

## THE EFFECT OF A LATITUDINAL TEMPERATURE GRADIENT ON GERMINATION PATTERNS

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**Premise of research.** Germination timing determines a plant's exposure to environmental factors and has strong impacts on fitness. Despite this key role in the life cycle, relatively little is known about variation in germination within species. This study examines whether geographic variation in local environmental conditions affects germination in populations of *Campanula americana* throughout eastern North America.

**Methodology.** We determined the temperature of spring and fall germination through a survey of 16 natural populations along a latitudinal transect over 4 yr. We then examined germination propensity and rate in a subset of these populations under controlled temperature conditions.

**Pivotal results.** Northern populations germinated at cooler temperatures than southern populations regardless of season, and fall germination occurred at warmer temperatures than spring germination across all populations. Northern populations germinated later in the spring than southern populations. Under controlled conditions, northern populations germinated more rapidly than southern populations, while warm temperatures accelerated germination, with all populations germinating at similar rates. However, under controlled conditions fewer seeds germinated from northern populations in comparison to southern populations, and the decrease in propensity to germinate at higher latitudes was pronounced under cool conditions.

**Conclusions.** Temperature influences germination timing in *C. americana*. In the field, germination occurs at a consistent time across a wide range of climates, but populations spanning the latitudinal gradient germinate under different temperatures. Under controlled conditions, warmer temperatures accelerate germination. Populations differ in plasticity of germination propensity to temperature. This reduced germination of northern populations, especially under cooler temperatures, may serve as an adaptive plastic response to the later arrival of suitable temperatures for growth. The influence of environmental variation on germination in natural populations and under ecologically relevant experimental conditions indicates that novel environmental conditions imposed by climate change may alter life-history patterns.

**Keywords:** *Campanula americana*, germination, latitude, population variation, temperature.

**Online enhancements:** appendix table and figure.

### Introduction

The timing of life-history transitions—which include germination, flowering, and fruiting—is critical for plant development (Kalisz 1986; Venable and Brown 1988; Elzinga et al. 2007). Such transitions are often sensitive to local environmental conditions (Bradshaw 1965; Badeck et al. 2004; Yang and Rudolf 2010). Because seasonal timing of growth affects fitness and local persistence (Steltzer and Post 2009), phenology may shift across a species' range such that transitions occur under

appropriate environmental conditions (Burghardt et al. 2016). These shifts include adaptation to local environmental conditions and plasticity in life-history responses to local climate. However, with climate change rapidly altering environments (IPCC 2007), changes in the growing season may create a mismatch between the local conditions suitable for phenological transitions and the timing of those transitions (Barua et al. 2011).

Germination, the earliest phenological transition, plays a major role in determining the conditions in which a plant will develop (Silvertown 1981; Hernández-Verdugo et al. 2001; Debieu et al. 2013) and is highly responsive to the environment (Simpson 1990; Baskin and Baskin 1998; Donohue 2002; Fenner and Thompson 2005; Sherry et al. 2007). Theory predicts a correlation between germination timing and environments that indicate favorable growing conditions (Cohen 1967; Venable and Lawlor 1980). The postgermination environment affects seedling establishment and survival, influences the onset of growth, and determines growing season length (Silvertown 1981; Cle-

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land et al. 2007; Inouye 2008; Wilczek et al. 2010). Germination timing is then likely to influence the selective environment and resulting life history (Donohue 2002). Therefore, understanding how the environmental conditions of germination vary across widespread species or disparate populations can provide valuable information about how germination strategies may be affected by changing climates (Donohue 2002; Vergeer and Kunin 2011; Hoyle et al. 2014).

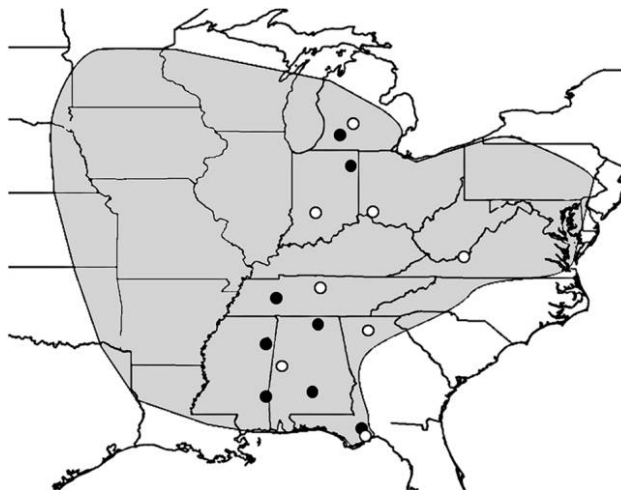
Geographic variation in environmental factors, such as temperature, influences the timing and success of germination (Donohue 2002; Daws et al. 2004). As a result, phenology may vary across latitudinal clines (Debieu et al. 2013; Burghardt et al. 2016). Geographic differences in environmental factors may contribute to population-level variation and local adaptation in germination (Thompson 1975; Meyer et al. 1989; Claus and Venable 2000; Pezzani and Montaña 2006; Higgins et al. 2015). However, we know little about the extent to which such variation in germination among natural populations is associated with specific environmental factors. Identifying environmental sources of variation in germination across a geographic range, such as local temperature, may elucidate causes of life-history variation (Donohue et al. 2005; Cleland et al. 2007; Prendeville et al. 2013; Burghardt et al. 2015). In addition, combining observational field studies with mechanistic controlled environment studies provides a basis for informing predictions of shifts in life-history variation in response to novel conditions.

This study examines germination patterns across the latitudinal range of a woodland herb in which germination influences life-history schedule and fitness. In *Campanula americana*, germination phenology determines whether plants grow as winter annuals or biennials. Because timing of life-history events is frequently associated with latitude (Reinartz 1984; Wilczek et al. 2009), we may expect differentiation in germination timing associated with environmental changes across a latitudinal cline. Using a combination of data from natural populations and controlled environments, we address the following questions: (1) How are the temperature conditions experienced across *C. americana*'s latitudinal gradient associated with germination timing of natural populations? (2) Has germination of *C. americana* populations differentiated in response to temperature across the latitudinal gradient? Using information from natural and controlled environment studies, we discuss the potential effects of climate change on the frequency of annual and biennial life histories in *C. americana*.

## Material and Methods

### Study System

*Campanula americana* L. (= *Campanulastrum americanum* Small; Campanulaceae) is a monocarpic woodland herb. The species has an extensive range throughout much of eastern and central North America (Barnard-Kubow et al. 2015; fig. 1). Phenology and life-history traits vary among *C. americana* populations (Kalisz and Wardle 1994; Prendeville et al. 2013) and are influenced by germination timing. However, germination timing is not affected by seed size (Galloway 2001). Individuals grow as an annual or biennial life form, flowering after a required period of vernalization (Baskin and Baskin 1984). Seeds that germinate in the fall overwinter as rosettes and flower in the summer as



**Fig. 1** *Campanula americana* populations included in field surveys (white circles, populations included in germination trials; black circles, populations not included in germination trials). Shaded region indicates the extent of the native range. For latitude and longitude of each population, see table A1, available online.

annuals; seeds that germinate in the spring, grow for a summer, overwinter, and flower during their second summer as biennials (Galloway 2002). Seeds of *C. americana* are nondormant at maturity and can germinate immediately following dispersal (Baskin and Baskin 1984). In the south, plants seed before germination, so seeds that germinate in the fall as annuals likely come from plants that flowered earlier that same year. In the north, while some seed dispersal occurs before fall germination, late-flowering individuals often complete their reproductive cycle after the window for fall germination, and those seeds will likely germinate in the spring as biennial plants. Under common garden conditions, annuals tend to be more common in populations from warmer southern areas, and biennials tend to be more common in populations from cooler northern areas (L. F. Galloway and H. R. Prendeville, unpublished data). This suggests that germination phenology may have differentiated in response to environmental factors that vary across a latitudinal gradient.

### Field Germination

We first determined whether environmental conditions were associated with geographic location in 16 populations spanning a latitudinal gradient extending from northern Florida to central Michigan (fig. 1; table A1, available online). Mean daily temperatures for a site close to each population (<15 km away) were obtained for 1953–2013 from the Climate Information for Management and Operational Decisions database (CLIMOD; Northeast Regional Climate Center 2011) and then averaged across the 60-yr period.

We then determined timing of germination in natural populations across latitudes. The 16 populations were visited for demographic surveys in the spring and fall every year for 4 yr (April and August, 2011–2014). At each survey, seedling number was recorded in at least 30 0.5-m<sup>2</sup> plots. To account for potential underestimation of germination in a single survey, occa-

sional new plants present in the subsequent survey were added to germination totals. The average number of true leaves per seedling was noted. The onset of both spring and fall germination in each population was estimated by subtracting 1 wk from the survey date per true leaf (typically less than three true leaves). This estimate was based on leaf development observed in greenhouse-grown seedlings from a variety of populations. The estimated dates were averaged over the 4 yr of the survey. To account for uncertainty in dating methods, dates were grouped into 2-wk blocks across the spring and fall. It should be noted that spring germination may occur earlier than our estimates indicate because seeds that germinate in the spring germinate under colder conditions than seeds that germinate in the fall, so leaves on spring seedlings likely develop at a slower rate than fall seedlings.

We determined average temperature during germination in the 16 populations. Daily mean temperatures for each population were also grouped into 2-wk blocks, and then temperatures in each block were averaged. This resulted in 26 average temperatures for each population, one for each 2-wk period of the year. We created temperature curves for each population using the average temperature during each 2-wk interval and mapped the estimated time of germination onto the temperature data (fig. A1, available online). The timing of germination in each population was then associated with the mean temperature in that time block.

#### Germination Trials

Seeds from eight of the study populations spanning the latitudinal range were used in a germination experiment (fig. 1). Field collected seeds were grown in the greenhouse, and crosses were conducted between plants from the same population in 2009 (methods described in Prendeville et al. 2013). This resulted in seed that had a common maternal environment for all populations. Seeds were then stored under cool dry conditions (5°C). Seeds in the controlled environment study were grown under four temperature treatments within the range observed during germination in natural populations (8–27°C; see “Results”): 8°C, 14°C, 20°C, and 26°C.

Germination experiments were conducted in two rounds because of space constraints. The two rounds were run consecutively, beginning in November 2014 and January 2015. In the first round, seeds from each of the eight populations were grown in three temperatures: 14°C, two 20°C treatments, and 26°C. One of the 20°C treatments was originally set to 8°C, but a cooling malfunction resulted in several days of fluctuation around 20°C before stabilizing at 20°C. Germination patterns of the two 20°C treatments were not different, so they were combined for analysis. In the second round, seeds from each of the eight populations were grown in all four temperatures.

For each round of the germination experiment, replicate seeds were planted for each population. We prepared germination trays using a soil mixture of 3:1 Metro-mix 200 to fritted clay. Seeds were planted on the surface of the soil. In the first round, two seeds were planted for each population in each of 15 randomly located cells in each temperature treatment, resulting in 30 seeds per population/temperature. We planted 240 seeds from all eight populations in each treatment. In the second round, two seeds were planted in each of 10 cells for each population, resulting in 20 seeds per population in each temperature treatment and

a total of 160 seeds per treatment. Trays were placed in growth chambers, each set to a 13-h day length and constant temperature, covered to maintain moisture, and watered as needed. Number of days from planting to germination, indicated by cotyledon emergence, was recorded daily for 30 d (round 1) and 50 d (round 2). We scored germination as 0 or 1. A single cell contained two seeds from one population; we tracked the germination of both seeds in a cell. A seed received a score of 1 when it germinated, with each germination event being considered a success.

#### Data Analysis

*Field germination.* First, latitude was correlated with mean annual temperature at each site using Pearson’s correlation test. ANCOVA was then used to determine the influence of season (fixed effect), population latitude (direct effect), and their interaction on temperature during field germination as well as on week of germination (R Development Core Team 2015, ver. 3.0.2).

*Germination trials.* To determine the effect of temperature on germination propensity in controlled environments, we conducted a generalized linear mixed model using a binomial distribution (SAS Institute 2011; PROC GENMOD). We included latitude (direct), temperature (fixed), and their interaction, and round was included as a random blocking factor. Seeds did not germinate in 8°C, except for a single individual from VA73, so the 8°C treatment was excluded from analysis. We used Tukey multiple comparison tests to compare germination propensity among temperature levels.

To determine the effect of temperature on germination rate (number of days until germination) under controlled conditions, we conducted a generalized linear mixed model using a Poisson distribution. We again included latitude (direct), temperature (fixed), and their interaction, and round was included as a random blocking factor (SAS Institute 2011; PROC GENMOD). Tukey multiple comparison tests were used to compare germination rate among temperature levels following a significant main effect of temperature.

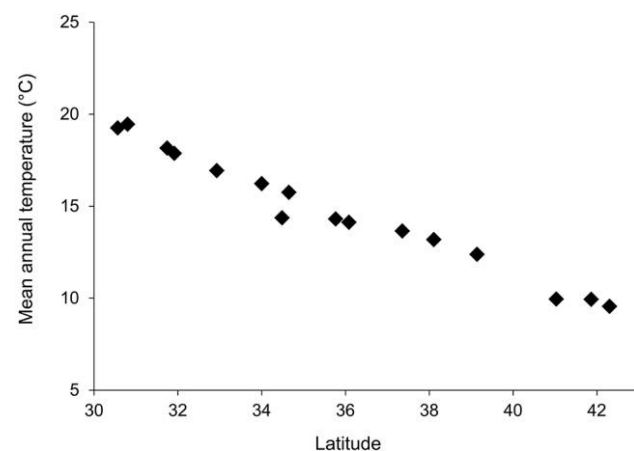


Fig. 2 Plot of mean annual temperature in 16 populations of *Campanula americana* sampled across its latitudinal range.

## Results

### Field Germination

Latitude was negatively correlated with mean annual temperature of *Campanula americana* populations, with northern populations cooler on average than southern ones (fig. 2;  $R^2 = 0.98$ ). Therefore, latitude can be used as a proxy for temperature in this system (cf. Prendeville et al. 2013). Temperature during field germination differed between seasons, with spring germination occurring at cooler temperatures (8°–20°C) than fall germination (16°–27°C; table 1; fig. 3A). Temperature during field germination also varied with latitude, where regardless of season, germination occurred at cooler temperatures in northern populations than southern populations (table 1; fig. 3A). Changes in the temperature during field germination across the latitudinal range were similar for fall and spring germination cohorts (table 1; fig. 3A; overall slope =  $-0.45$ ). In contrast, the effect of latitude on germination timing depended on the season (table 1; fig. 3B). The week of fall germination was consistent across the latitudinal range (fall:  $R^2 = 0.21$ , slope =  $-0.22$ ,  $P = 0.077$ ); however, northern populations germinated later than southern populations in the spring (fig. 3B; spring:  $R^2 = 0.47$ , slope =  $0.31$ ,  $P = 0.003$ ).

### Germination Trials

Temperature influenced germination propensity under controlled conditions. There was almost no germination at 8°C (not included in the analysis), and fewer seeds germinated at 14°C than in the other treatments (Tukey test for temperature,  $P = 0.003$ ; table 2; fig. 4A). On average, 19%  $\pm$  5% (mean  $\pm$  SE) seeds germinated at 14°C, 32%  $\pm$  3% germinated at 20°C, and 36%  $\pm$  6% germinated at 26°C, resulting in germination that was nearly double at 20°C and 26°C compared with 14°C. Germination propensity was lower in populations from northern latitudes, and this effect was greater at cool temperatures (table 2; fig. 4A).

Temperature influenced germination rate (table 2), with warmer temperatures accelerating germination (fig. 4B). Seeds placed in the 26°C treatment germinated more quickly ( $18.7 \pm 1.6$  d) than those at 20°C ( $27.8 \pm 2.7$  d) and 14°C ( $27.1 \pm 2.9$  d) across the latitudinal range (Tukey test for temperature,  $P < 0.05$ ).

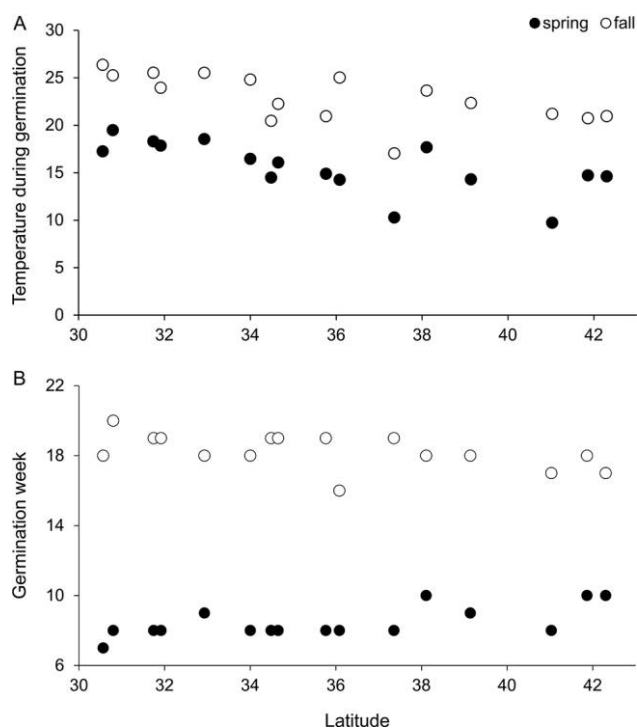
**Table 1**

**Comparison of Temperature during Field Germination and Week of Germination (ANCOVA) in 16 Populations of *Campanula americana* Sampled along the Latitudinal Extent of the Species Range**

Source	df	F	
		Temperature at germination	Week of germination
Latitude	1	21.47***	.46
Season	1	100.62**	1251.09***
Latitude $\times$ season	1	.08	13.53**
Error	28		

\*\*  $P < 0.01$ .

\*\*\*  $P < 0.0001$ .



**Fig. 3** A, Plot of average temperature at which spring germination ( $R^2 = 0.45$ , slope =  $-0.48$ ) and fall germination ( $R^2 = 0.41$ , slope =  $-0.42$ ) occur in 16 populations of *Campanula americana* sampled across a latitudinal gradient. B, Timing of spring and fall germination. Germination week indicates the 2-wk block of the year during which germination occurred in each population.

Germination rate was also more rapid for northern populations (table 2; fig. 4B; overall slope =  $-0.4$ ).

## Discussion

Temperature underlies differences in germination across populations of *Campanula americana* spanning a latitudinal gradient. Germination timing in the field demonstrates population differentiation in response to local temperature, with northern populations germinating under colder conditions and southern populations germinating under warmer conditions. Cool temperatures, typical of northern habitats, delayed germination across all populations under controlled conditions and may lead to later spring germination for field populations in the north. Northern populations demonstrate limited germination and potential within-season dormancy under cooler controlled conditions relative to southern populations. This differentiation in germination plasticity may serve as a potential adaptive response to colder local temperatures.

Northern *C. americana* populations germinated under cooler conditions in the field than southern populations, regardless of season. In contrast, week of germination was largely static, in particular for fall germination, despite changes in temperature across the latitudinal gradient. Concurrently, germination in natural populations occurs under a consistent, narrow range of daylight hours independent of latitude (12.5–13.9 h; L. F.

Table 2

Comparison of Germination Propensity (Generalized Linear Mixed Model [GLMM], Binomial Distribution) and Rate (GLMM, Poisson Distribution) in Three Controlled Temperature Treatments for Eight Populations of *Campanula americana* Sampled along a Latitudinal Gradient

Source	df	$\chi^2$	
		Germination propensity	Germination rate
Latitude	1	51.32***	33.82***
Temperature	2	11.70**	9.67**
Latitude $\times$ temperature	2	7.56*	3.16
Round	1	18.78***	147.34***
Error	411		

Note. Seeds were germinated in two temporal rounds, included in the analysis as a random factor.

\*  $P < 0.05$ .

\*\*  $P < 0.01$ .

\*\*\*  $P < 0.0001$ .

Galloway and M. A. Zettlemoyer, unpublished data). Consistency of germination time and photoperiod during germination means that seeds germinate at different temperatures in different populations. Variation in temperature during germination has been found across populations in other species (Meyer et al. 1989), as is seen in *C. americana*. Temperature during field germination may affect individuals' fitness and therefore create selective pressure on germination timing (Donohue 2005; Debieu et al. 2013).

Temperature underlies differences in germination timing in *C. americana*. Spring germination was delayed by 1 wk in the cooler northern populations, whereas the timing of fall field germination, when conditions were warmer, was consistent across populations. Unlike previous experimental work on *C. americana* (e.g., Kalisz and Wardle 1994; Baskin and Baskin 1998; Galloway 2002), we examined germination timing at a constant temperature in order to understand how seeds respond to specific temperatures, independent of fluctuations in temperature (cf. Barua et al. 2011). Warm temperatures accelerated germination across the latitudinal gradient when seeds were grown under controlled conditions. Therefore, germination rate in *C. americana* is plastic in response to temperature, and local temperatures influence germination timing in the field. Similarly, populations of *Arabidopsis thaliana* vary in germination timing in response to seasonal temperatures experienced during germination (Donohue et al. 2005).

Plasticity for germination propensity varies across latitudes. Under controlled conditions, seeds from southern populations had higher germination propensities than northern populations at all temperatures. Cooler temperatures reduced germination across populations, but this decrease was greater for northern populations. Such a reduction in germination is potentially an adaptive plastic response. There are at least two possibilities for why seeds from northern populations may reduce germination in comparison with those from southern populations. First, germination may be inhibited by the greater time required to accumulate sufficient growing degree days in northern populations. Second, spring germination could be delayed in northern popu-

lations because of greater within-season dormancy relative to southern populations (fig. A1). Within-season dormancy, resulting in delayed germination, could be a plastic response to a narrower range of permissive temperatures for germination and growth in the north. Late and reduced germination in colder conditions is a form of bet hedging to lower reproductive risk (Venable and Lawlor 1980; Clauss and Venable 2000; Tielbörger et al. 2012) and decrease risk of frost damage in northern areas with later snowmelt (Inouye 2008; Körner and Basler 2010; Way 2011). Previous studies have found that germination can be delayed where a harsher season imposes stronger selection (Kronholm et al. 2012; Debieu et al. 2013).

Acceleration of germination under warmer temperatures in *C. americana* suggests potential responses of germination to climate change. Warmer climates have been associated with earlier phenological transitions (Fitter and Fitter 2002; Franks et al. 2007; Wilczek et al. 2010; Munguía-Rosas et al. 2011; Anderson et al. 2012) and accelerate flowering and fruiting in *C. americana* (Haggerty and Galloway 2011; Galloway and Burgess 2012). However, because germination occurs during the season before flowering, the influence of warming temperatures on timing of germination is not expected to directly affect reproductive phenology.

Warmer conditions, however, may lead to an increase in the prevalence of fall germination in *C. americana*. In this species, seeds begin germinating following dispersal in late summer. In cooler climates there is often a limited window of time for ger-

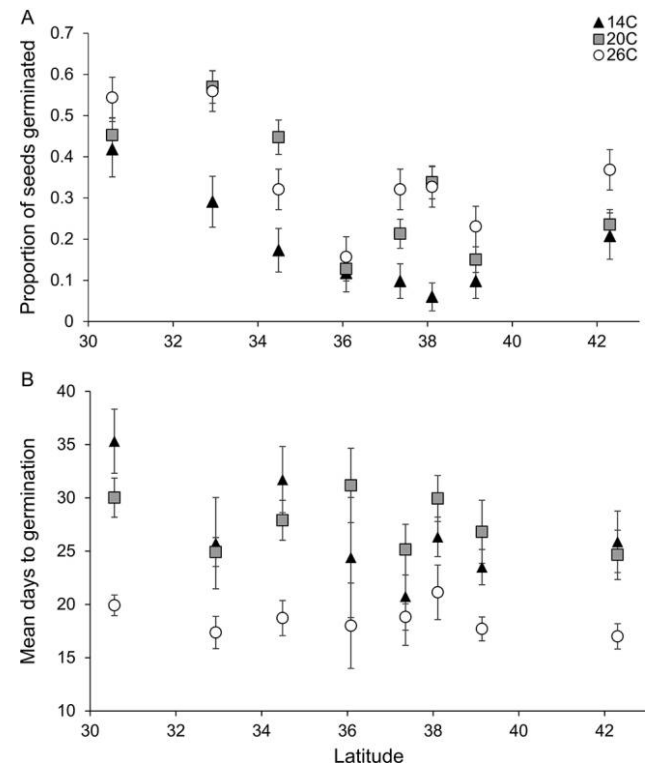


Fig. 4 Proportion of seeds germinated ( $\pm$  SE; A) and mean days to germination ( $\pm$  SE; B) when seeds of eight populations of *Campanula americana* spanning the latitudinal range were grown in three temperatures.

mination before the onset of prohibitive winter conditions (Galloway and Burgess 2009; L. F. Galloway and H. R. Prendeville, unpublished data). Under a warming climate the fall growing season is expected to last longer, especially at mid- to high latitudes (Menzel and Fabian 1999; Menzel et al. 2006; Ibáñez et al. 2010). Our results indicate that warmer temperatures will not only increase the window of time when germination is possible but also increase germination propensity and rate, resulting in increased fall germination and thus winter annuals, especially at northern latitudes. Such a shift in life history could affect natural populations of *C. americana*, as common garden experiments found that seeds from populations across a latitudinal gradient germinated more frequently as annuals when planted in the south and more frequently as biennials when planted in the north (L. F. Galloway and H. R. Prendeville, unpublished data). Therefore, climatic shifts will likely affect phenology, decreasing life span in the north, which is expected to affect plant fitness and population dynamics.

In conclusion, *C. americana* varies in both germination timing and plasticity for germination propensity in response to local temperatures across its latitudinal range. Delayed spring germination and limited germination under cold conditions in the

north are likely an adaptive plastic response to colder temperatures. Increased germination under warmer temperatures is expected under climate change and, combined with temperatures permissive for germination later into the fall, is expected to result in an increased frequency of annuals. In many species, including *C. americana*, understanding intraspecific variation in germination timing across geographic and environmental gradients is important for predicting how altered climatic conditions may affect life-history patterns in the future.

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### Literature Cited

- Anderson JT, DW Inouye, AM McKinney, RI Caloutti, T Mitchell-Olds 2012 Phenotypic plasticity and adaptive evolution contribute to advanced flowering phenology in response to climate change. *Proc R Soc B* 279:3843–3852.
- Badeck FW, A Bondeau, K Bottcher, D Doktor, W Lucht, J Schaber, S Sitch 2004 Responses of spring phenology to climate change. *New Phytol* 162:295–309.
- Barnard-Kubow KB, CL Debban, LF Galloway 2015 Multiple glacial refugia lead to genetic restricting and the potential for reproductive isolation in a herbaceous plant. *Am J Bot* 102:1842–1853.
- Barua D, C Butler, TE Tisdale, K Donohue 2011 Natural variation in germination responses of *Arabidopsis* to seasonal cues and their associated physiological mechanisms. *Ann Bot* 109:209–226.
- Baskin CC, JM Baskin 1998 Seeds: ecology, biogeography and evolution of dormancy and germination. Academic Press, San Diego, CA.
- Baskin JM, CC Baskin 1984 The ecological life cycle of *Campanula americana* in northcentral Kentucky. *Bull Torr Bot Club* 111:329–337.
- Bradshaw AD 1965 Evolutionary significance of phenotypic plasticity in plants. *Adv Genet* 13:115–155.
- Burghardt LT, CJ Metcalf, K Donohue 2016 A cline in seed dormancy helps conserve the environment experienced during reproduction across the range of *Arabidopsis thaliana*. *Am J Bot* 103:47–59.
- Burghardt LT, CJ Metcalf, AM Wilczek, J Schmitt, K Donohue 2015 Modeling the influence of genetic and environmental variation on the expression of plant life cycles across landscapes. *Am Nat* 185:212–227.
- Clauss MJ, DL Venable 2000 Seed germination in desert annuals: an empirical test of adaptive bet hedging. *Am Nat* 155:168–186.
- Cleland EE, I Chuine, A Menzel, HA Mooney, MD Schwartz 2007 Shifting plant phenology in response to global change. *Trends Ecol Evol* 22:357–365.
- Cohen D 1967 Optimizing reproduction in a randomly varying environment when a correlation may exist between the conditions at the time a choice has to be made and the subsequent outcome. *J Theor Biol* 16:1–14.
- Daws MI, E Lydall, P Chmielarz, O Leprince, S Matthews, CA Thanos, HW Pritchard 2004 Developmental heat sum influences recalcitrant seed traits in *Aesculus hippocastanum* across Europe. *New Phytol* 162:157–166.
- Debieu M, C Tang, B Stich, T Sikosek, S Effgen, E Josephs, J Schmitt, M Nordborg, M Koornneef, J Meaux 2013 Co-variation between seed dormancy, growth rate and flowering time changes with latitude in *Arabidopsis thaliana*. *PLoS ONE* 8:e61075, doi:10.1371/journal.pone.0061075.
- Donohue K 2002 Germination timing influences natural selection on life history characters in *Arabidopsis thaliana*. *Ecology* 83:1006–1016.
- 2005 Seeds and seasons: interpreting germination timing in the field. *Seed Sci Res* 15:165–187.
- Donohue K, L Dorn, C Griffith, E Kim, A Aquilera, C Polisetty, J Schmitt 2005 Evolutionary ecology of seed germination of *Arabidopsis thaliana*: variable natural selection on germination timing. *Evolution* 59:758–770.
- Elzinga JA, A Atlan, A Biere, L Gogord, AE Weis, G Bernasconi 2007 Time after time: flowering phenology and biotic interactions. *Trends Ecol Evol* 22:432–439.
- Fenner M, K Thompson 2005 The ecology of seeds. Cambridge University Press, Cambridge.
- Fitter AH, RSR Fitter 2002 Rapid changes in flowering time in British plants. *Science* 296:1689–1691.
- Franks SJ, S Sim, AE Weis 2007 Rapid evolution of flowering time by an annual plant in response to climate fluctuation. *Proc Natl Acad Sci USA* 104:1278–1282.
- Galloway LF 2001 The effect of maternal and paternal environments on seed characters in the herbaceous plant *Campanula americana* (Campanulaceae). *Am J Bot* 88:832–840.
- 2002 The effect of maternal phenology on offspring characters in the herbaceous plant *Campanula americana*. *J Ecol* 90:851–858.
- Galloway LF, KS Burgess 2009 Manipulation of flowering time: phenological integration and maternal effects. *Ecology* 90:2139–2148.
- 2012 Artificial selection on flowering time: influence on reproductive phenology across natural light environments. *J Ecol* 100:852–861.

- Haggerty BP, LF Galloway 2011 Response of individual components of reproductive phenology to growing season length in a monocarpic herb. *J Ecol* 99:242–253.
- Hernández-Verdugo S, K Oyama, C Vásquez-Yanes 2001 Differentiation in seed germination among populations of *Capsicum annuum* along a latitudinal gradient in Mexico. *Plant Ecol* 155:245–257.
- Higgins JW, N Ockendon, DJ Baker, J Carr, EC White, REA Almond, T Amano, et al 2015 Geographical variation in species' population responses to changes in temperature and precipitation. *Proc R Soc B* 282:20151561.
- Hoyle GL, H Cordiner, RB Good, AB Nicotra 2014 Effects of reduced winter duration on seed dormancy and germination in six populations of the alpine herb *Aciphyllia glacialis* (Apiaceae). *Conserv Physiol* 2:1–11.
- Ibáñez I, R Primack, AJ Miller-Rushing, E Ellwood, H Higuchi, SD Lee, H Kobori, JA Silander 2010 Forecasting phenology under global warming. *Philos Trans R Soc B* 365:3247–3260.
- Inouye DW 2008 Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. *Ecology* 89:353–362.
- IPCC (Intergovernmental Panel on Climate Change) 2007 Climate change 2007: the physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.
- Kalish S 1986 Variable selection on the timing of germination in *Collinsia verna* (Schrophulariaceae). *Evolution* 40:479–491.
- Kalish S, GM Wardle 1994 Life history variation in *Campanula americana* (Campanulaceae): population differentiation. *Am J Bot* 81:521–527.
- Körner C, D Basler 2010 Phenology under global warming. *Science* 327:1461–1462.
- Kronholm I, FX Picó, C Alonso-Blanco, J Goudet, J de Meaux 2012 Genetic basis of adaptation in *Arabidopsis thaliana*: local adaptation at the seed dormancy QTL *DOG1*. *Evolution* 66:2287–2302.
- Menzel A, P Fabian 1999 Growing season extended in Europe. *Nature* 397:659.
- Menzel A, TH Sparks, N Estrella, E Koch, A Aasa, R Ahas, K Alm-Kübler, et al 2006 European phenological response to climate change matches the warming pattern. *Glob Change Biol* 12:1969–1976.
- Meyer SE, E McArthur, GL Jorgensen 1989 Variation in germination response to temperature in rubber rabbitbrush (*Chrysothamnus nauseosus*: Asteraceae) and its ecological implications. *Am J Bot* 76:981–991.
- Munguía-Rosas MA, J Ollerton, V Parra-Tabla, JA De-Nova 2011 Meta-analysis of phenotypic selection on flowering phenology suggests that early flowering plants are favoured. *Ecol Lett* 14:511–521.
- Northeast Regional Climate Center 2011 Climate Information for Management and Operational Decisions (CLIMOD). Cornell University, Ithaca, NY.
- Pezzani F, C Montaña 2006 Inter- and intraspecific variation in the germination response to light quality and scarification in grasses growing in two-phase mosaics of the Chihuahuan Desert. *Ann Bot* 97:1063–1071.
- Prendeville HR, K Barnard-Kubow, C Dai, BC Barringer, LF Galloway 2013 Clinal variation for only some phenological traits across a species range. *Oecologia* 173:421–430.
- R Development Core Team 2015 R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <http://www.R-project.org/>.
- Reinartz JA 1984 Life history variation of common mullein (*Verbascum thapsus*). I. Latitudinal differences in population dynamics and timing of reproduction. *J Ecol* 72:897–912.
- SAS Institute 2011 SAS 9.3 for Windows. SAS Institute, Cary, NC.
- Sherry RA, X Zhou, S Gu, JA Arnone, DS Schimel, PS Verburg, LL Wallace, Y Luo 2007 Divergence of reproductive phenology under climate warming. *Proc Natl Acad Sci USA* 104:198–202.
- Silvertown JW 1981 Seed size, life span, and germination date as co-adapted features of plant life history. *Am Nat* 118:860–864.
- Simpson GM 1990 Seed dormancy in grasses. Cambridge University Press, Cambridge.
- Steltzer H, E Post 2009 Seasons and life cycles. *Science* 324:886–887.
- Thompson PA 1975 Characterization of the germination responses of *Silene dioica* (L.) Clairv., populations from Europe. *Ann Bot* 39:1–19.
- Tielbörger K, M Petrù, C Lampei 2012 Bet-hedging germination in annual plants: a sound empirical test of the theoretical foundations. *Oikos* 121:1860–1868.
- Venable DL, JS Brown 1988 The selective interactions of dispersal, dormancy, and seed size as adaptations for reducing risk in variable environments. *Am Nat* 131:360–384.
- Venable DL, L Lawlor 1980 Delayed germination and dispersal in desert annuals: escape in space and time. *Oecologia* 46:272–282.
- Vergeer P, WE Kunin 2011 Life history variation in *Arabidopsis lyrata* across its range: effects of climate, population size and herbivory. *Oikos* 120:979–990.
- Way DA 2011 Tree phenology responses to warming: spring forward, fall back? *Tree Physiol* 31:469–471.
- Wilczek AM, LT Burghardt, AR Cobb, MD Cooper, SM Welch, J Schmitt 2010 Genetic and physiological bases for phenological responses to current and predicted climates. *Philos Trans R Soc B* 365:3129–3147.
- Wilczek AM, JL Roe, MC Knapp, MD Cooper, C Lopez-Gallego, LJ Martin, CD Muir, et al 2009 Effects of genetic perturbation on seasonal life history plasticity. *Science* 323:930–934.
- Yang LH, VH Rudolf 2010 Phenology, ontogeny and the effect of climate change on the timing of species interactions. *Ecol Lett* 13:1–10.