulastrum americanum* seeds planted in cohorts earlier, at the same time, and later than the dispersal of native seeds.

Source	Year 1			Year 2		
	df	$\chi^2$	P	df†	F/Z	P
Cohort	3	51.29	<0.0001	5, 55	19.40	<0.0001
Life history	1	464.47	<0.0001	1, 11	323.89	<0.0001
Cohort × life history	3	20.32	<0.0001	5, 55	144.54	<0.0001
Family					0.08	<0.470
Cohort × family					0‡	...
Life history × family					1.26	<0.104
Cohort × life history × family					3.23	<0.001
Water	1	4.18	0.041	1, 28	0.02	<0.877
Block(water)	28	57.48	0.0008		2.99	<0.001

Notes: Life history reflects the frequency of germination as annuals (fall) and biennials (spring). Half of the blocks were watered to ensure that germination was not limited by rainfall. Log-linear analysis of germination in each replicate for seeds planted in year 1 is shown. For year 2, results of ANOVA of proportion germination for seeds planted are given, where F statistics and degrees of freedom are shown for fixed effects and Z values are shown for random effects.

† Degrees of freedom are given only for fixed effects.

‡ Covariance estimates of random effects have a lower bound of zero.

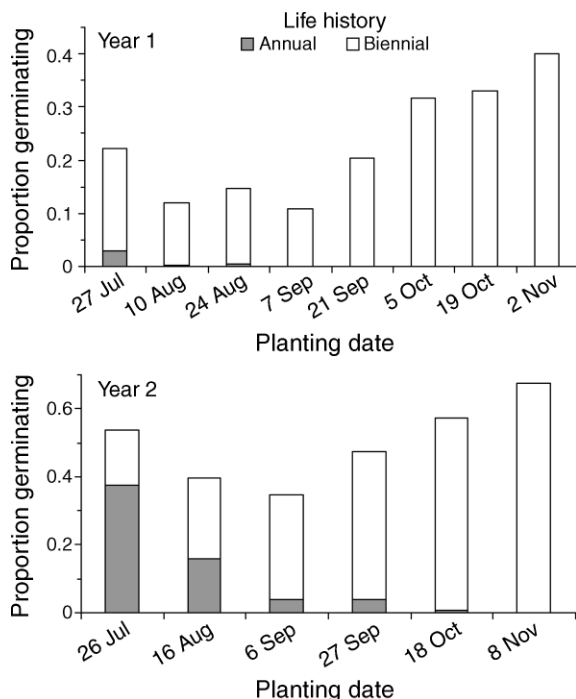


FIG. 5. The proportion of seeds germinating in the fall as annuals and the spring as biennials for seeds of *Campanulas-trium americanum* planted earlier, at the same time, and later than the natural dispersal period in two separate years. Natural seed dispersal typically peaks around 17 September.

et al. 2003, Sherry et al. 2007, Post et al. 2008). A previous investigation of response to growing-season length in *C. americanum* found that plants moved to a longer, warmer growing season flowered earlier, had an earlier average flower day, and matured fruit more rapidly (Haggerty 2006), revealing a reproductive phenology that also shifted earlier and became more compressed. While such changes in reproductive phenology may influence fecundity, especially if pollinator availability changes over the season or as climates change, the largest fitness effects in *C. americanum* are likely to be expressed as an altered frequency of annual and biennial growth forms.

In *C. americanum*, timing of flowering determines the timing of seed dispersal. Timing of seed dispersal, in turn, determines the probability that seeds will grow as annuals or biennials. Previous work in this species found earlier flowers on individuals were more likely to give rise to annual offspring (Galloway 2002). Here we demonstrate that maternal flowering time influences offspring life-history schedule. In fact, offspring life history was substantially more sensitive to changes in flowering time, in particular advances in flowering, than within-generation reproductive phenology. Using germination data collected in year 2, a two-week advance in flowering time would be expected to yield a fourfold increase in annual offspring and a four-week advance would be expected to yield a 10-fold increase. In

contrast, a two-week delay would not be expected to alter offspring life history but a month's delay in flowering would result in only one-eighth as many annuals. Similar to our findings, seed dispersal time determines the season of germination for *Daucus carota* (Lacey and Pace 1983) and whether offspring express a winter or spring annual life history in *Arabidopsis thaliana* (Donohue et al. 2005).

Because the wide range of flowering time and seed dispersal phenotypes reported here were created by manipulating environmental cues, this study demonstrates that the effect of maternal flowering time on offspring life-history schedule is due to the flowering time itself, not an underlying genetic correlation between flowering phenology and germination phenology. As a consequence of these phenotypic maternal effects, a change in the frequency of annuals and biennials in a population can be achieved by plastic responses to the environment that accelerate or delay flowering. Although the understory habitats used in the present study were dominated by biennials (Galloway and Etterson 2007), earlier flowering in understory *C. americanum* plants grown under a longer growing season (Haggerty 2006) suggests a plastic response in flowering initiation to warmer climates may increase the frequency of annuals in this population. Furthermore, we have shown that any increase in the frequency of annuals driven by climate change is expected to be even greater than that predicted from shifts in flowering time alone due to the compressed timing of flower deployment found in earlier-flowering plants. However, these ideas need to be formally evaluated as the plants in this study experienced similar photoperiods prior to placement in the field, an unlikely scenario if warmer environments altered flowering time.

Lack of strong cohort-dependent patterns suggest phenological traits in *C. americanum* are integrated and correlations are more important than external factors in determining the timing of reproductive traits. As a consequence, response to any selection on flowering time is expected to yield the evolution of the suite of characters that comprise reproductive phenology. Flowering time can evolve rapidly in *C. americanum*; three generations of artificial selection resulted in a 25-day difference in flowering initiation between plants selected to flower early and those selected to flower late (Burgess et al. 2007). Selection favoring either early or late flowering is also expected to yield an associated change in the frequency of annuals and biennials, extending the correlated response to selection across generations. Such a change of the ratio of annuals to biennials would, in turn, alter the generation time and therefore expected rate of evolution. Prediction of evolutionary response of flowering time to any selection from warmer climates therefore would need to consider the within- and between-generation fitness consequences of changes in flowering time (e.g., Kirkpatrick and Lande 1989, Donohue 1999).

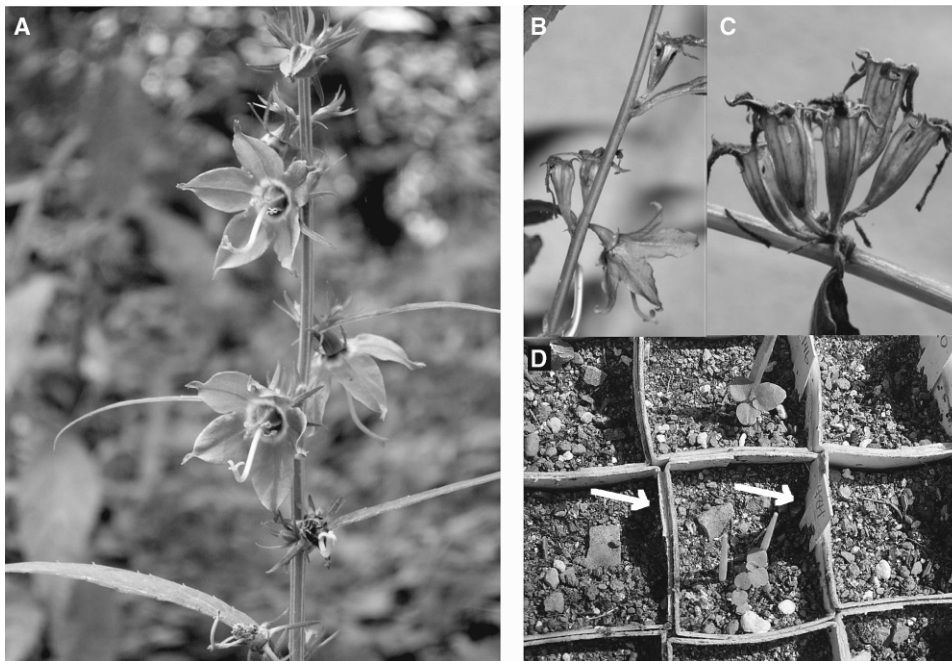


PLATE 1. Stages in *Campanulastrum americanum*'s reproductive phenology including (A) flowers, (B) immature fruit, and (C) mature fruit dispersing seeds. (D) The setup for the study in which seed dispersal time is manipulated showing different planting time treatments. The arrow on the right in panel (D) points to a tag indicating that seeds have been planted, while the absence of a tag by the left arrow indicates a later planting time for that replicate. Fall germinated *C. americanum* seedlings are present in the earlier planting time treatments. Photo credit: L. F. Galloway.

The expanded range of flowering and seed dispersal times created by the experimental treatments provided the opportunity to demonstrate a causal relationship between the timing of maternal flowering and offspring life-history schedule. In addition, the unusually early and late flowering and seed dispersal phenotypes reveal that reproduction and germination are possible outside of the typical window. We demonstrate that moderate changes in the initiation of flowering, similar to those observed in response to warming climates, will result in a shift in reproductive phenology, while more substantial changes are likely to occur in the offspring generation. While within-generation changes fit patterns found in other taxa, previous work has not explored transgenerational consequences of an altered flowering time. Across taxa, changes in flowering time may affect seed size, composition, and dormancy, as well as the timing of seed dispersal. Therefore, plastic changes in flowering time are likely to yield between-generation responses in many species and warrant further study as we seek to understand the effects of warming climates.

#### ACKNOWLEDGMENTS

We thank L. Lee, T. Nguyen, J. Starr, D. Stone, and H. Truong for field assistance, B. Barringer, C. Dai, L. Dierkes, and F. Kilkenny for comments on a previous version, NSF DEB-0316298 to L. F. Galloway for financial support, and Mountain Lake Biological Station for logistical support.

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#### APPENDIX

Mean flower production across the growing season for cohorts of *Campanulastrum americanum* that initiated flowering earlier, at the same time, and later than the native population (*Ecological Archives* E090-149-A1).