

The success of assisted colonization and assisted gene flow depends on phenology

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Abstract

Global warming will jeopardize the persistence and genetic diversity of many species. Assisted colonization, or the movement of species beyond their current range boundary, is a conservation strategy proposed for species with limited dispersal abilities or adaptive potential. However, species that rely on photoperiodic and thermal cues for development may experience conflicting signals if transported across latitudes. Relocating multiple, distinct populations may remedy this quandary by expanding genetic variation and promoting evolutionary responses in the receiving habitat – a strategy known as assisted gene flow. To better inform these policies, we planted seeds from latitudinally distinct populations of the annual legume, *Chamaecrista fasciculata*, in a potential future colonization site north of its current range boundary. Plants were exposed to ambient or elevated temperatures via infrared heating. We monitored several life history traits and estimated patterns of natural selection to determine the adaptive value of plastic responses. To assess the feasibility of assisted gene flow between phenologically distinct populations, we counted flowers each day and estimated the degree of temporal isolation between populations. Increased temperatures advanced each successive phenological trait more than the last, resulting in a compressed life cycle for all but the southern-most population. Warming altered patterns of selection on flowering onset and vegetative biomass. Population performance was dependent on latitude of origin, with the northern-most population performing best under ambient conditions and the southern-most performing most poorly, even under elevated temperatures. Among-population differences in flowering phenology limited the potential for genetic exchange among the northern- and southern-most populations. All plastic responses to warming were neutral or adaptive; however, photoperiodic constraints will likely necessitate evolutionary responses for long-term persistence, especially when involving populations from disparate latitudes. With strategic planning, our results suggest that assisted colonization and assisted gene flow may be feasible options for preservation.

Keywords: *Chamaecrista fasciculata*, climate change, flowering time, fruiting time, growing degree days, natural selection, phenology, plasticity

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Introduction

Environmental change wrought by increasing global temperatures can compromise the persistence of species in their current geographic locations. Although some species may possess sufficient developmental and physiological plasticity to tolerate novel conditions, others may be prone toward extinction (Thomas *et al.*, 2004; Carpenter *et al.*, 2008). Migration to newly suitable locations may alleviate some of the pressures imposed by a changing climate, and indeed, numerous taxa have shifted their ranges to higher latitudes and elevations over the past few decades (Perry *et al.*, 2005; Parmesan, 2006; Lenoir *et al.*, 2008). However, thermal conditions define only part of a species' niche, and migrating species may still experience unfamiliar conditions in the receiving habitat, including novel

community assemblages (Hellmann *et al.*, 2012; Nooten *et al.*, 2014) and photoperiodic regimes (Griffith & Watson, 2006). For some, survival will depend on adaptation to changing conditions at current localities, or on a combination of migration and evolution, as has been argued for the pole-ward migration of species following glaciation (Davis & Shaw, 2001). Limitations in dispersal abilities or adaptive capacities may necessitate the preemptive management of vulnerable or economically valuable species (Aitken *et al.*, 2008; Hobbs *et al.*, 2009).

Several conservation measures have been proposed to mitigate extinction risks in the face of climate change. Assisted colonization, or assisted range expansion, refers to the movement of a species beyond its current range boundary and has been recently suggested for species unable to adapt or migrate in response to global warming or for species of economic or ecologic importance (McLachlan *et al.*, 2007; Kreyling *et al.*, 2011; Loss *et al.*, 2011). Despite being hotly debated as a manage-

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ment strategy (Mueller & Hellmann, 2008; Ricciardi & Simberloff, 2009; Vitt *et al.*, 2010), formal trials to assess the prospects and limitations of assisted colonization have been few (Hewitt *et al.*, 2011). Additionally, academic, conservation, government, and industry sectors often differ in their management goals, and the focuses of existing studies of assisted colonization are not always applicable across disciplines (Pedlar *et al.*, 2012). For example, with regard to plants, the interests of forestry professionals (e.g. maximizing woody growth, Lu *et al.*, 2014) do not align with those considering assisted colonization as a species conservation strategy (e.g. maximizing population size, Willis *et al.*, 2009) or for ecosystem maintenance or restoration (e.g. maximizing primary productivity, Grady *et al.*, 2011; Lunt *et al.*, 2013). Whatever the motivation may be, long-term success will depend on the ecological and evolutionary responses of newly established populations to the selective pressures imposed by a continuously changing climate.

The feasibility of assisted colonization across latitudes is contingent upon the choice of relocation sites and source populations (Kreyling *et al.*, 2011; Leech *et al.*, 2011). Potential relocation sites should lie within areas projected to have similar climatic conditions found within the historic range (e.g. north of the current range boundary, Kreyling *et al.*, 2011). The choice of source populations, however, can present a conundrum. Those residing along the leading edge of a species' historic distribution may be best matched to the current thermal conditions just outside of it (Hill *et al.*, 2011), improving the chances of short-term success. However, rapid evolutionary responses may be necessary for long-term persistence as the climate continues to warm. Populations from lower latitudes may be better suited to the higher temperatures expected of the future conditions in the new site (Grady *et al.*, 2011), but may be unable to establish under current conditions. One challenge in implementing assisted colonization is the identification of a source population, or mixture of source populations, that ensures sufficient genetic variation in key traits to generate genotypes suited to future climatic conditions.

Many factors can limit the establishment of species in more pole-ward sites, and these restrictions will be particular to the species involved. However, there is one factor that changes with latitude in an absolutely predictable fashion – the annual photoperiodic cycle. Species that rely on photoperiodic and thermal cues for growth or development may experience conflicting signals if relocated further north (Bradshaw & Holzapfel, 2010). Evolutionary responses to changes in environmental conditions can proceed rapidly (Franks *et al.*, 2007), even in traits under photoperiodic

control (Bradshaw & Holzapfel, 2001). However, in the case of assisted colonization, immediate, plastic responses to novel temperatures or photoperiods may determine whether relocations are successful. While difficult to assess in a field setting, it may be important to consider the implications of photoperiodic mismatches when examining the responses of populations to increases in temperature (Bradshaw & Holzapfel, 2008) and when selecting candidate species or populations for assisted colonization.

Phenological traits can be influenced by both thermal (Blázquez *et al.*, 2003) and photoperiodic (Kobayashi & Weigel, 2007) regimes, and the timing of these traits may serve as indicators of environmental change (Fitter & Fitter, 2002; Parmesan, 2006). Despite their potential adaptive value (Stevenson & Bryan, 2000; Chuine, 2010), the fitness impacts of climate-induced shifts in development are seldom measured (but see Anderson *et al.*, 2012; Merilä & Hendry, 2013). Additionally, phenological traits are expressed sequentially (e.g. in plants, flowering onset precedes fruiting onset), and the plastic responses of individual traits are rarely measured independently of previously expressed traits (but see Haggerty & Galloway, 2011; Kim & Donohue, 2011). The monitoring of phenological traits may reveal whether relocated individuals are well suited to conditions in the receiving habitat, yet failing to examine the cumulative influence of environmental change across the life cycle may result in misidentifying the true targets or agents of selection or their capacity for evolutionary response (Ehrlén, 2015).

Relocating individuals from multiple, distinct populations, or their hybrids, may expand genetic variation in the newly founded population, increasing the chance that some individuals respond favorably to relocation and have reproductive rates high enough to sustain the population in the short term. Recombination among genetic variants would enable evolutionary adaptation to novel combinations of habitat and climate (Rice & Emery, 2003; Tallmon *et al.*, 2004; Aitken *et al.*, 2008; Loss *et al.*, 2011). However, population differences in reproductive phenology could limit, and bias, the potential for genetic exchanges among migrants (Weis, 2015). Implementing a combination of assisted colonization with assisted gene flow would require knowledge of the potential for natural genetic exchange among the populations relocated together under current and future conditions (Aitken & Whitlock, 2013). Experiments founded in proposed relocation sites can explore these patterns and can also help to reveal which traits contribute to fitness and how selection regimes will change as temperatures continue to warm (Aitken *et al.*, 2008; Lawler & Olden, 2011).

In this study, we examined phenotypic responses to warming and patterns of natural selection beyond the range and discuss our results as they relate to the feasibility of assisted colonization in combination with assisted gene flow. We planted seeds from latitudinally distinct populations of the annual legume *Chamaecrista fasciculata* north of its current range boundary and exposed them to present-day and future climatic conditions using artificial climate warming arrays. We assessed the responses of phenological traits in units of calendar days as well as growing degree days, and we also monitored growth, survival, and seed production to estimate patterns of natural selection. With an emphasis on comparing the feasibility of these management plans under current and future climatic conditions, we ask: (i) In an assisted colonization program, which source population(s) are likely to succeed? and (ii) In an assisted gene flow program, do phenological differences between populations impede genetic introgression?

Materials and methods

Study species

Chamaecrista fasciculata Michx. (Fabaceae, subfamily Caesalpi-noideae) is a frost-intolerant annual plant of tropical descent (de Souza Conceição *et al.*, 2009) distributed in North America across the Great Plains and eastward toward the Atlantic (Irwin & Barneby, 1982). Its upper range boundary includes all of the northern United States from Minnesota to New York, but it has not yet been known to occur in Canada (Fig. 1). It is

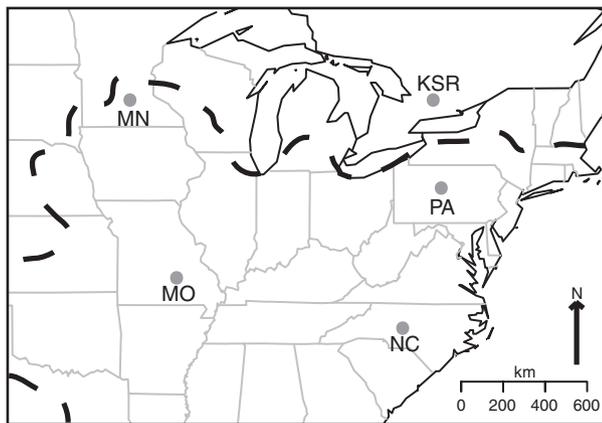


Fig. 1 A map of the eastern United States showing the northern range limit of *Chamaecrista fasciculata* (dashed line), as well as the seed collection sites in Minnesota (MN), Pennsylvania (PA), Missouri (MO), and North Carolina (NC). The experimental relocation took place in southern Ontario at the Koffler Scientific Reserve at Joker's Hill (KSR). The distribution of *C. fasciculata* was estimated from herbarium specimens, field observations, communications with other researchers, and the PLANTS database maintained by the United States Department of Agriculture.

frequently found in sandy soils and occupies prairie habitat or sites that have been recently disturbed (Foote & Jackobs, 1966). Growth and flowering are indeterminate, and an individual plant will produce anywhere from 1 to several hundred flowers in its lifetime.

During the summer of 2009, seeds were collected from populations located along two latitudinal transects in the United States (Fig. 1): one through the Midwest from Minnesota (MN, 44.8011°N, 92.9647°W) south to Missouri (MO, 38.4979°N, 90.5610°W) and the other along the East Coast from Pennsylvania (PA, 40.1790°N, 76.7248°W) south to North Carolina (NC, 35.8900°N, 79.0092°W). When possible, three fruit were collected from 50 to 100 individuals per population from plants spaced approximately 5 m apart (an estimated genetic neighborhood size for this species, Fenster, 1991). Any other differences between collection sites were not accounted for, and we acknowledge that maternal effects may play a role in the results we present here, as they would in any conservation strategy that involved seed collection from natural populations that did not also incorporate extensive breeding programs prior to implementation. Others have shown that populations of *C. fasciculata* are locally adapted across large geographic distances (Galloway & Fenster, 2000; Etterson & Shaw, 2001) and that the spatial scale of gene flow is limited via both pollen movement and seed dispersal (Fenster, 1991; Fenster *et al.*, 2003).

Experimental design

We used the Experimental Climate Warming Arrays at the University of Toronto's field station in southern Ontario, the Koffler Scientific Reserve at Joker's Hill (KSR, 44.0300°N, 79.5275°W), to expose plants to either present-day thermal regimes or those predicted of the area by mid-century (OMNR *et al.*, 2007). Each warming array consisted of a steel triangular structure anchored 1.25 meters above the ground with six infrared heaters mounted in a hexagonal configuration along the sides (design per Kimball *et al.*, 2008). Heating elements were angled inward and down from horizontal, producing a uniform heat shadow of 3 m in diameter. Six plots were heated by 1.5 °C during the day and 3 °C at night (Easterling *et al.*, 1997), while six identical plots remained unheated and experienced ambient temperatures. Any shading imposed by these structures was minimal (Kimball *et al.*, 2008). Temperatures were monitored at the plot level in three arrays per treatment using infrared radiometers (SI-111 infrared radiometer; Campbell Scientific, Edmonton, AB, Canada). Measurements were taken every 15 min, and a comparison of the average temperatures within a treatment were used to determine the degree of heat output necessary to maintain the target level of warming. Due to technical issues, data from one of the heated plots were dropped from all analyses.

In May of 2011, seeds were scarified, stratified for 3 days, and planted within the warming arrays in a hexagonal design with 20 cm spacing between plants. Individuals from each population were randomized within each plot. Due to record levels of precipitation in the area, all seedlings drowned and seeds had to be replanted in pots in the greenhouse adjacent

to the heating arrays while the experimental plots drained of water. To expose seedlings to different temperatures from emergence onward, half of the plants were moved just outside of the greenhouse to experience ambient thermal conditions, while the remaining plants inside the insulated greenhouse were exposed to elevated temperatures. Temperature measurements from several iButton Temperature Loggers (1992L; Maxim Integrated, San Jose, CA, USA) placed at soil level in random pots indicated an average temperature difference of 2.7 °C between locations inside and outside of the greenhouse. Plants were given 0.5 oz of fertilizer (20-20-20, 1 g l⁻¹) at 21 days after planting, and at 25 days, seedlings were transplanted into the warming arrays as outlined previously, with five plants per plot from each population. A ring of nonfocal plants was planted around the focal individuals in each plot to absorb any edge effects. Plots were watered every few days for the first 2 weeks after transplanting, after which they received natural levels of precipitation. Plots were weeded periodically throughout the experiment to minimize interspecific competition, and plants were harvested upon first frost, 178 days after planting.

We measured plants each day for several sequentially expressed phenological traits, or phenophases; the date of emergence, first flower bud, first open flower, and first mature fruit. The date of first bud was recorded when a flower bud first reached a length of 0.5 cm, while the date of first mature fruit was noted as the date when the first fruit pod browned and seeds rattled within. All fruit were collected as they reached this stage, before the pods explosively dehisced their seed. Flowers were counted daily on all individuals for 86 of the 93 days where flowers were present, allowing us to examine the effects of warmer conditions on the total number of flowers produced and the duration of flowering. We measured aboveground vegetative biomass after harvesting, and female fecundity was defined as the total number of seeds produced by an individual.

Our ability to assess responses to climate change can be dependent on the way that we measure the progression of life history stages. The growth and development of plants is frequently influenced by temperature, and in the absence of other limiting factors, a minimum accumulation of heat can be required before proceeding to the next developmental stage (Wang, 1960). Due to variable conditions among years, the number of days that elapse before this minimum heat sum is reached can vary. Accordingly, studies of the effects of warming on the timing of life history traits (in units of days) can be complimented by also examining the accumulation of heat across days, or growing degree days (GDD), upon the expression of those traits (in units of °C day, Neuheimer & Taggart, 2007). For example, a phenological trait that requires a fixed heat sum before developing will exhibit a constant value of accumulated GDD even if the timing of that trait shifts in response to changes in climate. In this way, temporal changes in phenological traits do not necessarily reflect plasticity to increasing temperatures; rather, plants development is contingent on specific patterns of heat accumulation. While this methodology is often overlooked outside of agriculture or entomology, phenological traits measured in units of GDD

can yield less variable results and can increase predictive power (Neuheimer & Taggart, 2007).

We calculated the accumulated GDD upon the onset of budding, flowering, and fruiting for each individual. Growing degree days are calculated by comparing the average daily temperature to a base temperature, T_{base} , below which growth does not occur: $GDD = (T_{max} - T_{min})/2 - T_{base}$, where T_{max} and T_{min} are the maximum and minimum daily temperatures, respectively (Miller *et al.*, 2001). T_{base} ranges from 5 to 10 °C in most commercial species, and T_{max} can be capped at 30 °C because growth often does not continue to accelerate at higher temperatures (Wang, 1960). However, many tropical species may require temperatures in excess of 30 °C for the development of certain traits (Trudgill *et al.*, 2005 and references therein). Due to *C. fasciculata*'s tropical origin, we calculated daily GDD in a variety of scenarios, with 0.5 °C increments of T_{max} capped from 30 to 35 °C and of T_{base} ranging from 5 to 10 °C (121 combinations in total). If average daily temperatures dropped below T_{base} , GDD was set to 0.

We used temperature data collected by Environment Canada at the nearby Buttonville Airport (43.8608°N, 79.3686°W) to calculate GDD for plants in ambient treatments in order to obtain weather data for the period of time prior to planting within the warming arrays. For heated treatments, we added the average of the daytime and nighttime increases in temperature due to artificial warming (2.25 °C) to ambient temperature data. For a given trait, accumulated GDD is calculated as the sum of daily GDD from the date of planting to the date of trait onset.

Statistical analyses

We confirmed that heated plots were maintained at a warmer temperature throughout the experiment by comparing temperature differences between treatments with a repeated measures linear model with thermal treatment, day, and their interaction as fixed effects. We incorporated an auto-regressive error structure of order 1 to account for any autocorrelation in observations among days, nested within plot (Zuur *et al.*, 2009). This analysis was performed using the nlme package (Pinheiro *et al.*, 2014) in R (R Development Core Team, 2014).

Differences in the timing of budding, flower, and fruiting onset, as well as flowering duration, the total number of flowers produced, aboveground vegetative biomass, and total seed production were analyzed via linear mixed models, again using the nlme package in R. To meet the assumption of residual normality, we analyzed the log of vegetative biomass +1. Population, thermal treatment, and their interaction were included as fixed effects, while plot was included as a random effect if it improved model fit. A significant effect of temperature is indicative of phenotypic plasticity in the trait of interest, a population effect reflects genetic differentiation among populations, and a significant interaction reveals genetic differences among populations in their plastic responses to warmer temperatures. Variance heterogeneity among populations or treatments was corrected using error variance covariates, if

necessary (Zuur *et al.*, 2009). The random term and error covariate components of the model structure were selected by minimizing AIC values, after which models fit via maximum likelihood were used to optimize the fixed effects. Here, we present final, optimized models selected via log likelihood ratio tests. We further assessed the influence of thermal treatment on development by repeating these analyses and substituting the accumulated GDD at the onset of budding, flowering, and fruiting in lieu of calendar days.

If a series of sequentially expressed phenological traits are accelerated or delayed by warmer temperatures, two factors may be involved. An early initiation of the first trait in the sequence will contribute to an early initiation of the remainder, while the intervals between these transitions, or phenophases, may show no change. However, trait-specific plastic responses can independently diminish or extend the phenophase intervals. We estimated the degree of independence for budding, flowering, and fruiting responses by repeating the analyses described above with the onset date of the previous phenophases as a covariate. In annuals, plant size is often inherently correlated to flowering onset (Bolmgren & Cowan, 2008; Weis *et al.*, 2014), and we included flowering onset as a covariate in the reanalysis of vegetative biomass. Similarly, we included flowering onset as a covariate in the examination of the independent effects of thermal regime on total flower number and flowering duration.

Phenotypic selection analysis

We estimated whether any temperature-induced changes in phenology or vegetative biomass were adaptive by assessing the magnitude of phenotypic selection. We used a hurdle model to separately examine the effects of phenotype on two components of fitness; survival to fruiting and total seed production. We first analyzed survival with a binomial generalized linear model with log link (the zero component) using the `glm` function in R, after which we modeled seed production (excluding zeros) with a zero-truncated negative binomial generalized linear model (the count component) using the `VGAM` package (Yee, 2010). Analyses estimating the strength of direct selection on individual traits include the date of flowering onset and vegetative biomass as fixed effects in both components, while fruiting onset was also included in the count component. Total selection was calculated with separate univariate analyses for each of the aforementioned traits. Trait values were rescaled to a mean of 0 and a standard deviation of 1 across populations and treatments, and we included temperature as an interacting fixed effect with all traits. A significant interaction between a trait and temperature would indicate that patterns of selection on that trait differ between thermal regimes. We also included population as a fixed effect to account for any unmeasured differences among populations that may affect survival or seed production. We report the significance of fixed effects from the final, optimized models via chi-squared values from analyses of deviance.

The coefficients from the saturated hurdle model will be reported as estimates of direct and total linear selection; however, we caution that they are not comparable to selection gradients and differentials as calculated by multiple regression

(Lande & Arnold, 1983). We chose statistically sound methodology to estimate relationships between phenotype and fitness, whereas linear multiple regression would have violated a number of assumptions (Mitchell-Olds & Shaw, 1987). For comparison, we report selection gradients and differentials as well, but our figures and discussion of selection will focus on the results derived from the hurdle model. To obtain gradients and differentials, we standardized traits and calculated relative fitness within each treatment, with relative fitness defined as the total number of seeds produced by an individual divided by the average number of seeds produced by all individuals in a given treatment.

Temporal reproductive isolation

We estimated the potential for gene flow between populations, as would occur under an assisted gene flow scenario, by analyzing the overlap in flowering schedules to determine the proportion of opportunities for pollen exchange between populations. For the few days with missing flower count data, we used a linear function running from the day before to the day after the missed count to interpolate the expected number of flowers (Wadgyamar *et al.*, 2015). Within a treatment, and for each population pairing, we used daily flower counts to construct and $n \times n$ matrix of pair-wise mating opportunities between all individuals. We calculated a correlation between the population of origin for pollen recipients (population scored as 0) and that of their potential pollen donors (population scored as 1), and we weighted this correlation by the mating probabilities produced in the mating matrix (see Weis & Kossler, 2004). Values of the resulting correlation coefficient, ρ , range from 0 to 1 and reflect completely random mating to complete reproductive isolation, respectively. We obtained 95% confidence intervals on ρ by bootstrapping 1000 \times with replacement (Weis & Kossler, 2004).

Results

Thermal environment

Average temperatures were consistently higher in artificially warmed plots than in ambient plots throughout the season (average daily temperature difference = 2.17 °C; Temperature, $F_{1,631} = 11.75$, $P < 0.001$; Day, $F_{1,631} = 346.71$, $P < 0.001$; Temperature*Day, $F_{1,631} = 0.46$, $P = 0.5$). Consequently, plants in heated plots had the opportunity to accumulate more GDD from the timing of planting to first frost than those in ambient plots (2036 °C-day \pm 22.8 SE vs. 1707 °C-day \pm 21.4 SE, respectively, across all combinations of T_{base} and T_{max}).

Population differences and responses to warming

Populations differed genetically in the average onset dates of budding, flowering, and fruiting according to their latitude of origin, with northern populations

progressing through developmental phases earlier and more rapidly than southern populations (Table 1, Population term, Fig. 2a). Warming advanced these traits in the MN, PA, and MO populations, with each phenophase advancing more than the last (Table 1, Tempera-

ture term). Ultimately, warming compressed the life cycle of plants in these populations. In the southernmost NC population, warmer temperatures advanced budding and flowering onset, but not fruiting. However, more NC individuals survived to produce fruit in the heated treatment than in the unheated (27% vs. 68%, respectively). Variation among populations in plasticity also increased in later-expressed traits, with the onset of fruiting responding to warming most variably among populations (Table 1, Population*Temperature term).

Table 1 Linear mixed-effects analyses of phenological traits, aboveground biomass, seed number, and growing degree day (GDD) accumulations for populations planted in both ambient and heated conditions. *F*-values are reported for fixed effects in the final, optimized model

Trait	Temperature	Population	Temperature* Population
Budding onset	74.88***	322.70***	2.11+
Flowering onset	112.82***	388.24***	2.98*
Fruiting onset	32.95***	281.54***	6.25***
Biomass	NS	51.39***	NS
Flowering duration	0.48	23.89***	NS
Flower number	NS	12.53***	NS
Seed number	7.40*	31.29***	NS
GDD at Budding onset	10.88**	338.92***	3.25*
GDD at Flowering onset	25.25***	367.19***	4.88**
GDD at Fruiting onset	506.66***	355.40***	24.45***

Significance: NS Not Significant, + $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Num. df: Treatment 1; Population 3; Treatment*Population 3.

Denom. df: 158–204.

To determine whether the time intervals between the phenophases responded to warming, we amended the analyses by including the onset date of the previous phenophase as a covariate (Table 2). Budding, flowering, and fruiting onset were influenced by warmer temperatures independently of shifts in the previous phenophases (emergence, budding, and flowering, respectively). However, the degree of independent response for flowering onset averaged just 1 day beyond shifts due to previous traits. Variation among populations in plasticity for budding and flowering onset disappear once the preceding responses to warming are accounted for. Only fruiting onset displayed significant independent variation in plasticity among populations.

Plants in the heated treatment had accumulated more growing degree days at the onset of budding, flowering, and fruiting than plants in ambient conditions, despite the temporal acceleration of most traits in all populations (Table 1, Fig. 2b). Additionally, we detected much more variation in plasticity when

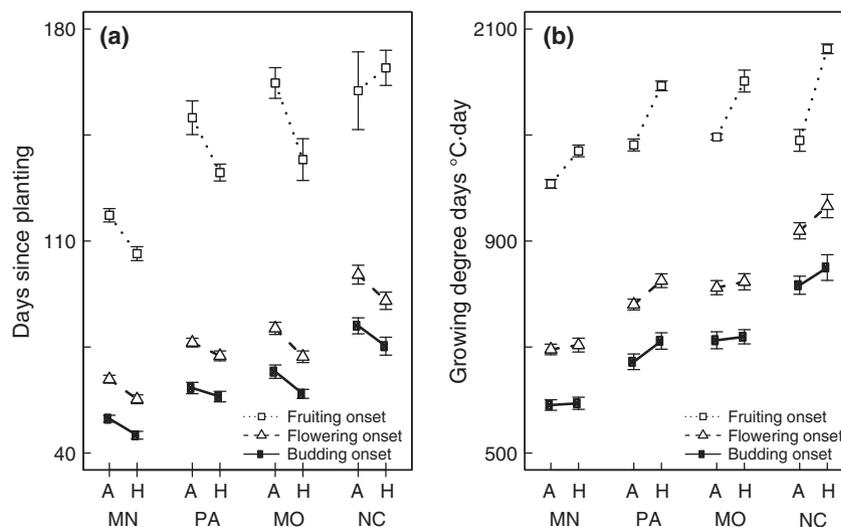


Fig. 2 Reaction norms showing the mean \pm 2 standard errors of (a) reproductive phenological traits and (b) growing degree day accumulations upon the expression of those traits in the Minnesota (MN), Pennsylvania (PA), Missouri (MO), and North Carolina (NC) populations in ambient (A) and artificially heated (H) conditions.

Table 2 Linear mixed-effects analyses of independent responses of phenological traits, aboveground biomass, and growing degree day (GDD) accumulations for populations planted in both heated and ambient conditions. Independent responses to warming are indicated by a significant temperature effect when the previous phenophase (included in parentheses) is included as a covariate in the model. *F*-values are reported for fixed effects in the final, optimized model

Trait (covariate)	Temperature	Population	Temperature* Population	Covariate
Budding onset (Emergence date)	75.90***	329.53***	2.30†	6.70*
Flowering onset (Budding onset)	455.28***	1198.63***	NS	565.93***
Fruiting onset (Flowering onset)	27.00***	318.42***	7.36***	11.53***
Biomass (Flowering onset)	NS	51.05***	NS	0.23
Flowering duration (Flowering onset)	6.74***	40.45***	2.47†	64.41***
Flower number (Flowering onset)	NS	12.85***	NS	12.27***
GDD Budding onset (GDD Emergence date)	10.66**	339.46***	3.42*	0.01
GDD Flowering onset (GDD Budding onset)	95.38***	1212.76***	NS	568.85***
GDD Fruiting onset (GDD Flowering onset)	689.36***	387.09***	22.89***	17.58***

Significance: NS: not Significant, † $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Num. df: Treatment 1; Population 3; Treatment*Population 3; Covariate 1.

Denom. df: 157–201.

phenological responses were assessed via heat sums than when analyzed using calendar days. These patterns remain when including the accumulated GDD of the previous phenophases in analyses (Table 2) and are a direct indication of thermal plasticity in phenological traits.

Heated conditions had no effect on the final, aboveground vegetative biomass of any population, although plants from the NC population were significantly larger than those from the other populations (Table 1, Fig. 3a). Unlike most annual plants, there seems to be no association between flowering onset date and plant size in *C. fasciculata* (Table 2), suggesting that the evolutionary potential of phenological traits may not be strongly affected by patterns of selection on growth.

In ambient conditions, the average number of seeds produced by a plant varied by population latitude of origin and was highest in the MN population and lowest in the NC population (Table 1, Fig. 3b). All populations produced more seed in the warmer environment, with proportionately larger increases in the southern populations. For the PA and MO populations, this warming-induced increase resulted in seed production levels equivalent to that of the MN population.

Phenotypic selection analysis

We examined the effects of temperature on the relationship between focal traits and two different fitness components; survival and the number of seeds produced. Despite responding to warming, the influence of flowering onset on survival was marginal and similar in both thermal environments (Table 3, Fig. 4a), with direct selection favoring early flowering (Table 4). In contrast, early flowering only increased seed

production under ambient conditions and was negligible in the warmer environment (Table 3, Fig. 5a). Thus, with regard to seed production, the advancement of flowering onset when heated was adaptive and may have ameliorated the maladaptive timing of flowering in the more southern populations.

Early fruiting onset similarly increased seed production in both thermal environments (Fig. 5b). Final vegetative biomass strongly influenced survival when warmed (Table 3, Fig. 4b) resulting in selection for larger plant size only in the heated conditions (Table 4), whereas larger plant size increased seed production similarly in both thermal environments (Fig. 5c). In this case, warming altered patterns of selection on a trait unaffected by temperature, but this is only apparent when examining the effects of trait variation on survival.

Temporal reproductive isolation

We calculated the strength of temporal reproductive isolation to gauge the potential for gene flow between pairs of populations. Mating opportunities were greatest between populations most similar in latitude of origin. In ambient conditions, ρ was lowest between the MN and PA populations and the PA and MO populations (Fig. 6a, below diagonal). In contrast, ρ was highest between the southern-most NC population and the northern MN and PA populations, indicating that these populations are almost entirely reproductively isolated.

The strength of ρ was significantly weaker for four of six cases when in heated (Fig. 6a, above diagonal) vs. ambient conditions, reflecting a greater potential for gene flow among populations as temperatures warm, and was significantly stronger in another comparison. Flowering duration was slightly variable among

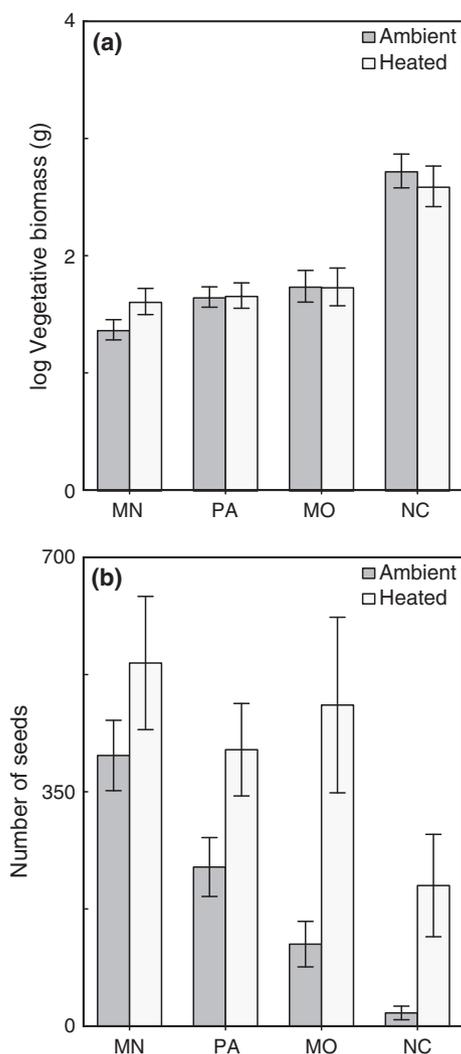


Fig. 3 The mean \pm standard error of (a) the log of above-ground biomass and (b) the number of seeds produced in the Minnesota (MN), Pennsylvania (PA), Missouri (MO), and North Carolina (NC) populations in ambient and artificially warmed conditions.

populations and thermal treatment, with the NC population flowering longer in ambient conditions than in heated and vice versa for the MO and MN populations (Table 1, Fig. 6b). Temperature had no effect on the total number of flowers produced in any population (Table 1, Fig. 6c). Although small, the differences in the degree of temporal isolation due to warming could be due, in part, to shifts in flowering onset date and the duration of flowering.

Discussion

We have presented an experiment to mimic the assisted colonization of a species to a pole-ward site beyond its historic geographic range under both current and

Table 3 A hurdle model demonstrating the effects of flowering onset, fruiting onset, and final plant size on survival and seed production in populations of *Chamaecrista fasciculata* planted in both ambient and artificially warmed conditions. This two-part analysis first models the probability of surviving to produce seed using a generalized linear model with a binomial distribution and logit link (zero component). Seed production, excluding zeros, is then modeled by a negative binomial generalized linear model with log link (count component). Chi-squared values are reported for fixed effects in the final, optimized model

	df	Survival	Seed number
Flowering onset	1	NS	3.90*
Fruiting onset	1	–	21.61***
Biomass	1	7.96**	100.94***
Temperature	1	13.98***	0.48
Population	3	58.42***	22.48***
Flowering onset*Temperature	1	NS	6.99**
Fruiting onset*Temperature	1	–	NS
Biomass*Temperature	1	6.77**	NS

Den. df: Zero 198–203, Count 318–321.

Significance: NS: not Significant, –: not included in model, † $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

anticipated future thermal regimes. We show that colonists from northern populations are the most fit under ambient temperatures and that mean fitness steadily declines for colonists from lower latitudes. Warmer temperatures alleviate the fitness decline for all but the southern-most (NC) colonists. Nearly, one in three plants from the NC locality failed to produce any seed in the heated treatment, and total seed production was only half of that of the northern-most population. Thus, the successful pole-ward colonization by a population that is expected to be preadapted to future thermal regimes may be limited by other environmental factors.

Our experiment also assessed the potential for assisted gene flow; that is, the potential for colonists from the south to interbreed with northern populations, and thereby introduce genes that may be adaptive under warmer temperatures. Differences in flowering time severely restricted mating opportunities between northern- and southern-most populations, regardless of thermal regime. Here we discuss the contributions of phenological and growth traits to fitness under ambient and warmed conditions and expand upon the implications for assisted colonization and assisted gene flow.

Phenotypic selection and responses to warming

Increased temperatures accelerated the onset of reproductive traits in all populations of *Chamaecrista fasciculata*, resulting in a compression of life cycle length for all but the southern-most population. Flexibility in life

Table 4 Estimates of direct and total phenotypic linear selection coefficients \pm SE for *Chamaecrista fasciculata* planted in ambient and artificially warmed conditions

Direct selection	Hurdle model				Multiple regression	
	Survival		Seed number		Seed number	
	Ambient	Heated	Ambient	Heated	Ambient	Heated
Flowering onset	-0.80 (0.65)	-1.10 (0.84)	-0.45* (0.23)	-0.19 (0.21)	-0.89*** (0.19)	-0.13 (0.21)
Fruiting onset	-	-	-0.69*** (0.14)	-0.51** (0.17)	-0.45** (0.15)	-0.58** (0.18)
Biomass	0.25 (0.33)	1.72** (0.64)	1.00*** (0.14)	0.90*** (0.10)	1.21*** (0.18)	0.81*** (0.10)
Total selection	Survival		Seed number		Seed number	
	Ambient	Heated	Ambient	Heated	Ambient	Heated
	Flowering onset	-1.19 (0.63)	-0.79 (0.64)	-0.68*** (0.25)	-0.35 (0.25)	-0.70*** (0.10)
Fruiting onset	-	-	-0.69*** (0.17)	-0.45** (0.16)	-0.50*** (0.13)	-0.33** (0.12)
Biomass	0.37 (0.31)	1.61** (0.51)	0.77*** (0.13)	0.97*** (0.09)	-0.04 (0.12)	0.52*** (0.52)

Significance: NS: not significant, † $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Coefficients were derived from a hurdle model that examined relationships between phenotypes and seed number (negative binomial distribution with log link) separately from those of phenotypes and survival (binomial distribution with logit link). Fruiting onset was not included in the survival analysis, as survival was scored as the production of at least one fruit. Significant differences in the strength of selection between treatments are indicated in Table 3. For reference, selection gradients and differentials derived by multiple regression per Lande & Arnold (1983) are also reported.

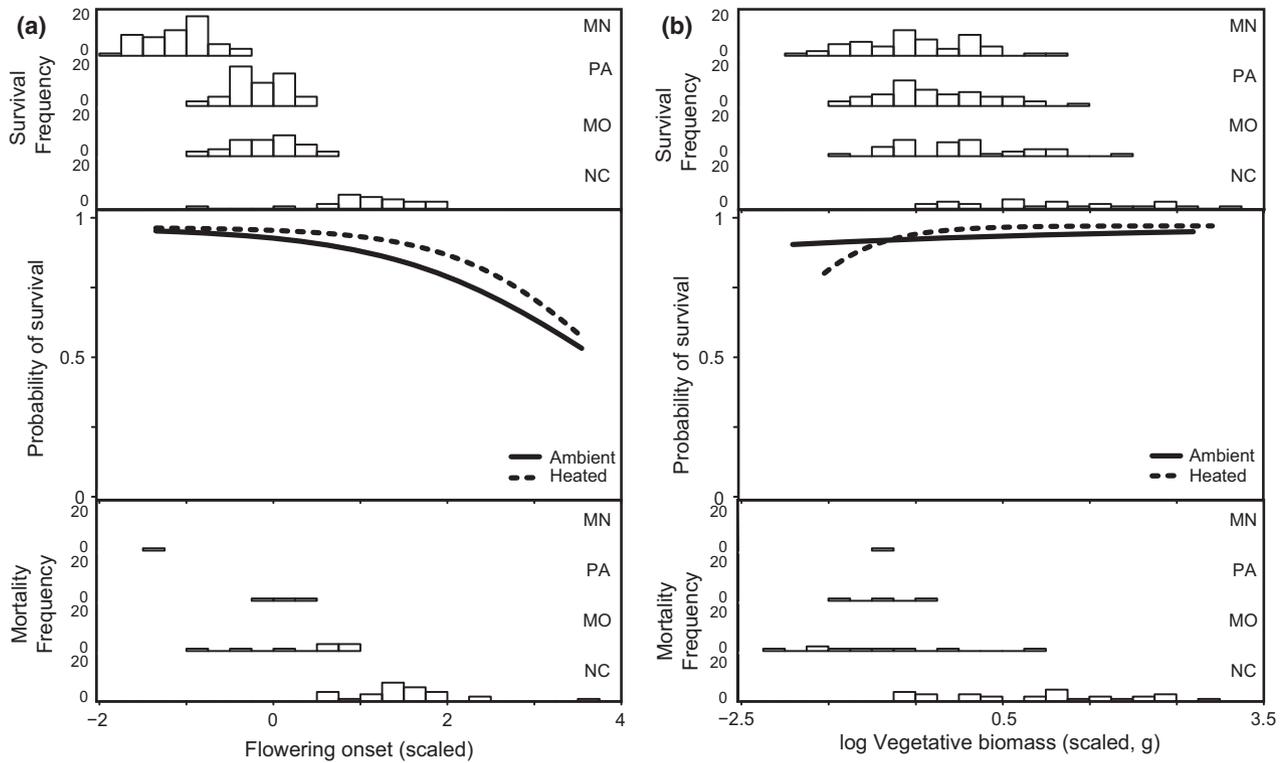


Fig. 4 Logistic regressions portraying the probability of surviving to produce seed in heated and ambient conditions as a function of (a) flowering onset or (b) aboveground vegetative biomass, scaled to a mean of 0 and a standard deviation of 1, per the zero component of the hurdle model (Table 4). Histograms depict the trait values of individuals that survived (upper panels) or died (lower panels) from each population.

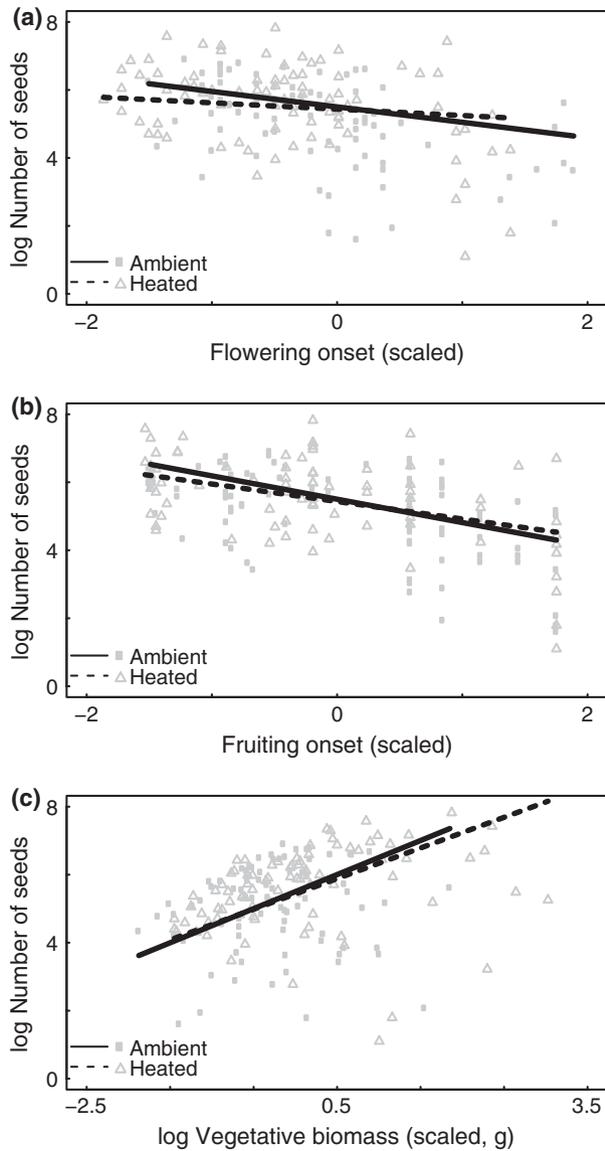


Fig. 5 Relationships between seed number and (a) flowering onset, (b) fruiting onset, and (c) the log of vegetative biomass per the partial regression coefficients obtained from the count component of the hurdle model in Table 4. Note that these relationships are linear on a log scale, and the response variable was log-transformed for ease of viewing and comparison.

cycle length can have profound consequences, including the potential to alter demographic processes (Galloway & Burgess, 2009; Zhu *et al.*, 2013), community structure or composition (Sherry *et al.*, 2007), interactions with pollinators (Elzinga *et al.*, 2007), or traits expressed in the offspring generation (Galloway & Etterson, 2007). Although seldom examined in plants, warming has elicited abbreviated periods of growth and reproduction in two arctic shrubs (Post *et al.*, 2008),

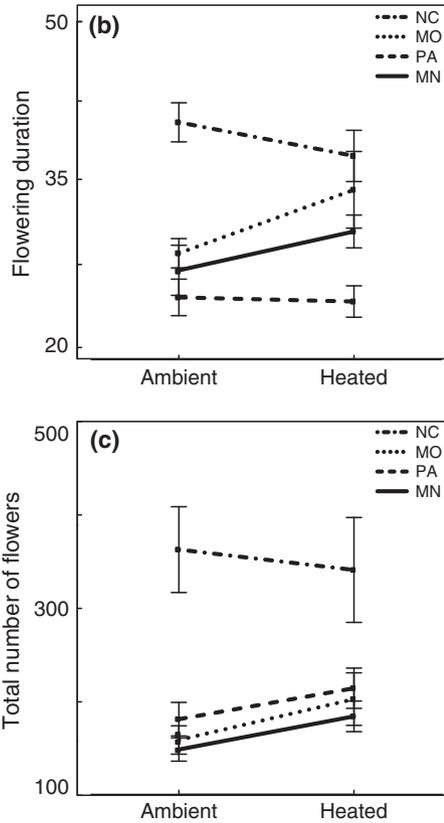
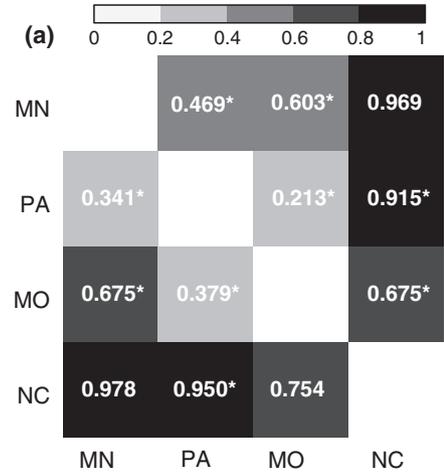


Fig. 6 (a) Estimates of the degree of temporal isolation, ρ , between populations of *C. fasciculata* in ambient (below diagonal) and artificially heated (above diagonal) conditions, and population and treatment differences in (b) average flowering duration and (c) total flower production \pm standard error. Estimates of ρ span from 0 (random mating between populations) to 1 (populations are reproductively isolated). We constructed 95% confidence intervals via bootstrapping 1000 \times with replacement. Estimates marked with an asterisk lie outside of the interval range of the corresponding population comparison in the opposing thermal regime.

a monocarpic herb (Haggerty & Galloway, 2011), and three perennial grassland species (Frei *et al.*, 2014), suggesting that this phenomenon may be more common than currently appreciated.

Plasticity in phenological traits can modify the timing of subsequently expressed phenophases (Donohue, 2002), with the potential for individual traits to shift in opposing directions (Sherry *et al.*, 2007). In *C. fasciculata*, we found that each phenological trait advanced more than the last, with the onset of fruiting displaying the greatest degree of plasticity in all populations. In herbaceous species, flowering onset date is the most commonly examined trait in studies of warming (Fitter & Fitter, 2002; Parmesan, 2006). However, our results suggest that fruiting onset can display higher sensitivity to temperature, is under stronger selection, and displays greater variation in plasticity among populations. These trends were amplified when examining phenological shifts in units of GDD. The temperature-driven responses revealed by the GDD analyses imply that the timing of life history traits is not entirely dependent on the accumulation of heat sums and that other factors, like photoperiod, may be influential in the expression of phenological traits.

Once flowering begins, plants must allocate resources between growth and reproductive functions, often producing a relationship between plant size and flowering onset date (Bolmgren & Cowan, 2008). Warming-induced shifts in flowering time may have consequences for competitive ability and reproductive capacity indirectly through their influence on size. In *C. fasciculata*, reproductive phenological traits responded to warming while final vegetative biomass and biweekly stem diameter measurements (data not shown) did not, suggesting that growth and development are not linked in this species. However, it has been demonstrated that accelerated growth in warmer conditions can compensate for the earlier onset of maturity in other species (Neuheimer & Grønkjær, 2012; Zhang *et al.*, 2012). Additionally, increased levels of carbon dioxide can differentially influence reproductive and vegetative traits (Reekie & Bazzaz, 1991), as demonstrated in *C. fasciculata* (Farnsworth & Bazzaz, 1995), and the effects of elevated CO₂ and temperature may interact synergistically to affect the expression of life history traits or allocation patterns between growth and reproduction (Morison & Lawlor, 1999).

Selection on flowering onset date and final plant size differed between thermal environments, while selection on fruiting onset was similar, demonstrating that patterns of selection may change as temperatures warm and that such changes are not always a result of plastic responses to warming. Additionally, the magnitude of direct selection imposed by warming differed among fit-

ness components. The plastic responses observed for all traits were either neutral or adaptive, and climate-related genetic variation in plasticity among populations could facilitate evolution and further bolster population performance. However, others have shown that the evolutionary responses of *C. fasciculata* to warming may be constrained by genetic correlations antagonistic to the direction of selection (Etterson & Shaw, 2001) or by low heritabilities in fitness related traits (Etterson, 2004). These previous findings should be interpreted with some caution (Bradshaw & Holzapfel, 2008); the elevated temperature regime was achieved by transplanting northern population to southern latitudes, thus confounding thermal and photoperiodic effects on the expression of loci contributing to phenology. Nevertheless, evolutionary restrictions like these may limit a species' ability to adapt in pace with a rapidly changing climate and should be considered in the decision-making criteria for assisted colonization.

Considerations for assisted colonization and assisted gene flow

Thermal and photoperiodic regimes vary by latitude. Adaptation to the local thermal and photoperiodic cycle results in a latitudinal cline in genes associated with circadian rhythms and development (Hut & Beersma, 2011). For species that flower after the summer solstice, like *C. fasciculata*, any photoperiods experienced at a particular latitude will occur later in the year in locations further north. Thus, relocating populations across latitudes could expose them to novel temperature and photoperiod combinations, which may elicit opposing developmental responses.

Here, we show that relocating populations across large spatial scales may impair attempts at assisted colonization across latitudes, although less so as temperatures warm. The northern-most population performed best, even when experiencing the thermal regimes typical of locations further south. The populations originating from intermediate latitudes displayed the highest reproductive efficiency (seeds per unit biomass) in the heated treatment. Although warmer temperatures increased seed production in the southern population by over 800%, reproductive output was still lower in comparison with all other populations. Warming-induced shifts in phenological traits were adaptive and most likely contributed to the increased fecundity seen in all populations. However, selection strongly favored early fruiting in the heated treatment despite phenological shifts, and photoperiodic constraints could limit further plasticity in life history traits.

In *C. fasciculata*, long day lengths may promote vegetative growth over reproductive development (Lee &

Hartgerink, 1986). In this experiment and in others, flowering onset for the NC population planted at KSR always occurred after August 28th, when the photoperiod in southern Ontario was 13.75 h, which is similar to the that experienced upon flowering at the NC home site on August 7th (data not shown). This occurred despite warming-induced advances in phenology (this experiment) and even when planting seeds 2 months ahead of the MN population (Wadgyamar *et al.*, 2015). This suggests that flowering onset date in *C. fasciculata* is under at least partial photoperiodic control and that the evolution of genes associated with photoperiodic responses would be necessary for successful long-term establishment of populations relocated to northern latitudes.

Scattering seed or planting individuals from multiple, distinct populations may inflate genetic variation and enhance responses to selection, but only if there is sufficient overlap in flowering periods among populations. We found almost no overlap in the flowering schedules of the northern- and southern-most population of *C. fasciculata*, with little potential for increases in mating opportunities as temperatures warm. For the northern populations, only the flowers produced during the end of the flowering season overlapped with open flowers from the NC population. As with many plants (Austen *et al.*, 2015), the probability of fruit set in *C. fasciculata* declines with later-produced flowers (Lee & Bazzaz, 1982), further reducing the likelihood that any mating opportunities will be realized between these populations. The introgression of genetic material from the southern to northern population is thus unlikely to occur naturally in the field. Efforts for assisted gene flow involving species with phenologically distinct populations may require captive breeding programs to create F2 (or later) generation hybrids in order to produce genotypes with combinations of thermal and photoperiodic responses that ensure successful establishment.

Many factors can influence the success of assisted colonization beyond those discussed here, including the availability of hosts species (Moir *et al.*, 2012) or mutualists (Keel *et al.*, 2011; Kranabetter *et al.*, 2012), novel species interactions (Hellmann *et al.*, 2012), competitive interactions (Stanton-Geddes *et al.*, 2012), or genetic constraints (Etterson & Shaw, 2001; Sheldon *et al.*, 2003; Both & Visser, 2005). Plants are at their most vulnerable when in the seedling stage, as we encountered with our attempt to plant this experiment from seed. As relocations will likely be carried out with seeds, emergence rates and seedling survival may increase if seeds are pre-treated (e.g. stratified, scarified, inoculated, etc.) prior to planting (McLane & Aitken, 2012). A lack of compatible rhizobia prevented individuals of *C. fasciculata* from

establishing beyond its northwestern range edge, and inoculations with known strains improved emergence and growth (Stanton-Geddes & Anderson, 2011). While we did not find such limitations in this experiment, examinations of factors influencing the ecology and evolution of range limits may further reveal the circumstances under which relocations are likely to succeed.

As the climate continues to warm, assisted colonization may prove to be a viable adaptation strategy to alleviate risks of extinction or decreased productivity. However, we have revealed several underappreciated complications in its implementation. For immediate relocations, populations originating from near the current range boundary may fare best, while those from too far within the range may do poorly, even in the future thermal regimes of the newly established site. Plasticity will likely make a strong contribution to initial survival and establishment, but the long-term success of adaptive relocations may ultimately depend on the capacity for adaptive evolution in the newly founded population, particularly when reproductive phenologies do not match the photoperiodic conditions of the receiving habitat. Additionally, among-population differences in flowering phenology may limit the potential for assisted gene flow in the field. With strategic planning, our results suggest that assisted colonization and assisted gene flow may be feasible options for preservation.

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