

The effect of maternal phenology on offspring characters in the herbaceous plant *Campanula americana*

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Summary

1 An individual's phenotype may influence the expression of traits in its offspring. I tested whether maternal flowering phenology influenced offspring germination and thus life history schedule in two populations of *Campanula americana*. Autumn-germinating *C. americana* grow as annuals whereas individuals that germinate in the spring are biennials.

2 Under near-natural conditions, early-season flowers produced seeds that were more likely to germinate in the autumn than late-season flowers, but spring germination was equally likely for all seeds.

3 Seed mass (two of three years) and percentage and timing of germination under constant environmental conditions (all three years) did not vary across the reproductive season. External environmental factors are therefore more likely to contribute to seasonal changes in the probability of germinating in the autumn than are the characteristics of the seeds.

4 The frequency of annual offspring varied among maternal plants. The association between the time a flower was open and offspring germination season suggests that evolution of life history schedule may be accomplished by altering maternal flowering phenology.

5 The association between the time of a flower's anthesis and its fruit maturation was weaker in two years when water was limiting. Limited water also resulted in earlier seed maturation, and consequently a greater proportion of a population's seed being produced during the period when autumn germination was likely. This, in combination with differences between populations, suggests that the magnitude of maternal phenological effects varies across years and populations.

Key-words: flowering phenology, fruiting phenology, maternal effects, seasonal effects, timing of germination

Journal of Ecology (2002) **90**, 851–858

Introduction

Maternal effects are ubiquitous across plant taxa (reviewed in Mousseau & Fox 1998). Many studies have focused on the influence of specific maternal environments (e.g. light, water, nutrient) on the offspring phenotype (e.g. Schmid & Dolt 1994; Lacey 1996; Sultan 1996; Galloway 2001a). However, the maternal phenotype may also influence its offspring. For example, maternal traits affect seed dispersal, and patterns of seed dispersal may influence the offspring growth environment and consequently character expres-

sion (Donohue 1999). Reproductive phenology is another example of a maternal trait that may have inter-generational effects. In *Daucus carota*, size and survivorship were greater for offspring of early flowering plants than those that flowered later (Lacey & Pace 1983).

Maternal resource status typically changes over the course of the flowering season. The modular nature of plant growth and reproduction results in the initiation of new reproductive investment (flowers) while investment in previous reproductive structures (developing fruits) is ongoing. Maternal resources are therefore divided among an increasing number of structures as the reproductive season progresses and, as a consequence, the probability of flower and fruit abortion increases (Stephenson 1981) and seed number per fruit and individual seed mass often decrease (Cavers & Steel 1984; Galen & Stanton 1991; Richardson &

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Stephenson 1991; Ashman *et al.* 1993; Vallius 2000). Seasonal declines may also be caused by architectural effects because later flowers are more distal and therefore produce fewer, smaller ovules, seeds and fruits (e.g. Herrera 1991; Wolfe 1992; Diggle 1995; Ashman & Hitchens 2000). Such seasonal changes, which reflect limitations within the maternal plant, may influence timing of germination (e.g. large seeds germinate earlier; Biere 1991; Simons & Johnston 2000). I refer to these as intrinsic aspects of maternal phenology that may influence the offspring phenotype.

Seasonal changes in the environment may also influence the offspring phenotype. For instance, temperature during seed production could affect offspring seed size, germination or seedling traits, and if it varies over the reproductive season, then maternal phenology may influence offspring trait expression (e.g. Lacey 1996). Similarly, herbivore levels (Brody 1997; Bishop & Schemske 1998; Agrawal 2001), water availability (Sultan 1996; Fox 1990) and day length (Case *et al.* 1996) may vary during reproduction and have cross-generation effects. These environmental effects associated with maternal reproductive phenology are a second source of intrinsic seasonal variation in the offspring phenotype.

Finally, external factors that vary over the dispersal season may have an effect after the seeds have ripened. For example, levels of seed herbivory may depend on when seeds are produced (Brody 1997; Bishop & Schemske 1998; Picó & Retana 2000). Temperature, light and moisture are also likely to vary seasonally. Maternal reproductive phenology may therefore influence the probability of germination by determining the environmental conditions that the seeds experience.

Maternal reproductive phenology is thus likely to influence the expression of offspring traits due to both intrinsic and external factors. These cross-generation influences will result in the complex evolutionary dynamics predicted by maternal effects theory (Kirkpatrick & Lande 1989; Wade 1998; Wolf *et al.* 1998). For example, Donohue (1999) demonstrated that plants grown in high density are relatively unbranched, enhancing dispersal. Widely dispersed seeds, in turn, grow at lower densities and have greater branching, reducing dispersal and increasing density in the next generation. Such negative relationships between the parent and offspring phenotype will reduce response to selection, whereas positive associations across generations may accelerate evolution (Kirkpatrick & Lande 1989). Despite the well-developed theory, there is little empirical information on the influence of specific maternal phenotypes on offspring trait expression in natural populations.

I investigated the effect of maternal flowering phenology on timing of seed maturation and offspring germination in *Campanula americana* L. (Campanulaceae), an understory herbaceous plant with a polymorphic life history schedule and long flowering

season (mean 43 days). Individual plants grow either as annuals or biennials depending on season of germination. Vegetative rosettes require a cold period to induce flowering (Baskin & Baskin 1984), and individuals that germinate in the autumn are vernalized immediately and flower as annuals the following summer. In contrast, individuals that germinate in the spring complete a growing season prior to vernalization and flower in their second summer as biennials. Maternal reproductive phenology may influence offspring life history schedule if seasonal factors affect the probability of a seed germinating in the autumn (see also Lacey & Pace 1983). To evaluate this hypothesis I used a combination of field and controlled-environment studies conducted over 3 years to address four questions. (1) Is there an association between the time a flower is open and the time seeds from that flower ripen? (2) Do seeds from flowers produced at different times in the season vary in their germination patterns? (3) If so, is this relationship due to intrinsic effects (i.e. seed provisioning or maternal environments) or to external factors? (4) Does germination schedule vary among offspring of different maternal plants?

Materials and methods

Campanula americana is a monocarpic insect-pollinated herb, flowering from mid-July to late-August. The primary pollinators are bumblebees foraging for nectar and halictids collecting pollen (Johnson *et al.* 1995; Galloway *et al.* 2002). The self-compatible protandrous flowers are located in compact inflorescences at reproductive nodes on the main stem and lateral branches. Typically, flowers open sequentially at adjacent nodes with only one flower open at each node. Between 1 and 90 flowers may be open at a time (Galloway *et al.* 2002). Most fruits contain 20–40 seeds.

Seasonal patterns of flower and seed production were evaluated over 3 years in two natural populations of *C. americana* 3.6 km from the Mountain Lake Biological Station (MLBS) in Giles Co. Virginia, USA. A population located in the understory of an oak–hickory forest was studied in 1997 and 1998. Because sequential drought years reduced population size, a roadside population 1.3 km away was studied in 1999. Twenty-five plants (30 in 1998) were randomly selected before the onset of flowering each year. Plants were censused every 5 days from the initiation of flowering in mid-July until the end of fruit production in late September. On every plant at each census up to four flowers were labelled, all open flowers were counted, and ripe fruits produced from previously labelled flowers were harvested and their seeds counted. Flowers typically remain open 2–3 days (Evanhoe & Galloway 2002), thus new flowers were marked at each census. Fruits were harvested during the 4–5-day period after the seeds had turned brown but before the pores through which the seeds disperse had opened. *Campanula americana* seeds have no after-ripening period

(Baskin & Baskin 1984), and can therefore germinate immediately following dispersal.

To assess whether flowering phenology influences season of germination, seeds from 17 plants from the 1999 census that flowered throughout the season were planted under near-natural conditions as they ripened. Three sowings were made, all within the natural dispersal period. Seeds from fruits harvested between 31 August and 9 September were planted on 25 September, seeds harvested on 14 and 19 September were planted on 5 October, and seeds from the final fruits to ripen (24 September–9 October) were planted 23 October. Because fruits were harvested before they dehisced and seeds in dehisced fruits disperse passively, planting time was likely to be similar to the natural dispersal date of seeds in comparably aged fruits. For each maternal plant, up to five tubular pots (4 × 14 cm, the unit of replication) containing Promix potting medium were planted with seeds initiated at each flowering date. A sibship of 20 seeds was sprinkled on the surface of each pot. Because seed production varied among plants, the number of pots per plant ranged from 9 to 27 with an average of 16. Replication also varied across planting dates (see Fig. 1). As a consequence of the association between the time a flower is open and the time the fruit from that flower ripens (see Results), seeds in the first planting were largely from flowers open in the first 15 days of the flowering season, the second planting from the next 10 days, and the final planting the last 15 days (correlation between planting time and the date a flower was open, with plant as the unit of replication: $r_s = 0.91$, $n = 73$, $P < 0.001$).

Pots were placed in a partly shaded clearing at MLBS, 3.6 km from the natural population but similar in elevation and light regime (see Galloway 2001b). Seeds were not watered, but dew and mist were common and augmented the moisture from rainfall. Pots were checked for germination every 10 days in the autumn, and again in the spring from snowmelt until

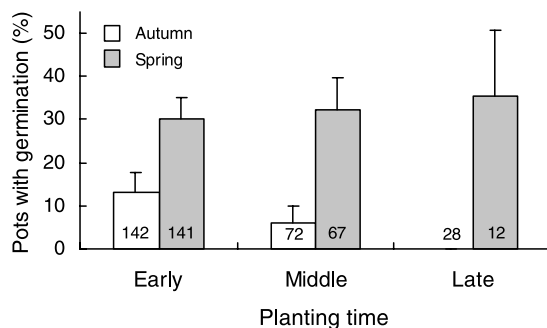


Fig. 1 The percentage of pots with autumn or spring germination for seeds of *Campanula americana* planted early, in the middle, and late in the seed-dispersal season. Seeds were planted as they ripened therefore differences in planting time reflect maternal reproductive phenology. Mean percentage germination (+ SE) of maternal plants is presented. Sample size is indicated within each column; disturbance by animals over the winter decreased sample sizes between autumn and spring.

there was no germination for two consecutive dates. Autumn germination began 8 days after all seeds had been placed in the field.

Variation in seeds produced over the season was determined by quantifying seed mass and patterns of germination under constant environmental conditions. In each year, the census plants having the longest flowering periods were selected to evaluate seed characters. Seventeen plants were used in 1997, and 10 in 1998 and 1999. Ten random seeds from flowers marked at each census date for each plant were chosen for germination and a subsample of six were weighed to the nearest 0.001 mg. In 1997 the seeds were individually placed in cells of tissue culture trays on moist filter paper. To enhance germination percentage (cf. Baskin & Baskin 1984; germination on sand vs. soil), each 1998 or 1999 seed was placed on the surface of a 1.5-mL microcentrifuge tube filled with Promix potting medium. In all years, seeds were arranged in a randomized design and placed in a growth chamber with 12-h days at 21 °C day and 14 °C night, near optimal germination conditions (Baskin & Baskin 1984). Seeds were kept moist and germination was recorded daily for 50 days. Each year the germination trial was conducted between the natural autumn and spring germination periods. Variation in the length of time between harvest and exposure to germination conditions among seeds from fruits initiated at different dates is not expected to influence results, since there is no after-ripening in *C. americana* seeds (Baskin & Baskin 1984).

STATISTICAL ANALYSIS

Rank correlations were used to evaluate the association between the date a flower was open and the date the fruit from that flower ripened. The mean harvest date was calculated for flowers marked at each census date for each plant. A Spearman rank correlation was then conducted between flowering date and mean harvest date, providing a measure of association between flowering and fruiting phenology in which individual plants were the experimental unit.

For seeds germinated under near-natural conditions, the numbers of pots with autumn germinating seeds and those with spring germinating seeds were compared across planting dates using a loglinear analysis. Two maternal families were dropped from analysis because they had few replicates ($n < 5$). Germination rates were small; no more than one seed germinated per pot in the autumn and in 76% of pots in the spring, two seeds germinated in most of the remainder. There was no association between the amount of spring germination and planting time, $\chi^2 = 0.07$, d.f. = 2, $P = 0.96$. Therefore I define germination per pot in each season as a dichotomous variable. Presence or absence of germination was analysed using a loglinear analysis assuming a binomial distribution and a logit link (PROC GENMOD, SAS Institute 2000). Planting time, maternal plant and season of germination were

Table 1 Loglinear analysis to determine the association between amount of germination and germination season, planting time, and maternal plant

Source	d.f.	χ^2	$P <$
Season	1	31.78	0.001
Planting time	2	9.05	0.011
Maternal plant	14	26.10	0.025
Season \times planting time	2	8.03	0.018

included as factors and likelihood-ratio tests were used to determine the appropriate model (Agresti 1996). Although the model reported in Table 1 was a good fit to the data (not significantly different from the saturated model, $\chi^2 = 72.83$, d.f. = 58, $P = 0.091$), there was a significant increase in model fit ($\chi^2 = 26.12$, d.f. = 14, $P < 0.025$) when the maternal plant–season interaction was added. However this maximum likelihood model did not converge due to the distribution of data among cells. I explored the potential interaction between maternal plant and germination season suggested by this result using G -tests to compare patterns of germination among maternal plants within each season. A G -test does not require as large a sample size as the maximum likelihood based loglinear analysis.

Seasonal changes in seed number per fruit, seed mass, and percentage and timing of germination under constant environmental conditions were analysed using analysis of variance (ANOVA). Separate analyses were conducted for each year. Flowering date (fixed) and maternal plant (random) were included as main effects. Seed mass was included as a covariate in initial analyses of days to germination; however, it did not explain a significant amount of variation in any year ($P > 0.15$, analyses not shown) and therefore was not retained in the analysis. The interaction between flowering date and maternal plant was not included in analyses of percentage germination because percentages were calculated for each maternal plant for each flowering interval. Repeated-measures ANOVA was not possible because most maternal plants did not produce flowers at all census dates. Seed number was square-root transformed and days to germination was natural-log transformed prior to analysis, to meet the assumptions of ANOVA.

Results

Maternal reproductive phenology may affect the timing of offspring germination if there is an association between the time a flower is open and the time the fruit from that flower ripens. Plants flowered during eight of the 5-day census intervals in 1997 and 1998, and 10 in 1999. Fruits matured during five intervals in 1997, two in 1998, and nine in 1999. The duration of fruit maturation was shorter in 1997 and 1998 because of drought during the flowering period (Fig. 2). There was a significant positive correlation between flowering date

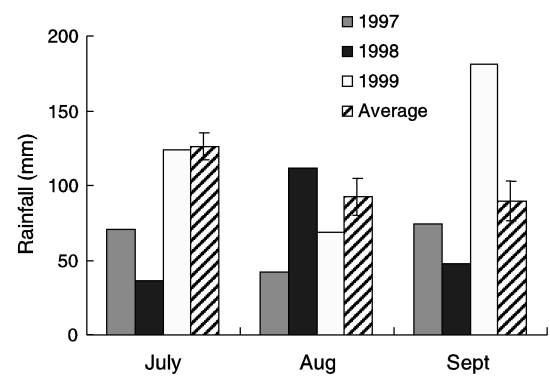


Fig. 2 Rainfall during *C. americana*'s reproductive season over the 3 years of study in comparison with the 22-year average (\pm SE). Data collected at MLBS (from National Climatic Data Center, Asheville, NC 1972–96 and MLBS 1997–99; 3 years have missing data).

and fruit maturation date in 1997 ($r_s = 0.26$, $n = 72$, $P < 0.03$), 1998 ($r_s = 0.63$, $n = 32$, $P < 0.001$), and 1999 ($r_s = 0.88$, $n = 134$, $P < 0.001$). In addition, there was a negative correlation between flowering date and the number of days it took a flower to become a mature fruit in 1997 ($r_s = -0.72$, $n = 72$, $P < 0.001$) and 1998 ($r_s = -0.97$, $n = 32$, $P < 0.001$). In these years, later-season flowers had little time to mature fruit in some individuals as a consequence of mortality due to drought. However, there was no such association in 1999 when rainfall was more abundant ($r_s = -0.13$, $n = 134$, $P = 0.12$).

Season of germination varied among seeds planted at different times in the autumn. Germination of seeds exposed to natural conditions occurred during three 10-day periods in the autumn and four periods in the spring. Germination rates were greater in the spring than the autumn (Table 1, Fig. 1). The percentage of pots with any germination dropped across the planting times from 43.2% of those planted early to 35.4% in the final planting time (Table 1, Fig. 1). However, a significant interaction indicated that the effect of planting time on germination differed between the seasons (Table 1). Autumn germination was greater in early-planted seeds (Fig. 1), but there was little difference in spring germination among seeds planted at any time. Therefore, different rates of autumn germination account for the change in total germination across planting times. Finally, rates of germination varied among maternal plants (Table 1), with significant heterogeneity for autumn ($G = 31.50$, d.f. = 14, $P < 0.001$) but not for spring germination ($G = 20.29$, d.f. = 14, $P = 0.12$), suggesting that only the number of offspring with an annual life history schedule varied among maternal plants.

Seasonal effects on seed mass varied among years, and among maternal plants in all years (Table 2a). In 1997, there was a significant decrease in seed mass across the flowering season (Fig. 3), but little variation was found in 1998 and 1999 when maternal plant

Table 2 Analysis of variance to evaluate seasonal changes in seed mass, timing of germination, and percentage germination in *C. americana* over 3 years. Seeds were from flowers open at 5-day intervals throughout the reproductive season; maternal plant was included as a blocking variable

Source	1997			1998			1999		
	d.f.	MS	<i>F</i>	d.f.	MS	<i>F</i>	d.f.	MS	<i>F</i>
Flower interval	5,37	0.065	13.41***	6,8	0.0047	0.96	9,52	0.0072	1.75†
Maternal plant	16,37	0.049	10.14***	9,8	0.019	4.03*	9,52	0.050	12.29***
Interval × plant	37,287	0.0049	6.04***	8,118	0.0048	7.47***	52,284	0.0041	6.57***

Source	1997			1998			1999		
	d.f.	MS	<i>F</i>	d.f.	MS	<i>F</i>	d.f.	MS	<i>F</i>
Flower interval	5,63	0.27	1.40	6,3	0.09	0.57	9,53	0.12	0.84
Maternal plant	16,45	0.65	3.40***	6,4	1.15	7.02*	9,52	0.93	6.46***
Interval × plant	32,166	0.19	0.85	4,37	0.16	0.70	52,556	0.14	1.75**

Source	1997			1998			1999		
	d.f.	MS	<i>F</i>	d.f.	MS	<i>F</i>	d.f.	MS	<i>F</i>
Flower interval	5,41	0.08	1.77	6,8	0.05	1.32	9,52	0.00	0.59
Maternal plant	16,41	0.14	3.05**	9,8	0.20	5.53*	9,52	0.05	6.07***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, † $0.1 > P > 0.05$.

accounted for the largest amount of variance explained (Table 2). Under constant environmental conditions the number of days to germination and percentage germination was always influenced by the maternal plant (Table 2), but not by the date during the season that a flower was open. Overall, there is little evidence for seasonal changes in *C. americana* seeds when grown under constant environmental conditions.

Seasonal patterns of flower, fruit and seed production suggest that the majority of seeds are produced early enough to allow germination in the autumn as well as the spring. A total of 71%, 71%, and 37% (1997, 1998, and 1999, respectively) of the seasonal flower production occurred during the first four census dates (Fig. 4). Of these early-season flowers, 73%, 40%, and 79% set fruit in 1997, 1998, and 1999, respectively, compared with 19%, 6% and 46% of later flowers. Seed number per fruit declined significantly over the season in two of the years (Fig. 5). The combined effect of seasonal changes in these reproductive variables can be seen in the fraction of seasonal seed production for flowers open at each census date (Fig. 6). The number of seeds produced at each date was estimated by multiplying the (number of flowers) × (proportion of those flowers that set fruit) × (mean number of seeds per fruit). In the two dry years, 1997 and 1998, over half of the total seeds were initiated in the first 10 days of the reproductive period and over 80% in the first 15 days. This contrasts with 1999 when only 41% of the total seeds had been initiated 15 days into the reproductive

season (Fig. 6). However, the 1999 censuses were conducted in a different population which received more light (roadside vs. under canopy). Although the combination of greater rainfall and light availability resulted in plants that had larger floral displays (Fig. 4) and more seeds per fruit (Fig. 5), reproductive phenology of the two populations is similar (personal observation).

Discussion

Maternal reproductive phenology may influence offspring life history schedule in *C. americana*. In 1999, when conditions were most favourable for reproduction, flowers were produced over 50 days and fruits ripened over 45 days. There was a strong association between the time in the season a flower was open and the time the fruit from that flower ripened. Reproductive timing, in turn, influenced germination timing, with seeds from early flowers having the greatest rates of autumn germination and those from late season flowers having none, and therefore no annual offspring. In contrast, seeds from all fruits germinated with equal frequency in the spring when they would grow as biennials.

Differences in germination timing between seeds from early- and late-season flowers may be due to intrinsic factors. Maternal resource limitation and architectural constraints often increase over the season (reviewed in Diggle 1995), consistent with seasonal declines in the fraction of *C. americana* flowers that

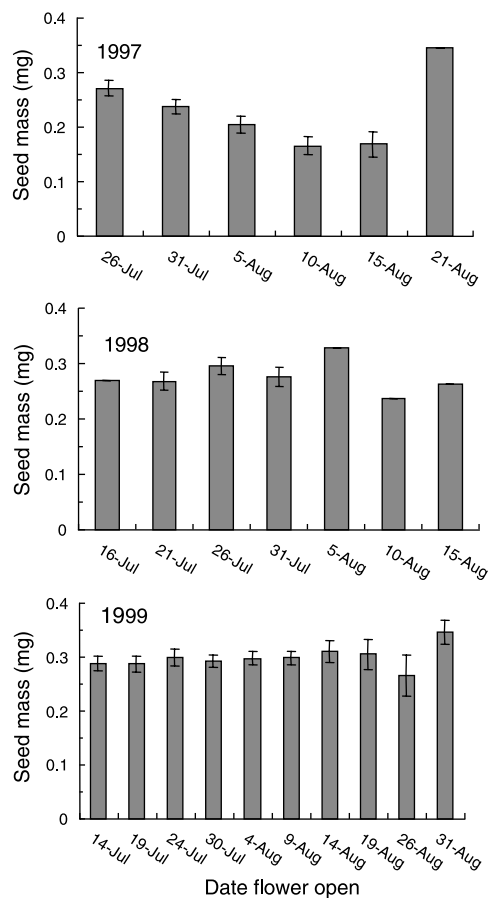


Fig. 3 Mass of seeds from flowers open at 5-day intervals across the reproductive season for 3 years in *C. americana*. Means (\pm SE) of maternal plants are given; at some dates seeds from only a single plant were available. See Table 2 for analysis.

produced mature fruit and the number of seeds per fruit. Although late-season pollinator limitation may create the same patterns (e.g. O’Neil 1999), resource limitations are a more likely explanation, since the length of time that the flowers were functionally male, an index of pollinator visitation, changed little over the season (personal observation). Seasonal decreases in fruit production and the number of seeds per fruit are often associated with a decrease in seed size (e.g. Cavers & Steel 1984; Roach 1986; Wolfe 1992). Seed traits may also be influenced by environmental changes (e.g. in temperature) over the long flowering season. However, in two of three years timing of seed initiation in *C. americana* did not affect seed mass (see also Richardson & Stephenson 1991), and the timing of germination or percentage germination under constant environmental conditions was not affected in any year. Therefore, there is little evidence that seasonal change in intrinsic factors such as maternal resource status or maternal environments underlie the association between reproductive phenology and autumn germination.

Greater autumn germination of seeds from early-season flowers is therefore probably due to external factors. Most autumn seed germination was scored 3 weeks after the last group of seeds had been in placed

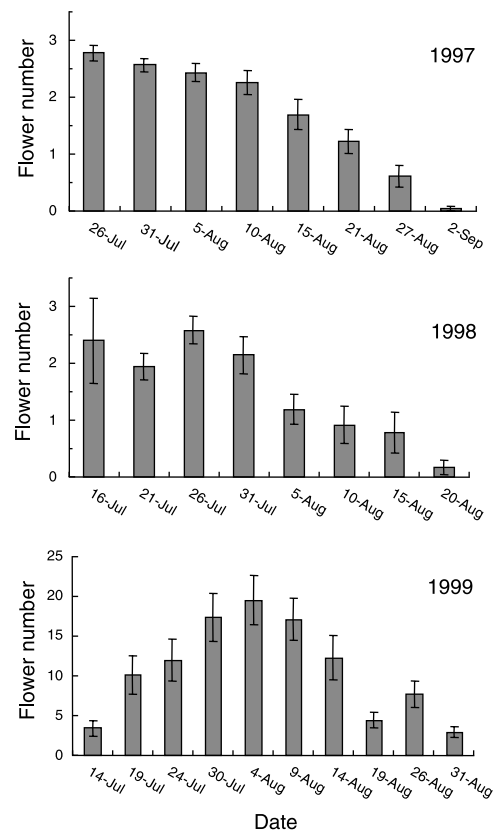


Fig. 4 Mean flower number (\pm SE) per plant at 5-day intervals across the reproductive season for 3 years in *C. americana*.

in the field. Emergence under the near-optimal controlled conditions took an average of 16 days (minimum 9 days), suggesting germination of late-planted field seeds was possible. However, germination may progress more slowly under cooler field conditions and late-planted seeds may not have had sufficient time to germinate before the onset of winter. Alternatively, the greater autumn germination of seeds from early-season flowers may have been triggered by seasonal environmental events (e.g. rainfall). Autumn germination in *C. americana* is typically episodic following rains (unpublished data); there was rainfall within 10 days of each planting time. However weekly precipitation decreased over the autumn (MLBS weather data), therefore early-dispersed seeds received more rainfall before the period of peak germination than late-dispersed seeds. In either case, conditions conducive to germination, rather than intrinsic differences among seeds, appear to determine whether seeds germinate in the autumn and grow as annuals.

The association between annual offspring and flowers open early in the season suggests that selection favouring the annual life cycle may act through the parental generation. In dry summers, plants with an annual life cycle may be favoured by selection since the majority of mortality in the study populations is due to water stress of rosettes in early to mid-August when annual *C. americana* have already flowered (Galloway, unpublished). The proportion of annuals may be

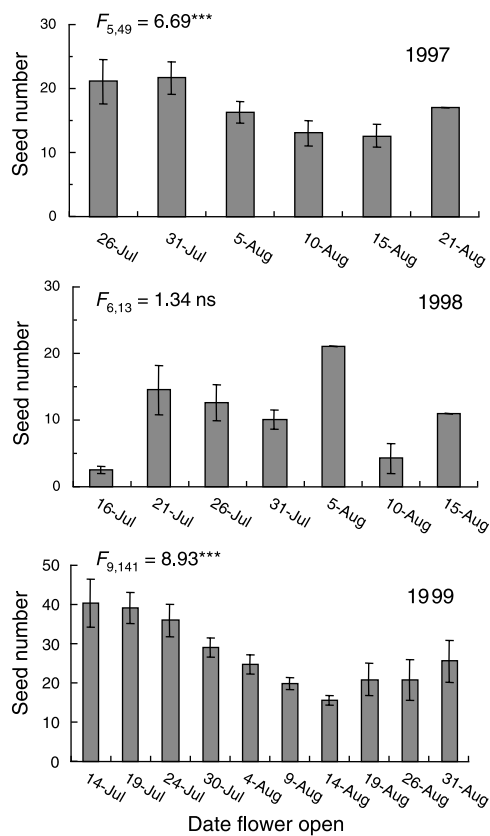


Fig. 5 Seed number per fruit for fruits produced from flowers of *C. americana* open during 5-day intervals across the reproductive season for 3 years. Means (\pm SE) of maternal plants are given; at some dates seeds from only a single plant were available. *F*-values from ANOVA indicate seasonal changes in seed number per fruit. *** $P < 0.001$.

changed by direct selection on the timing of germination. Alternatively, a plant that accelerated its flowering phenology would have a larger portion of its flowers open during the time period in which autumn-germinating seeds are produced. Therefore, the frequency of annual plants may be increased by selection on maternal flowering phenology (i.e. maternal inheritance, Kirkpatrick & Lande 1989). Variation in the proportion of annual offspring among maternal plants coupled with autumn germination of early-produced seeds suggests that selection on life history schedule may act through maternal reproductive phenology. A population of *C. americana* located 9 km from the study populations, but 655 m lower in elevation, also suggests an association between timing of flowering and of offspring germination. Plants in this population flower earlier, seeds germinate earlier, and the population includes a greater proportion of annuals (Galloway 2001a; unpublished).

However, the extent to which maternal reproductive phenology influences offspring life history schedule may vary among years and populations. In two years rainfall during the flowering season was substantially below average and drought influenced patterns of reproduction and mortality. In drought years the time a flower was open was not as tightly correlated with the

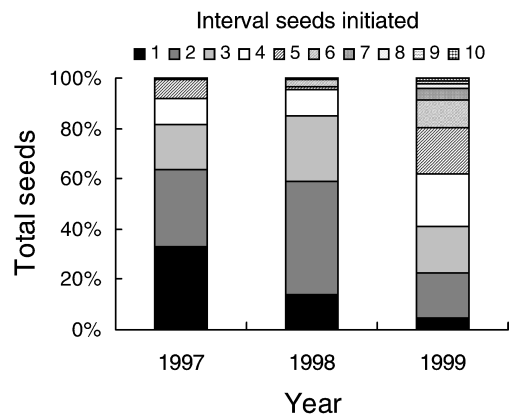


Fig. 6 The estimated percentage of seasonal seed production in *C. americana* initiated at 5-day intervals throughout the reproductive season for 3 years.

time the fruit from that flower ripened as in years with abundant rainfall. In fact, in drought years many fruits ripened synchronously when plants died, and as a consequence the potential for maternal reproductive phenology to influence offspring life history schedule was reduced. However, in dry years a greater proportion of the seeds were produced early in the season, and if these have a greater potential to germinate in the autumn, as found in 1999, then more of the seed pool may germinate as annuals than in a moist year. However, the 1999 data were collected from a different population and other environmental and genetic differences between the populations may influence the comparison in addition to rainfall levels. Yearly and population-level variation in environmental factors and the proportion of annual *C. americana* (e.g. Wardle 1995; Galloway unpublished data) suggest that multiple-year studies across several populations are necessary to understand the contribution of maternal phenology to offspring life history schedule. More generally, seed and seedling traits may be influenced by intrinsic and external factors associated with maternal reproductive phenology in a number of species, and therefore the inter-generational effects of phenology reported here may be widespread.

Acknowledgements

I thank A. Birkhead, S. Freeman, M. Katz, and E. Miller for help with seed germination; the Mountain Lake Biological Station for logistical support; T.-L. Ashman, J. Etterson and two anonymous referees for comments on previous versions of the manuscript; and the National Science Foundation for financial support (DEB-9752947 and DEB-9974126).

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Received 7 February 2002

revision accepted 26 June 2002