

Examining the Comparative Fitness of the American Bellflower (*Campanulastrum americanum*)
in a Novel Environment.

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Abstract

Global climate change has altered habitat suitability for many species. I examined populations of American Bellflower (*Campanulastrum americanum*) from throughout their range to determine if populations have the ability to adapt to a novel environment. The comparative study was undertaken spring-fall 2014 at Pierce Cedar Creek Institute. Specifically, to determine if populations are able to persist in a novel environment, I examined the survival and reproductive success of plants. To understand the mechanisms affecting persistence, I noted phenological traits, pollinator visitation, and pollinator identities. In addition, I quantified selection on flowering phenology by pollinators in the novel environment. The phenology data showed no difference in days to bolt among populations and also no difference in days to fruit maturation. However, floral phenology displayed differences in the days to first flower and displayed differences correlated with latitude. The fitness results showed lower fecundity for populations in the south as opposed to populations from the central and northern parts of the range. It seems that for pollination, the number of visitations a population receives is related to floral phenology, with most visitations occurring on plants that flowered within a certain timeframe. This study is useful to understand how this species will persist and adapt as local habitats change due to global climate change.

Introduction

The global climate is changing at an unprecedented rate effects strong selective pressure on populations (Freer-Smith et al. 2007, Bertin 2008). Numerous studies have quantified selective pressures on plant populations and shown plants ability to adapt (Kang 1989, Mitton 1997). Some populations will not be able to adapt in time to a changing environment, while others may adapt (Cleland et al. 2007). If genetic variation exists within a population then natural selection will favor those individuals in a population that survive better and are more fecund in the new climate. Climate change has affected plant species distributions in that plants move to a more suitable habitat (Cleland 2007, Freer-Smith et al. 2007). Using alpine and nival (growing under-snow) plants, Engler showed that habitat suitability is temperature dependent and as temperatures rise the area of suitable habitat decreases significantly (Guisan and Theutillat 2000; Engler et al. 2008). The species distribution model predicted that over 40% of species were to lose ~90% of their suitable habitat, resulting in 40-50% of plant species becoming extinct due to the increased temperatures and inability to effectively move (Engler et al. 2008). In a separate study conducted by Kelly and Goulden, the upward shift in temperature, snow-line, frost free-period, plus a severe drought made it more difficult for plants to survive in the warmer, dryer areas of its range (Kelly and Goulden 2008). Vegetative shifts were observed in numerous species of different growth forms and life history types that favored wetter, cooler, upper portion of its range (Kelly and Goulden, 2008)

The ability of a plant species to establish in a new location depends on various traits: pollinator availability, self-pollination ability, and reproductive rate among others (Caplet et al. 2013). Upon moving to a new location, suitable pollinators may not be present (Berg et al. 2010), which results in a strong-selective pressure to attract novel pollinators or increase the degree of self-compatibility (Barret et al. 2008). Higher reproduction rate provides for a greater number of individuals with the ability to extend towards a new location (Lenior et al. 2008; Caplat et al. 2013). Plants exhibit expedited life

cycles as a method of producing more offspring. If two plants produce the same net number of offspring per generation, but one plant has shorter periods between generations than plants it will create more individuals. For this reason it was found that plants with expedited life cycles (less time between generations) migrated to higher elevations because of climate change faster than plants with slow life cycles (more time between generations; Caplat et al. 2013).

Plant adaptations often occur through phenology (Cleland 2007). Phenology of plants at various locations can be related to its fitness throughout its respective range. Meaning, a specific phenology could be beneficial (improve fitness) in one location and harmful (lower fitness) in another location. In populations of *Campanulastrum americanum* phenology varies across the range with northern populations germinating and maturing fruits faster in a controlled environment in comparison to southern populations. These faster phenological traits are likely adaptive as the growing season is shorter with less precipitation in northern populations in comparison to southern populations (Prendeville et al. 2013). The American Bellflower's range is large covering approximately 13 degrees of latitude and around 18 degrees of longitude, which includes most of the United States Midwest region. A large range has allowed for different populations to adapt different phenology based on each population's location. Many populations' local adaptations may hinder them from persisting in a novel environment with a shorter growing season and less precipitation because they may not develop fruits in time in short season or may dry out without a certain amount of rain. *Campanulastrum americanum* can grow as a biannual or annual depending on when seeds germinate. This difference can affect the fitness of the individual because, depending on the region, it may be advantageous for an individual to be of a certain life history (Prendeville et al. 2013). Seeds that germinate in the fall become annuals, getting vernalized in the winter and then bolting and flowering in the spring/summer. The seeds that germinate in the spring become biannuals, growing as rosettes during the summer and getting

vernalized the following winter before bolting and flowering during the next growing season (Prendeville et al. 2013).

Many species are global climate change winners, meaning they have a suitable habitat readily available as climate change progresses or they are able to adapt to fit their new environment accordingly. Thus, to determine *C. americanum* populations have the ability to adapt to a novel location I addressed the following questions: 1) Does phenology—specifically, bolting date, date of first flower, and days to fruit maturation— differ among populations? 2) Does survival and fitness differ among populations? To understand the limiting factor on plant population establishment, I addressed the following question: 3) Does selection on floral phenology differ among populations? Michigan is on the northern edge of the *Campanulastrum americanum*'s range and has both lower temperatures and precipitation than southern portion of the range (Prendeville et Al. 2013). I predict that populations that are adapted to warmer temperatures and higher rainfall amounts will be less fit in the cooler, dryer climate of Michigan.

Methods

To examine *Campanulastrum americanum*'s (American Bellflower) ability to adapt to a novel environment, 26 native populations throughout the range (Figure 1) were grown from seed in a greenhouse at the University of Virginia. Five weeks after germination, rosettes were vernalized for seven weeks at 5C. Using a random block design, 30 replicates of each population were transplanted into a common garden at Pierce Cedar Creek Institute (PCCI) in Delton, Michigan. The location or “planting block” was taken into consideration of a possible effect on phenology or overall fitness. Michigan is on the northern range edge of the American Bellflower (Figure 1). The plants were placed within fenced plots to protect them against mammalian herbivory. The plants were also treated once a week with *Slug Magic*, starting when planted and ending when the plants were big enough to resist the

slugs themselves (June 27). They were also periodically treated with Monterey B.t. an organic insecticide to ward of caterpillars.

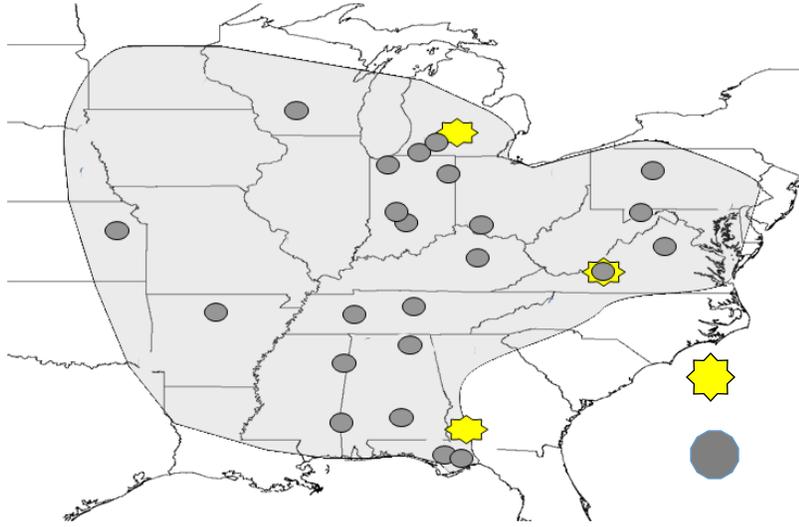


Figure 1: Locations of native populations and common gardens used in the experiment. Yellow stars represent a common garden, light grey shading represents the range, and dark grey circles represent a native population location.

Phenology and fitness

To determine if populations differed in phenological traits, bolting (growth of a stalk) was scored once a week, day of first flower once every three days, and days to fruit maturation once per week. Fitness was measured through rosette and adult survival, fruit number per plant, and number of seeds per fruit per plant. Fruit maturation was observed and scored in a uniform area on every plant that included

four nodes on the flowering stem, which included the first flowering node on the main stem and three above it. Two fruits just prior to maturation were collected from each plant to estimate seeds per fruit. Fruits were collected outside the observation area for fruit maturation phenology to not affect the

Latitude	Pop
30.564683	FL83
30.800483	FL42
31.74407	MS55
31.91573	AL69
32.929317	AL79
34.00155	MS70
34.487222	GA22
34.65048	AL29
35.76525	TN19
35.8595	AR58
36.08207	TN34
37.35495	VA73
37.89993	KY51
38.10725	IN77
38.38052	VA71
39.06617	KS60
39.13838	OH11
39.3355	IN68
39.550133	IN21
39.61382	MD5
41.00795	PA27
41.02975	IN37
41.59265	IN28
41.862917	MI43
42.30103	MI44
43.35098	WI14

phenology data. Fruits were collected when the calyx was enlarged and light green to yellow in color, but prior to pores opening and seed dispersal.

To determine if phenological and fitness traits differed among populations, we used a generalized linear model with population as a fixed effect and planting block as a random effect (PROC GLIMMIX, SAS 2010). For bolting date, date of first flower, and days to fruit maturation, as well as fruits per plant, we used a negative binomial distribution, and for seeds per fruit, we used a Gaussian distribution.

Selection on floral phenology

To determine if selection on floral phenology differs among populations, I observed the number of pollinator visits and identity of pollinator (to genus). Pollinator observations were conducted five days per week, for one hour per day (weather permitting). I noted if flower stigmas visited by pollinators were open and available to receive pollen. I observed five rows of plants at a time for ten minutes before moving on to the next five rows. An alternative method was also employed where individual pollinators were followed from plant to plant and similar data recorded. Every plant was observed five times using either method. Using a generalized linear model with a negative binomial distribution, I examined the fixed population and the random effects of planting block, and observation day on the number of pollinator visits.

Results

Phenology

Phenology was examined as the mechanism by which *C. americanum* may adapt to a novel climatic environment. We found that almost all populations bolted around May 22 or day 70 (Figure 3); populations of *C. americanum* did not differ statistically in days to bolt ($F_{25, 680}=0$, $P=1.000$) or any difference among blocks ($F_{1,680}=0.03$, $P=0.8704$). Furthermore, there was no statistical evidence of differences over a latitudinal cline for populations ($F_{1,704}=0.01$, $P=0.9287$) or blocks ($F_{1,704}=0.02$, $P=0.8753$)

Populations of *C. americanum* did show differences in flowering phenology among populations ($F_{23,485}=46.28$, $P<0.0001$). The planting block was also found to have a statistical difference between populations ($F_{23,485}=15.66$, $P<0.0001$). Each population displayed a certain flowering phenology and this difference was also found to be correlated with latitude ($F_{1,507}=26.14$, $P<0.0001$). The planting block showed no difference in flowering phenology when latitude was taken into account ($F_{1,507}=2.49$, $P=0.1150$). In addition the plants from southern populations took far longer to flower than those from populations in the north (Figure 4). The growth rates of plants after bolting were found to have no difference between populations (Figure 6).

Survival and Fitness

Survivorship did not differ among populations ($F_{25,745}=0.67$, $P=0.8860$), there was also no difference in planting block between populations ($F_{1,745}=1.71$, $P=0.1908$). Survivorship over a latitudinal cline was also found to have no difference ($F_{1,769}=0.79$, $P=0.3338$) nor the planting blocks ($F_{1,769}=1.67$, $P=0.1964$). We found that the southern populations had a lower fecundity than the northern populations, producing fewer fruits than the northern and central populations. While some of

the central populations matured more fruits than northern populations, it is apparent that southern populations fecundity is far lower than both northern and central populations. (Figure 7).

Pollinator Availability

It was found that plants from the northern and central populations received more visitations than those from the south (Figure 8). The plants that flowered within a certain timeframe, had optimal pollinator availability and received the most visitations. Those that flowered early or late did not receive as many visitations (Figure 9)

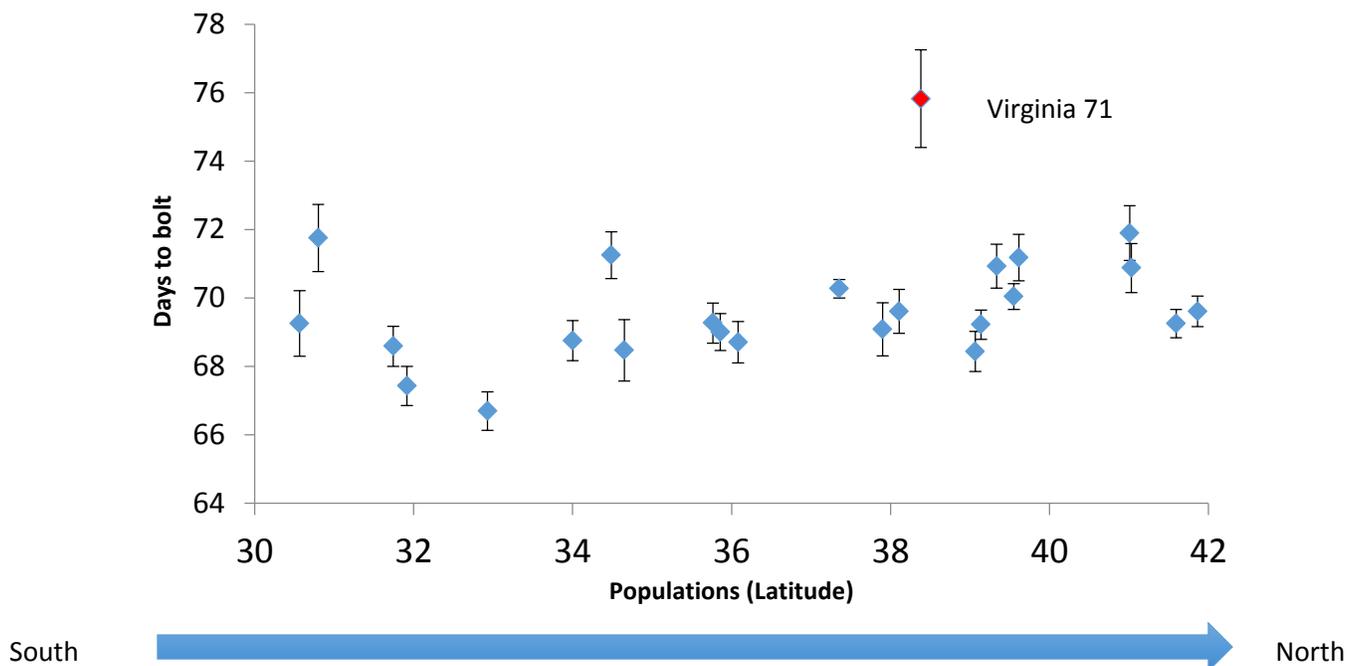


Figure 3: Plants from 24 distinct populations do not differ in mean number of days to bolting. Sample size is 30 plants per population, bars display standard error. Note the y axis starts at 64 days. Virginia 71 is in red and is the only population that showed any variance (not statistical).

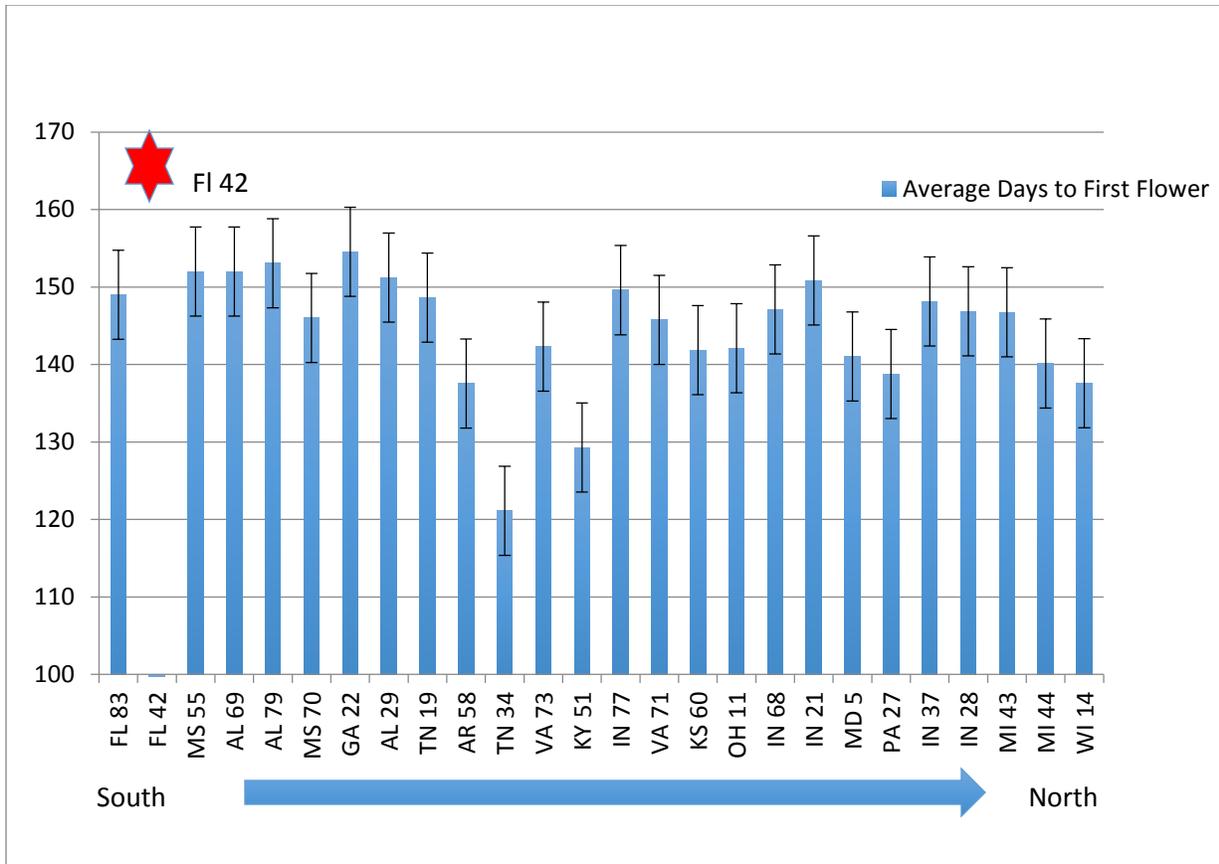


Figure 4: Displays days to first flower from April 14, 2014 for 26 populations from the entire range.

There is no difference over a latitudinal cline from south to north. Note FL 42 has not flowered yet and is denoted with a red star. It should be noted that TN 34 flowered before all other populations, and is a good example of population adaptation because it's native population is located on limestone, which drives quick flowering to avoid desiccation.

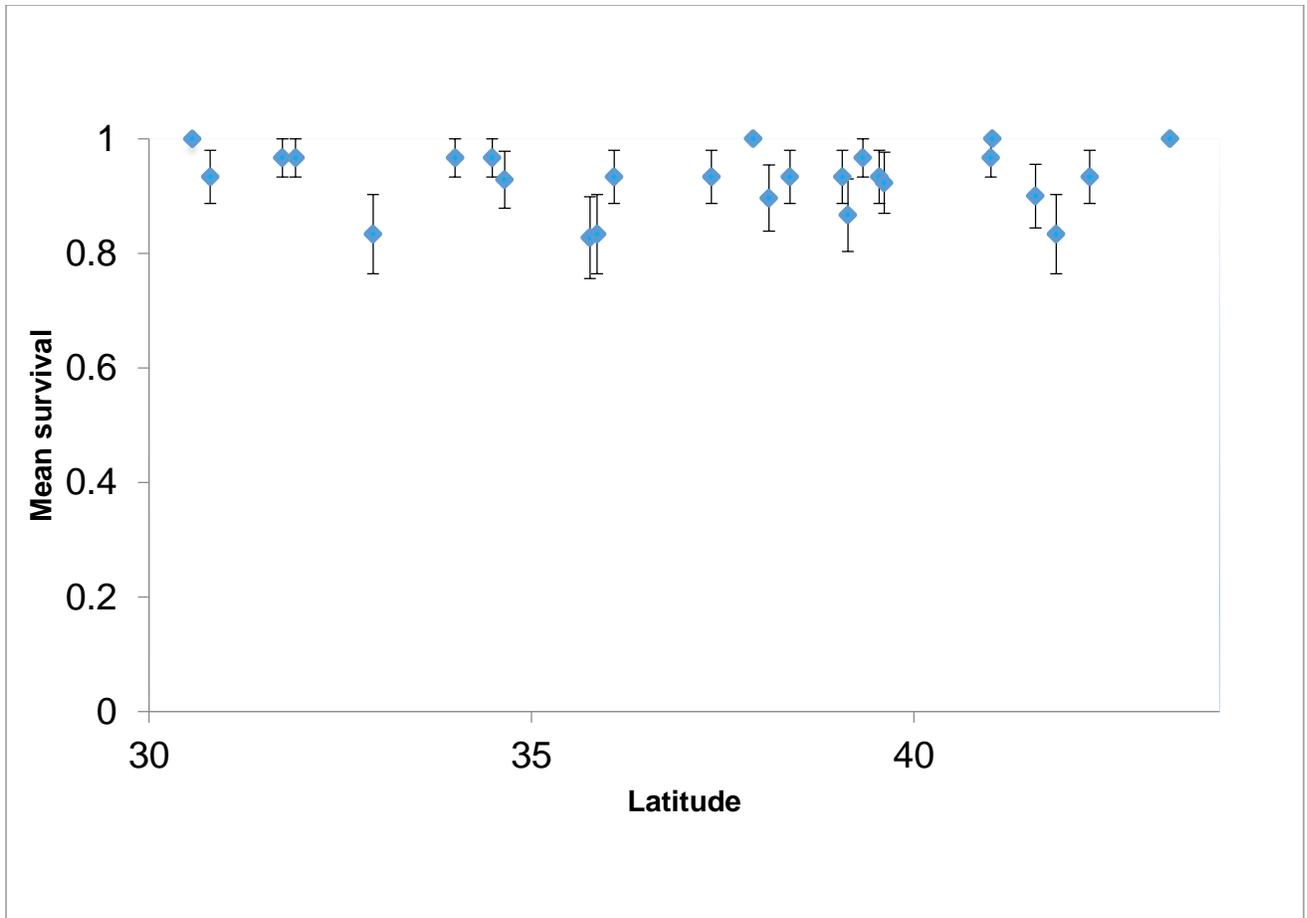


Figure 5: Survival data from each population shows no difference in survival between populations or any latitudinal pattern of any kind. Each bar represents the average for each population which consists of 29 or 30 plants.

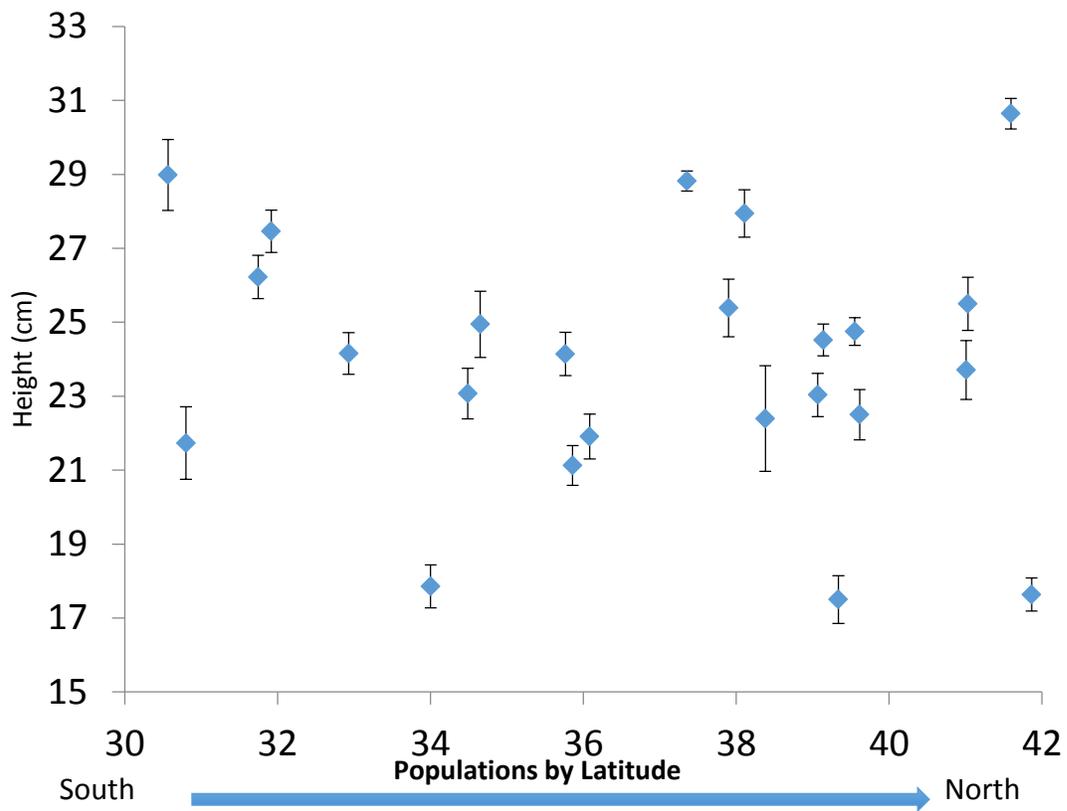


Figure 6: Growth rates for all populations had no difference, when all measured on the same day (June 15) Each population consists of 29 or 30 plants that are averaged and the bars are standard deviations for each averaged population.

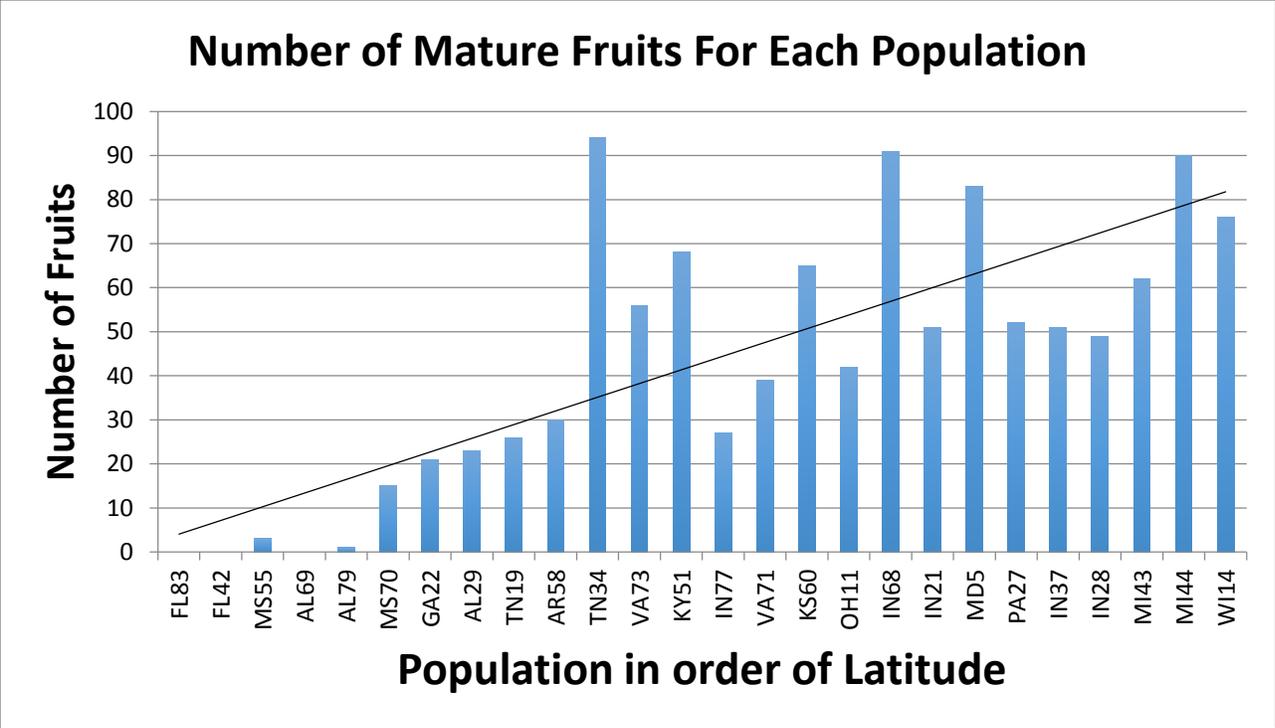


Figure 7: Displays a difference in the amount of mature fruits over a latitudinal cline. Southern plants produce less mature fruits and northern and range center plants produce more mature fruits.

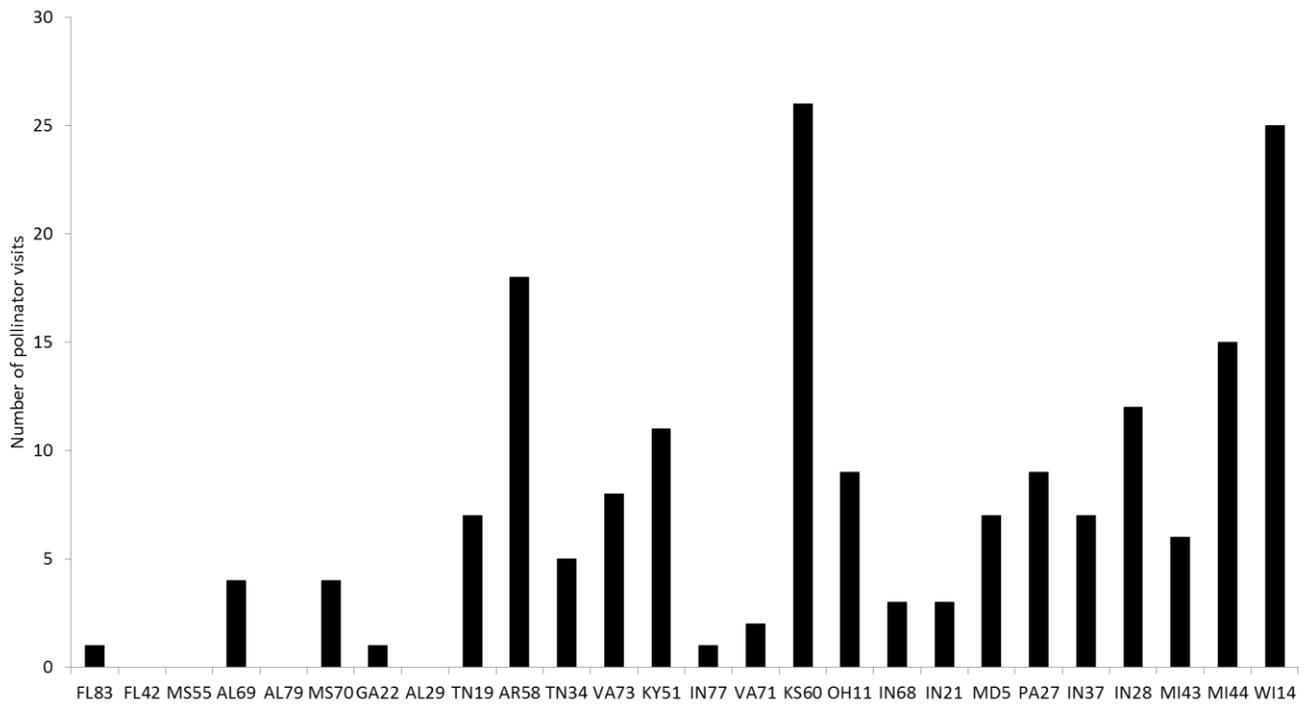


Figure 8: Displays pollinator visitations for each population over a latitudinal cline. There seemed to be a preference for both Wisconsin and Kansas populations.

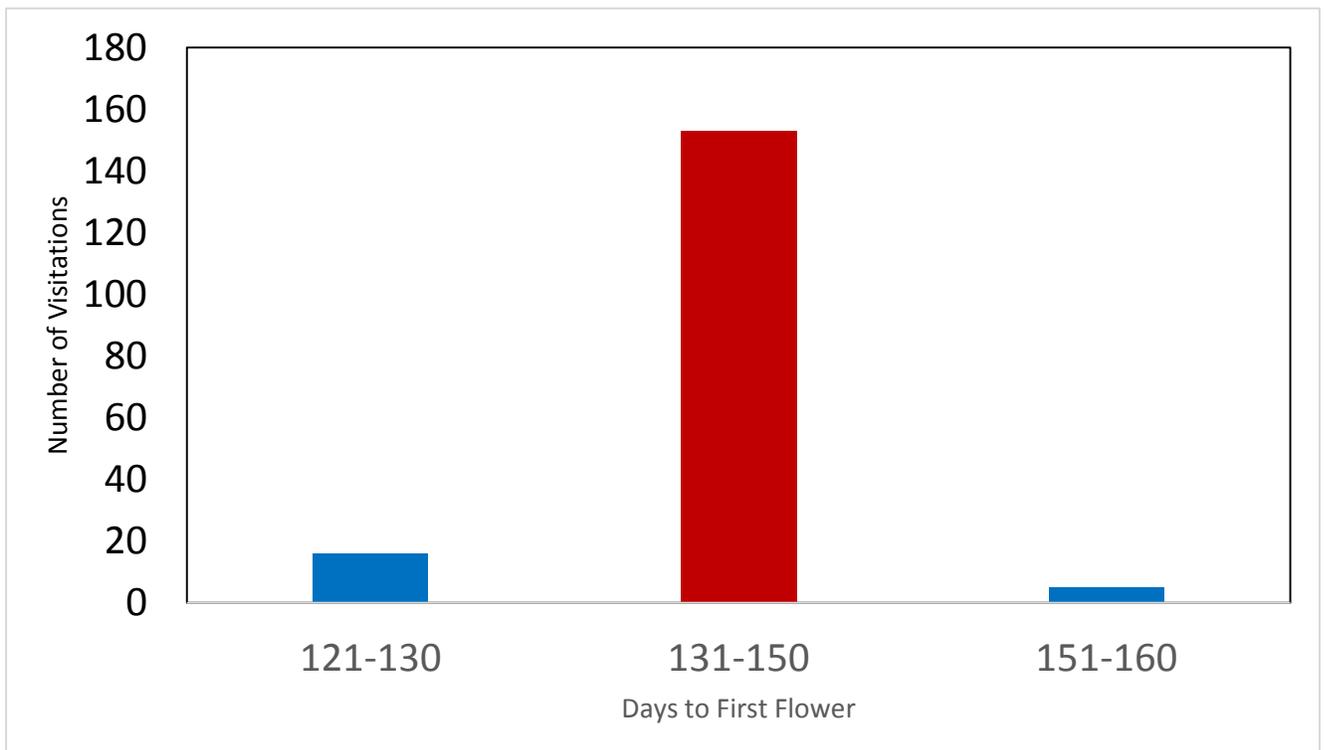


Figure 9: Displays the highly increased number of pollinator visits for populations that flowered in 131-150 days compared to flowering earlier or later.

Discussion

Phenology

Phenology in *Campanulastrum americanum* has been noted to be adaptive in individual populations from across its range (Prendeville et. al 2013). When each population was analyzed in novel environment populations of *C. americanum* showed very little difference across a latitudinal cline while maintaining some of their specific phenology adaptations. Populations all bolted at the same time, flowered according to their unique population and developed fruits all in approximately a month after flowering.

The plants bolting at the same time is an interesting find. This means that any phenology adaptation that occurs in the American Bellflower is not related to bolting in Michigan. I originally thought that each population could have its own particular strategy by way of bolting. I thought that northern latitudes would have to bolt earlier to counteract the short growing season, while southern latitudes would need to bolt later due to the longer growing season in the south. The results showed that regardless of the population most *C. americanum* bolted within a week window around the third week of May. This begs the question, why did every population bolt at the same time? The answer could possibly lay in environmental cues. Anything from temperature, photoperiod to precipitation could all be affecting when plants bolt (Purves et al. 2004). Perhaps in Michigan all populations of *C. americanum* experienced their particular environmental cue at the same time, The large

difference in photoperiod across the latitudinal cline (Florida to Michigan) leads me to believe that this could be the reason for uniform bolting amongst varying populations. There are “three main photoperiod response types: short-day plants (SDP) in which the response is induced when the photoperiod is shorter than the critical daylength (CDL); long-day plants (LDP) in which the response is induced when the photoperiod exceeds the CDL; and day-neutral plants (DNP) which do not respond to photoperiod (Jackson, 2008).” For example, *Xanthium strumarium* has a long CDL (15.5 h), while *Lolium perenne* and *Lolium temulentum* have low CDLs (9 hrs). Michigan has longer days than most other populations, meaning the American Bellflower could be a LDP in response to photoperiod. Since all populations were placed in the same location at the same time it could suggest that the plants all experienced “the American Bellflower’s” photoperiod cue for bolting, simultaneously. Photoperiod and other possible environmental cues should be further examined within the American Bellflower to pin point exactly what caused uniform bolting in this experiment.

The flowering phenology of the various populations indicated more phenological adaptation potential than both bolting and fruit maturation traits. Each population seemed to have its own specific timing for when it flowered. In addition there was a statistical correlation between latitude and flowering phenology. The Tennessee 34 population flowered before any other population and all of them flowered within about a week. While its neighbor, Tennessee 19 did not flower until late August and once more did so in a synchronized fashion. The population adaptations through phenology seemed to be linked to the specific site more than the population’s latitude. The population TN 34 is on a limestone bed, this is most likely the reason for their quick flowering adaptation. If they do not flower and develop fruits quickly the

plants will have a fitness of zero because they will dry out and die before they can develop fruits (Figure 4). In addition there was a statistical correlation between latitude and flowering phenology. This means that from south to north the amount of time it took for a population to flower decreased. The reason for this is almost certainly, the longer growing season and the increased precipitation in the south. Although, the range center populations seem to have flowering dates close to many northern populations, on a whole the days to flower do differ with population latitude. The extremes from north to south is interesting because it shows the dynamic ability that plants even within the same species possess. These differences in flowering phenology may be a mechanism for the American Bellflower to adapt to a novel environment as global climate change progresses.

Fruit maturation showed very little variance among populations. All populations developed mature fruits in about a month. With both fruit maturation and bolting having no phenological variance when planted in Michigan, it can be assumed that any variance in phenology must be attributed to flowering phenology. The results showing no variance in either bolting or fruit maturation show a potential limitation of the American Bellflower. With the only phenology mechanism available for local adaptation being floral, becomes more difficult for the American Bellflower to persist or adapt to a novel or changing environment.

Survival and Fitness

We predicted that there would be a much lower survivorship in the southern populations due to the climate differences (i.e. precipitation, temperature, photoperiod) in the south in comparison to the north. Statistically, there was no difference among populations in

survival. The transplanted *C. americanum* were able to tolerate the foreign climate well enough to survive. However, plants from the southern populations on the range edge were reduced. Plants from Florida might not have survived as well because of the decrease in temperature and precipitation that comes with higher latitudes. However, this slight reduction in survival could also have been due to a difference in soil chemistry, a Michigan nematode or even a fungus native to Michigan (Wilson, 2009) A separate experiment would need to be conducted to understand the reason for diminished range edge survival.

Fitness was affected by the latitudinal cline. The range edge in Michigan housing these 26 unique populations showed great difference in number of mature fruits per plant. The populations that flowered later (generally southern populations) had less time to develop fruits and as a result were unable to produce as much fruit as the range center and higher latitude populations. It seems that even if survival is not a problem, reproduction could be challenging for a population in a novel environment. When moving to a new location, the adaptation that almost certainly helped at their native location could harm the population significantly in a new environment. For example, flowering in Florida populations occurs late; benefiting the population because the long growing season in their native location allows for larger, taller, healthier plants with plenty of time to still mature fruits after flowering. However, when placed on the northern range edge the adaptation to flower late harms their fitness greatly, because the short growing season will not leave enough time for fruits to mature. Almost all populations were able to survive and flower, but even though southern populations flowered, the stress of the climate and the late flowering date caused lowered fecundity.

Pollinator Availability

We found that that pollinators have no specific population preference. In addition to the quantitative data collected, observations using the secondary method-- following individual pollinators- revealed no preference of pollinators amongst populations. Each pollinator would fly down each row going from flower to flower regardless of the population. Efficiency of the pollinator seemed to be the main concern of each individual. The data reflects very low pollination in southern populations, this could lead one to believe pollinators purposefully skipped southern plants. However, pollinator availability is strongly linked to floral phenology. This is important because many southern plants floral phenology causes them to flower very late, or never, in the novel Michigan environment. This fact transitively affects the amount of pollinators available to the populations that flower late or never flower at all. When answering the question, is there a difference among populations in pollinator availability? The answer, would be almost certainly. In regards to plant population establishment, though pollinators do not seem to prefer certain populations, natural selection may select upon populations whose floral phenology allows for them to expand to a novel environment with a pollinator community readily available.

Implications

This study is useful in understanding plant population establishment due to global climate change. The bolting data collected showed that latitude can alter phenology from many populations. The evidence could mean phenology may be a viable mechanism for plant adaptation to an ever changing climate. In addition, populations all having unique flowering times reinforces the notion that phenology may be used by plants to adapt to their specific

environment. In terms of survival this study's data reflect plants' ability to move across a range and tolerate the new climate enough to survive. While a mechanism of dispersal was not tested the ability of plants to survive in a new location once they arrive showed that survival is possible in a novel environment. More studies should look particularly at *C. americanum's* dispersal ability and the mechanisms by which it may arrive in a new location. "Gaining basic dispersal information is a critical step for understanding species' geographical distributions and for predicting the likely impacts of future climate change (Thomson, Fiona J., et al., 2010)" and is something that this study has not covered adequately. According to our study plants have the ability to cope with a much different climate allowing movement to a new location possible. However, having the ability to survive in a new location and persist there are two different things. For plants to persist populations fitness comes into question. Fitness showed definite differences in populations across a latitudinal cline. Therefore, some plants may be able to move to a new location but the challenge of reproduction could still cause extinction due to climate change. Another factor in a plants ability to move to a more suitable habitat is pollinator availability. This study found that a population's adapted floral phenology (flower timing) may deter certain plant populations from moving while aiding others. Other plant species should be studied in relation to pollinator availability and plant population establishment.

Global Climate change affects many plants in many different ways. This study and studies like it help to understand more about specific plants and the trends that are found amongst all plants, which in turn, can help craft a plan to deal with the altering climate in particular species. *Campanulastrum americanum* may be able to use the flexibility found within

floral phenology as an adaptive tool to changing climate. The American Bellflower seems to only possess one avenue for adaptation through phenology. However, other plants may be able to adapt using more than just floral phenology and in turn improve their species fitness as global climate change proceeds.

Works Cited

Barret, Spencer C.H., Colautti, Robert I., Eckert, Christopher G. 2008. *Plant reproductive systems and evolution during biological invasion*. *Molecular Ecology* 17 (1): 373-383.

Berg et al. 2010

Bertin, Robert I. 2008. *Plant Phenology and Distribution in Relation to Recent Climate Change*. *Journal of the Torrey Botanical Society* 135 (1): 126-142.

Broadmeadow, Mark S.J. Freer-Smith, Peter H. Lynch, J.M. *Forestry and climate change*. Wallingford: CAB International, 2007.

Caplat, P., Cheptou, P.O., Diez, J., Guisan, A., Larson, B.M.H., Macdougall, A.S., Peltzer, D.A., Richardson, D.M., Shea, K., Van Kleunen, M., Zhang, R., Buckley, Y.M. 2013. *Movement, impacts and management of plant distributions in response to climate change: insights from invasions*. *Oikos* 122: 1265–1274.

Cleland, E.E., Chuine, I., Menzel, A., Mooney, H.A. & Schwartz, M.D. (2007). *Shifting plant phenology in response to global change*. *Trends in Ecology and Evolution* 22: 357-365.

Engler, R., Guisan, A, Normand, S., Pearman, P.B., Randin, C.F., Thuiller,

W., Vittozs, P., Zappa, M., Zimmermann, N.E. 2009. *Climate change and plant distribution: local models predict high-elevation persistence*. *Global change biology* 15: 1557-1569

Freer-Smith et al. 2007

Guisan and Theutillat 2000;

Jackson, S. D. (2009), Plant responses to photoperiod. *New Phytologist*, 181: 517–531.

doi: 10.1111/j.1469-8137.2008.02681.x

Kelly, A.E. and Goulden, M.L. *Rapid shifts in plant distribution with recent climate change*. 2008
PNAS 105 (33) 11823-11826.

Lenior et al. 2008

Prendeville et al. 2013

Purves, William K., Sadava, David., Orians, Gordon H. , Heller, Craig. *Life Part 6: The Biology of Flowering Plants (7th ed.)* New York, NY: Macmillan.

Sherry, Rebecca A., Xuhui Zhou, Gu, Shiliang, Arnone, John A. III, Schimel, David S., Verburg, Paul S., Wallace, Linda L. *Divergence of reproductive phenology under climate warming*. *Yiqi Luo Proc Natl Acad Sci U S A*. 2007 January 2; 104(1): 198–202. Published online 2006 December 20.
doi: 10.1073/pnas.0605642104 PMID: PMC1713188

Thomson, Fiona J., et al. *Chasing the Unknown: Predicting Seed Dispersal Mechanisms from Plant Traits*. *Journal of Ecology* 98.6 (2010): 1310-8. ProQuest. Web. 29 Oct. 2014.

Wilson, Michael J, Kakouli-Duarte, Thomais, ebrary, Inc. (2009). *Nematodes as environmental indicators*. Wallingford, Oxfordshire: CABI.