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Latitudinal Gradient Facilitates Adaptive Population Differentiation of Campanulastrum americanum (American Bellflower)

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LATITUDINAL GRADIENT FACILITATES ADAPTIVE POPULATION DIFFERENTIATION OF CAMPANULASTRUM AMERICANUM (AMERICAN BELLFLOWER)

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The College of Letters and Sciences

The Graduate Program in Natural Sciences (Biology Track)

Latitudinal Gradient Facilitates Adaptive Population Differentiation of

Campanulastrum americanum (American Bellflower)

A Thesis in

Natural Science

By

Marisa L. Naciuk

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I have submitted this thesis in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

Local adaptation is characterized by higher reproductive fitness by individuals present in their native habitats relative to nonlocals. This study utilized *Campanulastrum americanum* as a model for assessing whether an environmental gradient can facilitate local adaptation. In particular, I determined if a latitudinal gradient across eastern United States has influenced the phenology and reproductive fitness of five northern and four southern *C. americanum* populations. These populations were reciprocally transplanted into two common garden sites positioned in the most northern and southern extent of the species distribution: Hastings, MI and Columbus, GA. I observed adaptive population differentiation of northern and southern populations. Both reproductive and phenological traits of plants of southern origin were differentiated from plants of northern origins and under selection. There was also significant selection towards earlier flowering initiation. Earlier flowering, along with faster bolting rate and delayed fruit maturation has assisted in maximizing reproductive fitness of southern populations in the south. While, earlier flowering, delayed fruit maturation, and delayed bolting has facilitated higher fitness of northern populations in the northern part of the species range. This study demonstrates that selection may occur across a species distribution, creating locally adapted populations.

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INTRODUCTION

Local adaptation has an influential role in maintenance of genetic and phenotypic diversity, facilitating ecological speciation, and expanding species ranges (Levene 1953; Felsentein 1976; Hendrick 1986; Kirpatrick & Barton 1997; Tiffin & Ross-Ibarra 2014). It is characterized by a higher level of Darwinian fitness expressed by individuals present in their native habitats relative to foreigners (William 1966). The occurrence of local adaptation is dependent on the interactions between evolutionary forces (ie: selection, gene flow, drift, and mutations), and the presence of heterogeneous environmental conditions that create variation in selection pressures (Blanquart *et al.* 2013). Studies have utilized geographical variations, such as latitudinal clines to address how local adaptation can relate to environmental variation (Mitchell-Old *et al.* 2007; Svetec *et al.* 2015).

Environmental factors can differ throughout a species distribution, creating spatial heterogeneity among populations; thereby exerting selective pressures that can drive divergent changes in population genetic structure among populations (Hunter 2006; Coop *et al.* 2010; Eckert *et al.* 2010; Tanja *et al.* 2013). These changes reflect adaptive gene complexes that confer benefits (higher fitness) for a phenotype in particular set of environmental conditions (Levene 1953; Felsentein 1976; Hedrick *et al.* 1976; Hedrick 1986; Galloway & Fenster 2000; Kawecki & Ebert 2004; Riis *et al.* 2010; Anderson *et al.* 2011; Luquet *et al.* 2015). Overtime, the frequency of these changes accumulates, forming genetically and physiologically distinct populations (Hunter 2006). Thus, environmental variation has major implications on genetic and subsequent phenotypic differentiation of organisms (Miller & Fowler 1994; Ward *et al.* 2012). Phenotypic

variation due to underlying genetic differences among individuals can serve as evidence of populations adapting to their local environments (Clausen *et al.* 1940; Linhart & Grant 1996; Miaud & Merila 2001; Garcia *et al.* 2007; Feder & Nosil 2010; Blanquart *et al.* 2012; LeCorre & Kremer 2012).

Phenotypic variation can also be a by-product of differences in genotype expression in response to environmental heterogeneity, also referred to as phenotypic plasticity (Scheiner 1993; Scheiner & Lyman 1991; Agrawal 2001; Pigliucci 2005; Gratani 2014). The advantage of plasticity is that multiple phenotypes can be expressed depending on the environment, allowing organisms to deal with unpredictability (Fordyce 2006; Ghalambor *et al.* 2007; Fusco & Minelli 2010; Scheiner 2014). Most importantly, it provides a variety of phenotypes for natural selection to act upon (Lazzaro *et al.* 2008). Phenotypic plasticity becomes adaptive when a phenotype confers higher fitness within a particular environment that has no associated genetic differentiation (Kawecki & Ebert 2004; Kleunen & Fischer 2005; Volis *et al.* 2005; Beldade *et al.* 2011; Ward *et al.* 2011). The environment drives genetic changes in populations that promote adaptation as well as promote variable gene expression that has the potential to become adaptive as well.

Genetic differentiation of populations may also reflect coordination of phenology with their environment to maximize their fitness (Rathcke & Lacey 1985; Reeekie & Bazzaz 1987; Kozlowski 1992; Levin 2006). Organisms, such as plants have some indication of conditions present in their environment by relying on specific environmental elements as cues such as temperature and amount of light (Karban 2008). Growth and reproduction phenology is in accordance with these cues to ensure that reproduction occurs in the most appropriate set of environmental conditions. In the case

of a plant, flowering, fruiting and seed dispersal are key aspects of reproduction, and a vital determinant of an organism's fitness (Kim *et al.* 2009).

Gene x environment interaction not only facilitates the occurrence of local adaptation (Kawecki & Ebert 2004), but also influences the fitness of hybrids between locally adapted populations. Hybrids may have fitness higher than either parent (hybrid vigor or heterosis) or their fitness can be lower, outbreeding depression (Darwin 1867; Dobzhansky 1951; Lynch 1991; Muller 1942; Arnold *et al.* 1999; Whitlock *et al.* 2000; Truelli et al. 2001; Lippman & Zamir 2007; Orr 1995; Willet 2012). In many plant species, the positive effects of heterozygosity on fitness are usually observed during the F1 generation, due to overdominance or masking of recessive deleterious alleles (Waser & Price 1994; Fenster & Galloway 2000; Waser *et al.* 2000; Willi & Van Buskirk 2005). Outbreeding depression is often more evident in later generations due to epistatic incompatibility (Levin 1978; Lynch 1991; Edmands 2007). This form of genetic incompatibility is described as a reproductive failure, but it is formally referred to as hybrid breakdown (Oka *et al.* 2004; Yasumoto & Yahara 2008; Burton *et al.* 2013). Dobzhansky-Muller model offers an explanation of how and why this genetic incompatibility arises within later generations (Orr 1995; Coyne & Orr 2004; Fitzpatrick 2008). When populations are isolated, some mutations are favored by natural selection and increase in frequency. When populations mate, these mutations interact during recombination and contribute to a reduction in offspring fitness (Felsenstein 1974; Muller 1964; Edmands 2007).

The American Bellflower (*Campanulastrum americanum* (L.) Small is a plant species with a wide distribution and exhibits diverse morphological and phenotypic traits

(Galloway & Etterson 2005; Etterson *et al.* 2007). This makes this species an ideal model for assessing the relationship between geographic (latitude) and phenotypic variation, and if phenotypic variations indicate adaptive population differentiation. Given the potential for adaptation to occur across its range facilitated by differences in environmental conditions, latitudinal variation in phenological and reproductive traits can serve as lines of evidence of local adaptation for this species. Variation in flowering time has been observed within and among populations of *C. americanum* (Burgess *et al.* 2007; Galloway & Burgess 2009). This variation has likely evolved in accordance to cues present in their local environment that reduces the risk of floral damage and incomplete seed development, and increases the availability of potential mates (Anderson *et al.* 2011).

The overall goal of my study is to determine if environmental differences that reside along a latitudinal gradient has facilitated local adaptation of *C. americanum* populations. The four specific objectives of my study are as follows: 1) Assess if populations of *C. americanum* are locally adapted by comparing reproductive traits across common garden sites, 2) Assess if phenological traits vary across sites and if they are adaptive, 3) To determine if variations in reproductive and phenological traits are consistent with adaptive differentiation, and 4) Determine the fitness of hybrids and magnitude of hybrid breakdown.

METHODS

Study System

Campanulastrum americanum (L.) Small (=Campanula americana L., Campanulaceae) is an autotetraploid herb that has an expansive distribution across eastern and central North America (Prendeville et al. 2013). Populations are typically found in disturbed habitats and occupied by deciduous forest (Galloway et al. 2003, Galloway 2005). Populations exhibit diverse morphological and phenotypic traits such as number of days to seed emergence, number of days to flower initiation, number of branches, number of fruits, and plant size (Kalisz & Wardle 2004; Galloway & Etterson 2005; Etterson et al. 2007; Burgess et al. 2007; Haggerty & Galloway 2011). Flowering phenology and timing of germination are closely associated with one another in this species. Timing of germination influences when rosettes are vernalized which must occur to initiate bolting, and thus also impacts when flowering initiation will occur.

Creating experimental seeds for Parents and Offspring

Seeds were sampled from nine populations of *C. americanum* along a latitudinal gradient in 2008 (methods noted in Prendeville et al. 2013). Five populations were located in the northern (MI, MN, NE, OH, and WI) region of the species range, and the remaining four resided in the southern (AL, MS, OK, and TN) region (Figure 1, Appendix A). Seed collected from each population contained 15-20 families, to account for genetic variation that may exist within each population. In 2011, ten seeds from family per were sowed in planting trays containing 3 Metromix: 1 Turface. Trays were placed in a growth chamber at University of Virginia with 25 °C day/ 14°C night, 12-hr

days for four weeks. If multiple seeds germinated then seedlings were thinned to one. Trays were then placed in a cold room set at 5 °C and 12-hr days for 7 weeks. Seedlings were transplanted to containers and move to a greenhouse. Plants were watered on daily basis and exposed to a 16-hr light cycle. Once individuals developed three flowers or one week had past since the day of first flower, hand pollination occurred. Within a population individuals were crossed between different families to establish a line of parents. A F1 hybrid generation was established by crossing individuals belonging to different populations noted by state abbreviation: WIxMS, MNxOK, NExAL, OHxOK, TNxMN, and MSxMI (Figure 1). Then reciprocal crosses of these F1 population cross-types were also performed using the same set of pollen donors and pollen recipients to generate F2 hybrids. Generations associated with reciprocal crosses were noted with an "R". Fruits were collected when mature and placed in cool storage until seeds were ready to be used for the seed germination experiment in July 2012. A subset of these seeds was also planted in plug trays, following the above procedure in December 2012. After germination seedlings were exposed to 5 °C for 47 days and then utilized in the rosette transplant experiment.

Seed germination experiment

To address questions of whether populations exhibit higher fitness within their native range and if phenological patterns are indicative of adaptive differentiation, a reciprocal transplant experiment was performed. Seeds from each population were planted on 4-5 & 10-11 August 2012, in two common garden sites, respectively: the Pierce Cedar Creek Institute in Hastings, Michigan and Columbus State University's

Oxbow Meadows Environmental Learning Center in Columbus, Georgia. Each site contained 30 blocks (26.7 cm by 34.3cm) consisting of nine sleeves. There is some variation in the number of seeds per replicate amongst the populations. In one set of populations (AL, MI, MS, TN, and WI), 10 seeds were planted in each replicate and represented once in each block. A total of 30 replicates in each site contained a representative of this set of parents (10 seeds/replicate x 1 replicate/block x 30 blocks = 600 seeds/population/site). The remaining population set (NE, OK, MN, and OH) had five seeds per sleeve and one replicate per block (5 seeds/replicate x 1 replicate/block x 30 blocks =150 seeds/population/site). The total number of germinants present in each replicate and number of days to germination was monitored in Georgia and Michigan on a bi-weekly basis until 31 May & 2 June 2013, respectively. Another round of planting occurred on 21 August 2013 at Pierce Cedar Creek Institute and 24 August 2013 at Oxbow Meadows, and germination was monitored until 25 April 2014 at Oxbow Meadows and 28 May 2014 at Pierce Cedar Creek Institute. During the second year, blocks consisted of five peat pot trays (nine pots per block). Seeds were planted and observed over a similar period as the previous year's experiment.

Two response variables were scored. Percent germination was calculated by dividing the maximum number of germinants that emerged in a germination replicate by the total number of seeds planted. Minimum time to germination was the number of days between when the seeds were planted and when the germinant(s) were first observed.

Rosette transplant experiment

I also transplanted rosettes to assess phenology and reproductive traits independent of any effects of local adaptation on germination. A total of 317 rosettes were transplanted to each common garden site on 3-4 March 2014 at Oxbow Meadows and 11-13 April 2014 at Pierce Cedar Creek Institute. They were randomly assigned to one of eight blocks and planted 25 cm apart. There is an unbalanced design due to uneven germination and survival. AL, MI, MS, TN, and WI had 17-24 replicates per site, populations NE, OK, MN, and OH had 14-16 replicates/site, and MN had 8 replicates/site. Additionally, there were 11-15 replicates for the F2 associated with each parental cross. Survival was monitored weekly until bolting was evident at which point plants were checked daily for initiation of flowering.

Traits were measured to describe reproductive phenology. Height prior to flowering was determined by conducting a census of plant height prior to the opening of the very first flower of the season (19 June 2014 at Oxbow Meadows and 16 July 2014 at Pierce Cedar Creek Institute). A plant was measured from the base to the tip of the meristem. Height at first flower was taken on the day that the first flower on an individual plant was observed as just opened. The ratio of height prior to flowering and height at flowering is an index of bolting rate. Once the very first flower of the season opened, day of first flower was scored every three days. Number of days to first flower was difference between when rosettes were transplanted and when the first flower was observed on an individual. To monitor fruit maturation, a segment of the mainstem was marked off with twist ties on day of first flower. The first open flower was the first node of this segment, and the next four nodes above this node made up the segment. Fruit maturation was

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monitored on a weekly basis in this marked segment until 31 October 2014 at Oxbow Meadows and 2 November 2014 at Pierce Cedar Creek Institute. Minimum number of days to fruit maturation was the difference between when a flower first appeared and when maturation was first noted in the marked node region.

I also measured a suite of size and reproductive traits. Total branch length was calculated by adding the length of all the branches on an individual plant measured on the day of first flower. Flower production was monitored on a weekly basis. The total number of flowers was calculated by summing the total number of opened flowers scored each week until 31 October 2014 at Oxbow Meadows and 2 November 2014 at Pierce Cedar Creek Institute. Two near-mature fruits were collected, i.e. brown in color but pores not open. Fruits were collected at random from one or two nodes above or below the marked node region. The total numbers of seeds per fruit was determined by taking the average of seeds produced by two fruit samples.

Plants were harvested when 80-100% of fruits in the marked node region were mature. Plants were cut at the very base of the stem, just above the ground. If conditions were wet, the plants were placed in a drier (85°F) for 3-5 days. On the other hand, if conditions were very dry, drying was not required. Biomass was determined by weighing dried harvested plants. Fruit number was determined by counting fruits on harvested plants as well as any fruits that fallen off and present at the bottom of collection bags.

If a weekly census was missed, it was conducted a day after the designated census date. If a three day census was missed it was conducted on the next scheduled date, and it was noted that data reflected flowering activity that had occurred over two census

periods. Lastly, any measurements of individual plants that were not taken on the day of

first flower were collected on the next scheduled three day census.

Statistical Analyzes

Assessing local adaptation

Phenological, size and fitness traits were compared across common garden sites and

populations using an ANCOVA (JMP Statistical software Version 12, SPSS Statistics

Version 23). The model included fixed effects of "origin," whether populations

originated from the north or south, "site" as planting location, and random effects

population nested in origin and block nested in site. For germination traits, "year" was

also included as a fixed effect and block was nested in year and site. An origin x site

interaction would provide evidence that performance of populations of each origin

differed between sites. Local adaptation would be supported if populations native to the

region exhibit higher reproductive fitness than populations from different latitudes.

Tukey HSD was used to assess which traits means were statistically different within and

across sites. To meet assumptions of normality, days to germination and percent

germination were log-transformed. All remaining reproductive and phenological traits

were log+1-transformed.

I also assessed local adaptation by calculating cumulative fitness of individuals

from different source origins by multiplying the number of seeds per fruit by total

number of fruits. An ANOVA was conducted to compare cumulative fitness associated

with each origin within and across sites. Site, origin, and site x origin were fixed effects,

while population nested in origin and block nested in site were random effects.

Phenotypic selection analysis

A phenotypic selection analysis was performed to assess if phenotypic plasticity observed in each common garden site was adaptive (Lande & Arnold 1983), and if patterns of selection varied across sites. Nine parental populations were included in this analysis. Both phenological (number of days to first flower and bolting rate), size (biomass, total branch length, and height at flowering), and reproductive (total number of flowers, seeds/fruit) traits were included. All of these traits were first standardized to a mean of zero and unity of variance prior to conducting the analysis. I also assessed for correlations and multicollinearity among the traits. Pearson product-moment correlation coefficients revealed that total number of fruits was significantly correlated with biomass, total number of flowers, and height at flower (Appendix B1, B2). Variance inflation factors generated were all less than three, indicating that results of this analysis was not influenced by multicollinearity that exists among these traits

Total number of fruits was used as measure of relative fitness. Fruit counts at each site were divided by the associated site mean. Standardized linear selection gradients (β), were generated by performing a multiple regression of relative fitness on standardized traits to obtain partial regression coefficients. Standardized nonlinear selection gradients (γ) are doubled parameter estimates generated from a multiple regression of relative fitness on standardized traits and their squares. Population was included as blocking term for both multiple regression analyzes. To test whether each gradient (selection) significantly varied across sites ANCOVAs were performed, in which standardized phenological traits were assigned as covariates and common garden site was a fixed effect.

Assessment of hybrid breakdown

To test whether hybrid breakdown occurred in between population crosses, an ANOVA (JMP Statistical software Version 12.0) was performed with generation, cross, site, generation x site, generation x cross, and cross x site as fixed effects. A linear contrast was performed on the L.S. means of each level of generation associated with a particular trait. Fitness traits total number of flowers, total number of fruits, seeds/fruit, and biomass were included in this assessment. I am most interested in testing whether parents (P1 & P2) in an associated cross outperformed (higher fitness) their hybrid offspring (F2s). Reciprocal crosses were also included and were noted by placing a capital R in front of the generations associated with the cross. A linear contrast: **(P1+P2)//2=(F2+rF2)/2** was used to test whether the performance of F2 is lower than the average performance of parents.

RESULTS

Germination

Percentage of seedling emergence and time to germination significantly varied between northern and southern populations in the Michigan site but not the Georgia site. Timing of seedling emergence of northern populations was significantly delayed at the Michigan common garden site (mean=160 days), taking an additional 83 days to germinate (Table 1, Figure 2a). Plants of northern origin also took an additional 44 days to germinate than southern populations at the Michigan site (Table 1). Southern populations on average took 66 days to germinate at both sites (Figure 2a). A greater percentage of seedlings emerged from populations of southern origin (mean~13%)

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compared to northern origin (mean~10%; Table 2, Figure 2b). The Michigan site exhibited a significantly higher percentage of seedling emergences (mean ~15%) relative to Georgia (mean~7%). At the Michigan site, approximately 5% more southern seedlings emerged compared to seedlings of northern origin (Figure 2b).

Phenology

All phenological traits exhibited a significant site effect and site x origin interaction (Table 1 & 2). Phenology of plants of southern and northern origin varied significantly across sites, suggesting plasticity. Within sites, not all phenological traits exhibited significant variation, for example bolting rate. A significant difference in bolting rate was only observed in Georgia, where northern plants bolted significantly slower than southern plants (N mean~0.21, S mean~0.36). In Michigan, bolting rates of plants from different origins were very similar (N mean~0.44, S mean~0.47; Table 2, Figure 3a). Across sites, populations of northern origin were on average only 0.30 of their height at flowering relative to the 0.41 for southern plants, indicating slower bolting in northern plants.

Unlike bolting phenology, distinct trends were observed in flower initiation and fruit maturation within both sites. Significantly earlier flowering and delayed fruit maturation was exhibited by populations present in their native range (Table 2, Figure 3b,c). Native populations flowered six days sooner. There was variation in how delayed fruit maturation occurred in populations native to each site. Fruits of populations native to the southern common garden site matured 17 days later, while fruits of populations native to the northern site took an additional seven days to mature. Furthermore, there was a notable difference in timing of flowering of plants of southern origin across sites.

Southern plants took longer to flower in Michigan by approximately 10 days, while flowering of plants of northern origin varied by four days across sites (Figure 3b). Additionally, as observed with flowering time, fruit maturation of southern plants varied significantly across sites (Table 2). Fruits of plants of this origin took additional 22 days to mature, while fruit maturation of northern plants varied by four days.

Reproductive Traits

Size traits revealed greater variation between populations of different origin in the Georgia site. Size of plants were greater in Georgia (mean biomass=2.07g, mean height=73cm, mean branch length=35cm) compared to Michigan (mean biomass=0.53g, mean height=54cm, mean branch length=0.02cm). Furthermore, height at flowering and total branch length displayed a significant site x origin interaction (Table 2). Plants of southern origin were 0.24g heavier and 14 cm taller at flowering than northern plants at the Georgia site, and total branch length of plants of this origin was 34.98cm longer (Table 2, Figure 4). In Michigan, size of plants from different source origins was comparable.

Reproductive traits revealed significantly greater reproductive output of populations native to common garden locations. All reproductive traits significantly varied across sites. Approximately 3x more flowers, 3x more seeds, and 6x more fruits were produced by plants from both source origins in Georgia than Michigan (Figure 5). Within sites, native populations produced approximately double the number of seeds and flowers compared to nonlocals. Fruit production of native populations did not significant differ from foreign populations (Figure 5c).

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Total fruit number and seeds per fruit were used to estimate cumulative fitness of each source origin. Cumulative fitness of each origin significantly varied across and within sites (Table 3, Figure 6). Populations native to the region, where the common garden resided, had a cumulative fitness that was 1.3x higher than the nonlocals; same for both sites. The fitness levels found in Georgia were 9x higher than the levels found in Michigan.

Phenotypic selection analyzes

There was stronger direct selection (β) on the phenological and reproductive traits of *C. americanum* populations in the Georgia common garden site (Table 4). Earlier flowering time was favored at the Georgia site but no selection on flowering time was found in the Michigan site. There was significant selection towards a larger number of flowers, and biomass at the Georgia site but not in Michigan. Michigan displayed selection for greater height at flowering and smaller seed number, whereas no significant selection on these traits was evident in Georgia. Both Georgia and Michigan displayed significant selection on biomass, though the selection for biomass in Georgia was 1.3x greater (Table 4).

Standardized nonlinear selection was evident in both sites (Table 4). A positive quadratic selection gradient is present for the number of flower produced, total branch length, and biomass at the Georgia site. This suggests that relative fitness (fruit production) is an increasing function of greater flower production and larger plant size (branch length and biomass). There is also negative quadratic selection gradient associated with number of days to first flower (Table 4). Individuals in Georgia that

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flower earlier and yield a larger reproductive output will have higher fitness. In the Michigan common garden site, there are also positive quadratic selection gradients evident. Relative fitness is a function of greater height at first flower and biomass, and thus larger size would be associated with higher fitness. The number of seeds per fruit is the only trait that has a negative quadratic selection gradient at the Michigan site, indicating a decelerating relationship between relative fitness and seed number (Table 4). The phenotypic distribution of each trait was reviewed by analyzing graphs. Number of days to first flower and seeds per fruit are under stabilizing selection, indicated by a higher frequency of individuals expressing phenotypes in the middle of the distribution. Height at first flower, total number of flowers, total branch length, and biomass are under disruptive selection, evident by greatest number of individuals expressing a phenotype towards one end of the distribution.

Hybrid breakdown

Biomass indicated that there was a significant generational effect, while the other traits did not. (Table 5A, Figure 7). Linear contrast revealed that there was significant variation in number of seeds per fruit for each level of generation, which indicates hybrid breakdown. Furthermore, there is significant variation in biomass for each generation level that indicates heterosis (Table 5B). When analyzing variation across levels of generation by site and population cross-type, mean differences were consistently insignificantly for majority of the traits (Appendix C, D).

DISCUSSION

Local adaptation

Reproductive fitness clearly suggests both northern and southern populations are locally adapted. In this study, fitness is indicated by flower, seed, and fruit production, along side traits tied to size (height at first flower, biomass, and total branch length). Fitness of native plants was significantly higher at both sites, and thus they are outperforming individuals that are not native to their range. Furthermore, differences in plant size may also be related to reproductive success in a particular environment (Primack 1987; Wesselingh et al. 1997). Greater size was observed by both origins at the Georgia site, implying that size of plant is more indicative of climatic differences. Overall larger plant size in the southern common garden, along with heritable phenotypic variation selected by natural selection within southern plants, has resulted in higher fecundity and higher reproductive output. Estimated cumulative fitness values serve as further evidence of local adaptation revealed by reproductive traits, illustrating higher fitness levels by natives as well.

Percent germination was also used an indicator of fitness. Plants of southern origin had the highest percentage germination across sites, displaying higher fitness than northern populations at both sites. Higher fitness of southern plants at the Georgia site and lack of evident of higher fitness of northern plants at the Michigan site is inconsistent with the other reproductive traits.

Underlying differentiation in phenology may facilitate local adaptation. In my study, phenological traits varied significantly across sites for plants of southern and northern origins. This suggests that phenological traits for both source origins are plastic.

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Earlier flowering and delayed fruit maturation were exhibited by both origins within their native range. On the other hand, variation in bolting rate was only observed in the Georgia site, in which native populations bolted significantly earlier. Similar to my study, delayed fruit maturation and delayed bolting have been reported in *C. americanum* populations native to regions of northern latitude and higher elevations (Haggerty & Galloway 2011; Prendeville *et al*. 2013). Other plant species such as *Arabidopsis thaliana* demonstrate a positive correlation between number of days to bolt and latitude, where plants from northern latitudes bolted later than plants from southern latitudes. Temperature fluctuates in a systematic fashion along a latitudinal gradient (Johanson *et al*. 2000; Stinchcombe *et al*. 2004). Thus, cooler temperatures may support later bolting in this species. Whether plasticity of phenological traits of southern and northern populations is adaptive can be addressed by relating phenology to reproductive fitness. Fitness is higher for populations present in their native range. It can be concluded that earlier flower, earlier bolting, and delayed fruit maturation may have been selected for in southern extent of the distribution, because they maximize the reproductive output of southern plants. While, earlier flowering, delayed fruit maturation, and delayed bolting may assist with increasing the fitness of northern plants.

The role of time to germination in reproductive fitness is unclear. Earlier germination in the Georgia common garden site suggests that southern plants would complete their reproductive cycle faster. Timing of germination may be a reflection of a suite of environmental factors that could influence seedling emergence; light, soil moisture, salinity, and temperature (Tanveer *et al*. 2012). Temperature has been shown to significantly increase phenological progression in warmer temperatures (Gordo & Sanz

2010; Hulber *et al.* 2010; Galloway and Burgess 2009; Haggerty & Galloway 2011), and thus could promote shorter germination time at southern latitudes.

Phenotypic selection

There is selection towards greater number of flowers, biomass, and length of branches in the southern common garden site. While, only a few reproductive traits (height at flower, biomass, and seeds) are under significant selection at the northern site. This shift towards higher reproductive output (flowers) and overall size (biomass, branches) in the southern site is also observed, indicating that plasticity is adaptive. Unlike my study, previous experimental research on *C. americanum* have report no relation between biomass and latitude gradient. However, what studies have found that are consistent with my results is that there are significant variations in branch lengths and final plant size (Kalisz & Wardle 1994; Prendeville *et al.* 2013). In other species such as *Arabidopsis thaliana,* biomass has been shown to negatively correlate with elevation with smaller plants associated with cooler environments (Montesnios-Navarro *et al.* 2011). Length of growing season, amount of rain, temperature, and characteristics of vegetation cover can influence plant size (Crauford & Wheeler 2009; Rajasekar *et al.* 2013). A combination of environmental factors existing along a latitudinal gradient may be contributing to overall selection for larger size.

Furthermore, I observed significant selection for earlier flowering in the southern populations in the southern site but not the northern site. The length of time to flower initiation can influence how many flowers or fruits an individual can produce during a growing season (Ollerton & Lack 1998). Earlier flowering may be beneficial in that there

is a longer flowering duration, higher likelihood of fertilization and visitation of pollinators, and reduction in competition with other flowering species; contributing to reproductive success (Mosquin 1971; Rathcke & Lacey 1985; Gentry 1974; Heinrich 1975; Stiles 1975; Alonso 2004). Longer growing seasons present in the south would allow an individual plant to produce more flowers (Haggerty & Galloway 2011). Greater flower production in the south could possibly be facilitated by a longer growing season and earlier flower initiation. This is in accordance with the expectation that longer growing season and warmer temperatures would encourage earlier flower initiation (Fitter & Fitter 2002; Etterson 2004; Griffith & Watson 2005; Parmesan 2007; Gordo & Sanz 2009; Galloway & Burgess 2012). This behavior has also been observed in *C. americanum* at lower elevations, warmer conditions (Haggerty & Galloway 2011). Delayed flowering initiation has also been observed by other species native to southern latitudes such as *Lythium salicaria* (Olsson & Agren 2002).

It has been demonstrated that flowering time can be a plastic, responding to environmental cues such as photoperiod, vernalization, and resource availability that indicate when conditions are best to reproduce (Levy & Dean 1998; Gordo & Sanz 2009). However, flowering time may also be reflective of internal cues (Levy & Dean 1998; Burgess *et al.* 2007; Haggerty & Galloway 2011). The results of this study suggest that flowering time is an adaptive response of southern and northern populations. When populations were closer to their home sites displayed quicker flowering initiation but when placed in a location outside of their native range their flowering period was delayed. *C. americanum*, along with *Melandrium* and *Aradidopsis*, exhibit distinct flowering genotypes across a specified geographic range (Lawrence 1963; Westerman

1971; Stincombe *et al*. 2004). Thus, variation in flowering time could potentially be reflected of genotypes selected in a particular environment.

The magnitude of selection and the traits under selection appear to differ across sites. To confirm whether selectional gradients differed across sites, analysis of covariance was performed to assess if selection gradients differed across the common garden sites. There was significant site x gradient interaction for the total number of days to first flower, total number of flowers, seeds per fruit, height at first flower, total branches, and biomass. This indicates that magnitude of selection on traits at each site varies and this is influencing relative fitness of populations. For majority of these reproductive and phenological traits, expect for biomass, there was evidence of local adaptation suggested by higher fitness of populations native to each site and significant site x origin interaction. All of these traits also exhibited a significant site effect. This confirms that the site interaction being observed is due to differences in magnitude of selectional gradients that exist at each site.

Hybrid Breakdown

There is little evidence to suggest that genetic differentiation between northern and southern populations has resulted in hybrid breakdown. Across population cross-types and sites, F2 generations did yield reproductive outputs as large as their associated parents, suggested by linear contrast. This also holds true within each site (Appendix C, D). The results of this study are in accordance with the common observation that few postzygotic barriers to hybridization develop polyploidy. In polyploids, there is a genetic buffer against allele fixation. Any mutations that form in a population are not likely to

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become fixed in a population (Stebbins 1950). *C. americanum* is an autotetraploid and if

genetic differentiation occurred among populations, a genetic buffer would prevent new

mutations from becoming fixed. This could either slow down the formation of a

reproductive isolation barrier or limited the possibility of epistatic incompatibility.

Adaptive population differentiation

Adaptive population differentiation occurs when natural selection acts upon

heritable phenotypic variation (Linhart & Grant 1996; Miaud & Merila 2001; Garcia *et*

al. 2007). Selective forces have acted upon reproductive and phenological variations

present within *C. americanum* populations of different origins, resulting in genetic and

phenotypic differentiation. Varying environmental conditions present along a latitudinal

gradient has facilitated differentiation. Furthermore, magnitude of selection was not

uniform across the gradient, creating differences in selection across sites. Higher fitness

levels at home sites suggest that differentiation between these source origins is adaptive.

This study demonstrates that utilizing geographical variations such as latitude can assist

in investigating whether locally adapted populations have formed across a species range.

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Table 1. Nested ANOVA of minimum number of days to germinant emergence and percent germination across nine parental *C. americanum* populations, five from a northern origin and four from a southern origin, planted into common gardens at sites in Georgia (south) and Michigan (north). The experiment was repeated in two years. F-values are listed for each fixed and random effects. Block(Site, Year), Population(Origin) are the random effects in this model. There are seven degrees of freedom associated with Population[Origin] and 104-111 degrees of freedom for Block(Site, Year). Degrees of freedom are one for each fixed effect. Denominator d.f. are in brackets. (*)P<0.10, *P<0.05, **P<0.01, ***P<0.001

Response Variable	Site	Year	Origin	Origin x Site	Origin x Year	Site x Year	Pop (Origin)	Block (Site, Year)
Days to germination	28.98*** (381)	0.06 (385)	5.08 (391)	12.36*** (378)	0.58 (379)	66.94*** (384)	6.81*** (271)	1.57** (271)
% germination	77.06*** (1220)	0.15 (1214)	12.93** (1229)	0.15 (1218)	0.07 (1214)	0.02 (1220)	1.30 (1113)	1.48** (1113)

Table 2. ANOVA of reproductive and phenological traits of *C. americanum* populations of northern and southern origin planted at Georgia and Michigan common garden sites (2014). F-values are listed for each fixed and random effects. Block[Site], Pop[Origin] are the random effects in this model. There are seven degrees of freedom associated with Pop[Origin] and 36 degrees of freedom for Block[Site]. Degrees of freedom are one for each fixed effect. Denominator d.f. are in brackets. (*)P<0.10, *P<0.05, **P<0.01, ***P<0.001

	Trait	Site	Origin	Origin x Site	Pop[Origin]	Block[Site]
A. Phenological Traits	**Bolting rate**	105.33*** (503)	0.55 (503)	8.78** (447)	4.07*** (447)	1.68** (447)
	Number of days to first flower	11.19** (249)	0.14 (249)	20.43*** (204)	16.89*** (204)	0.63 (204)
	Min number of days to fruit mat	14.45** (242)	1.04 (242)	6.77* (197)	0.71 (197)	1.19 (197)
B. Reproductive Traits	**Seeds per fruit**	18.65** (296)	0.58 (297)	23.21*** (290)	5.07*** (252)	1.04 (252)
	Total number of fruit	5.21* (272)	0.02 (273)	1.17 (228)	2.43* (228)	3.68*** (228)
	Total number of flowers	38.26*** (558)	1.21 (452)	21.89** (557)	0.34 (385)	4.53*** (385)
C. Size Traits	**Height at flowering**	0.03 (249)	0.90 (249)	15.19*** (204)	3.42** (204)	2.78*** (204)
	Total branch length	10.84** (356)	6.07* (356)	10.95** (311)	0.97 (311)	0.59 (311)
	Biomass	50.4967*** (237)	4.13 (299)	0.29 (290)	2.45* (254)	4.93*** (254)

Table 3: ANOVA of cumulative fitness of northern and southern origins planted at Georgia and Michigan common garden sites (2014). F-values are listed for each fixed and random effects. Block[Site] and Pop[Origin] are the random effects in this model. There are seven degrees of freedom associated with Pop[Origin] and 38 degrees of freedom for Block[Site]. Degrees of freedom are one for each fixed effect

Effect	d.f. denominator	F-value	p-value
Site	369	9.52	0.0276
Origin	369	0.18	0.6813
Site x Origin	241	7.29	<0.0001
Pop[Origin]	302	4.94	<0.0001
Block[Site]	302	1.71	0.0022

Table 4. Standardized selection differentials (S), linear gradients (β), and quadratic gradients (γ) of northern and southern *Campanulastrum americanum* populations planted in common garden sites located in Columbus, GA and Hastings, MI. To detect differences in selection across common garden sites, site and gradient interactions were analyzed. The F values are reported for these ANCOVAs. Site means: GA (29.58), MI (16.09). (*)P<0.10, *P<0.05, **P<0.01, ***P<0.001

	Trait	B			γ		
		GA site	MI site	Site x β	GA site	MI site	Site x γ
A. Phenological Traits	Days to first flower	-0.844***	-0.266	7.034***	-0.248(*)	-0.175	1.940
	Bolting Rate	-0.174	0.196	1.727	-0.047	0.176	34.43***
B. Reproductive Traits	Total # of flowers	1.26***	0.136	88.305***	0.558***	0.062	9.38**
	Seeds per fruit	0.008	-0.432*	8.805***	0.063	-0.123*	6.59*
C. Size Traits	Height at first flower	-0.334	1.074***	97.61***	-0.058	0.511***	3.83(*)
	Total Branches	0.636**	0.044	14.94***	0.116**	0.121	8.27**
	Biomass	1.10***	0.842***	133.78***	0.375***	0.634***	20.19***

Table 5A. ANOVA testing the effects of generation on expression of reproductive traits of *C. americanum* populations. The numerator degrees of freedom is one. F-ratio is listed for each effect.

Trait	Site	Gen	Cross	Site x Gen	Cross x Gen	Cross x Site
Total # of seeds	19.65***	3.04(*)	0.81	0.00	0.36	0.70
Total # of fruit	15.16***	2.32	0.47	0.04	0.31	0.99
Total # of flowers	9.98**	1.16	1.85	0.32	0.42	0.78
Biomass	24.20***	7.56**	2.64*	1.97	0.84	3.28**
d.f numerator	1	2	5	1	3	5

**d.f. denominator: 545-673

Table 5B. Linear Contrast of L.S means generated by ANOVAs for reproductive traits that quantifies the level of reproductive fitness exhibited by each generation (P, F2, RF2) of *C. americanum* populations. Reciprocal crosses were also included these analyzes and were noted by placing a capital R infront of the generations associated with the cross. P-values indicate whether F2 (F2 +RF2) means are significantly different from parents. The numerator d.f. is one. An arrow in the mean comparison column indicates if F2s had means lower or higher than the parents.

Traits	d.f. denominator	F-ratio	p-value	Mean Comparison
Number of Seeds/fruit	547	8.83	0.003	↓
Biomass	555	7.94	0.005	↑
Total number of fruits	507	0.13	0.777	=
Total number of flowers	675	0.11	0.746	=

Figure 1. Location of nine *Campanualastrum americanum* populations (AL, NE, MI, TN, OK, MN, MS, WI, and OH) planted in common garden sites, Pierce Cedar Creek Institute in Hastings (MI) and CSU's Oxbow Meadows Environmental Learning Center in Columbus (GA). Common garden site locations are noted with stars. Red lines indicated which populations were crossed to generate F1s and F2s.
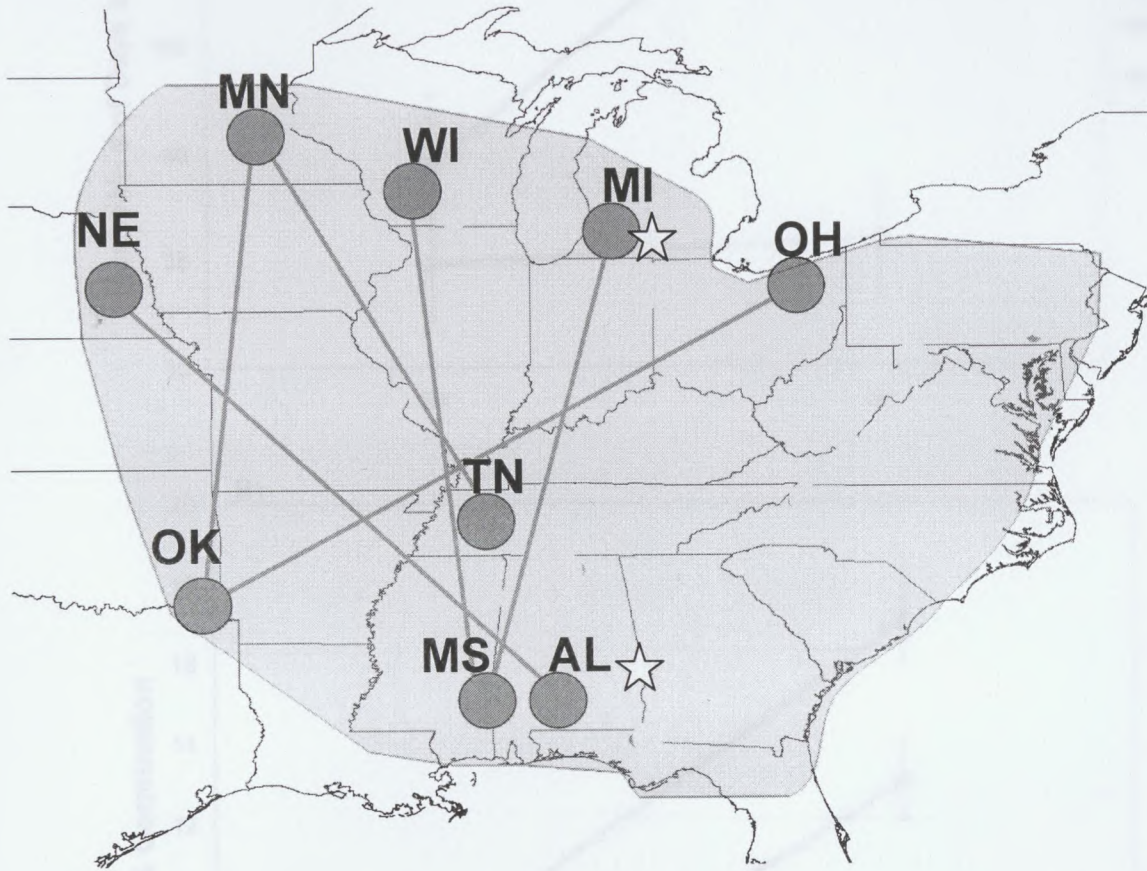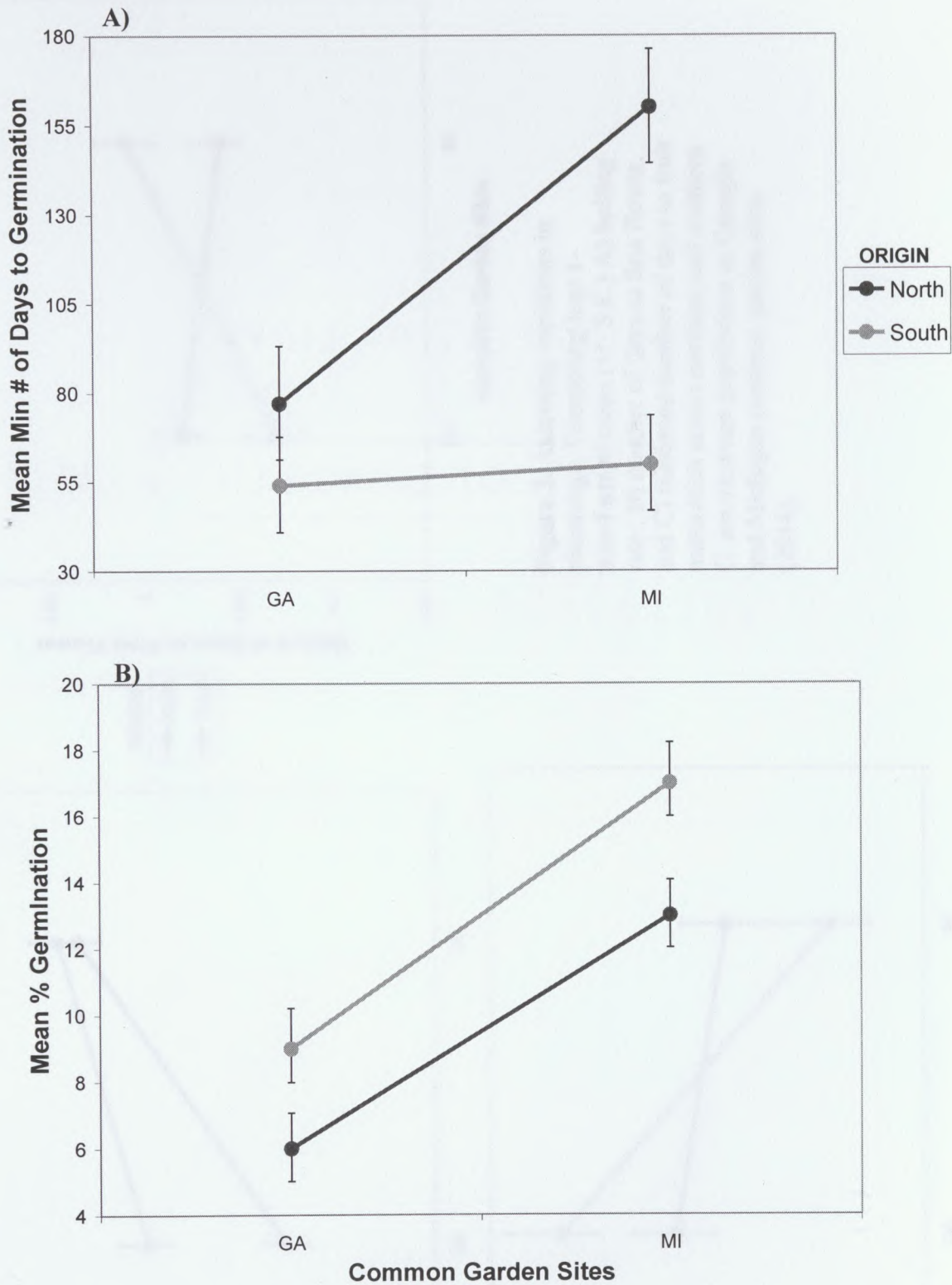
Figure 2. Comparing L.S. mean (+/- S.E.) A) minimum number of days to germination, B) percent germination across northern and southern *C. americanum* populations at Georgia and Michigan common garden sites. Means reflect germination data that was collected for a period of two years (2012-2014).
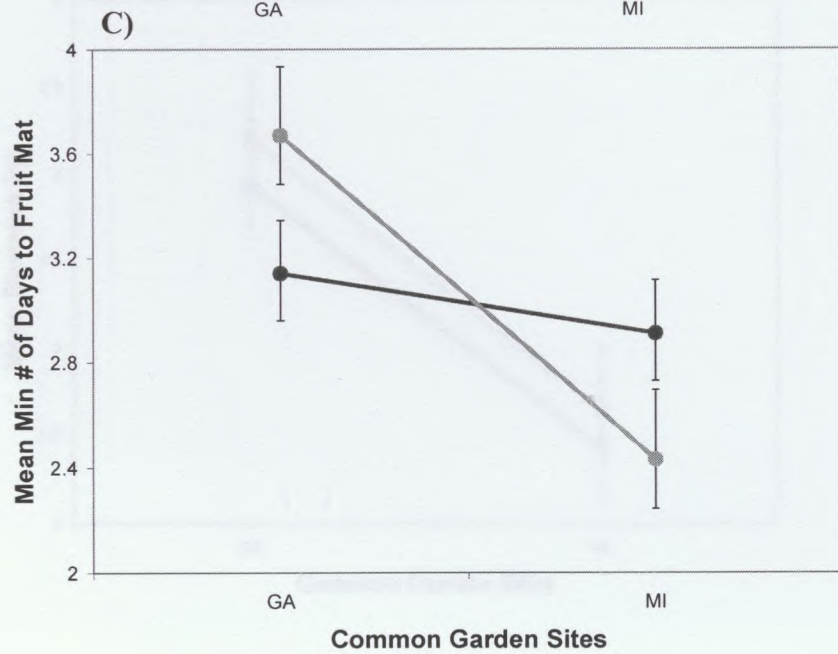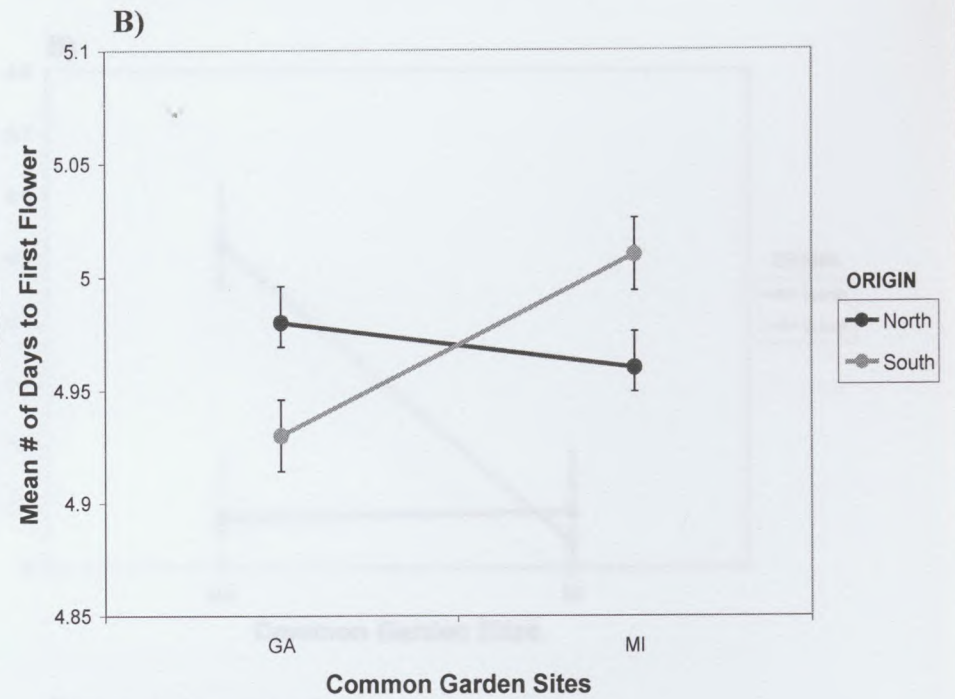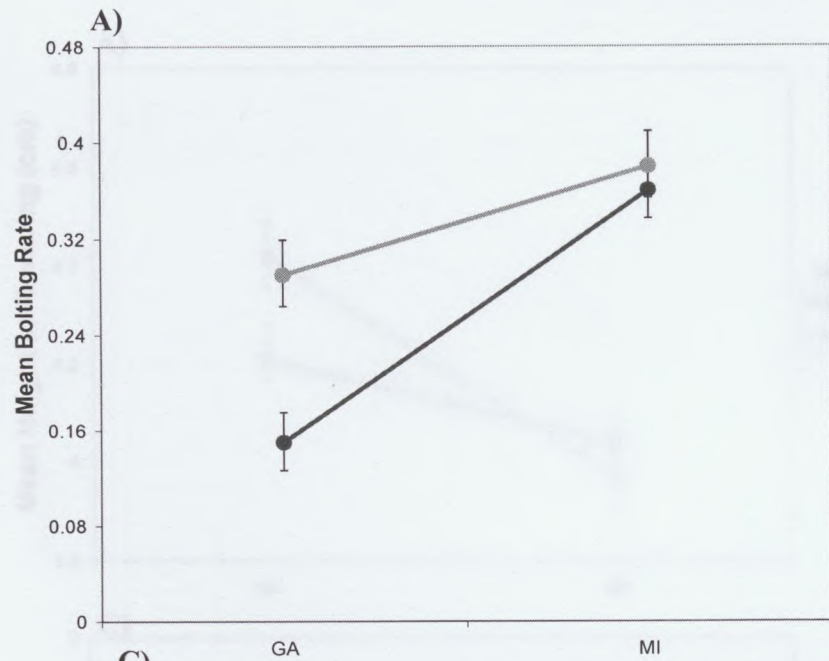
Figure 3. Assessing variations in phenology. Comparing log+1-transformed mean (+/- S.E.) A) bolting rate , B) number of days to first flower, and C) minimum number of days to fruit maturation across northern and southern *C. americanum* populations at Georgia and Michigan common garden sites (2014).
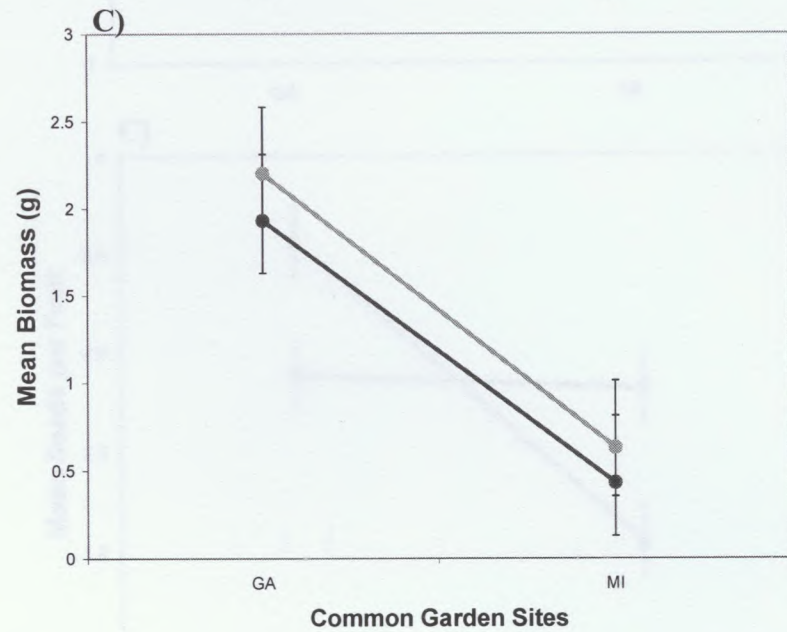
Figure 4. Assessing traits indicating size. Comparing log+1-transformed mean (+/-S.E.) A) height at flowering, B) total branch length, and C) biomass across northern and southern *C. americanum* populations at Georgia and Michigan common garden sites (2014).
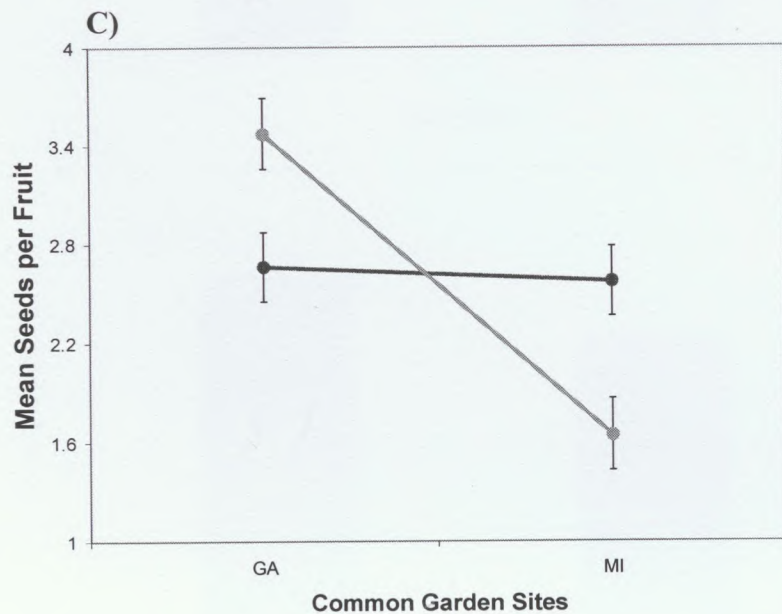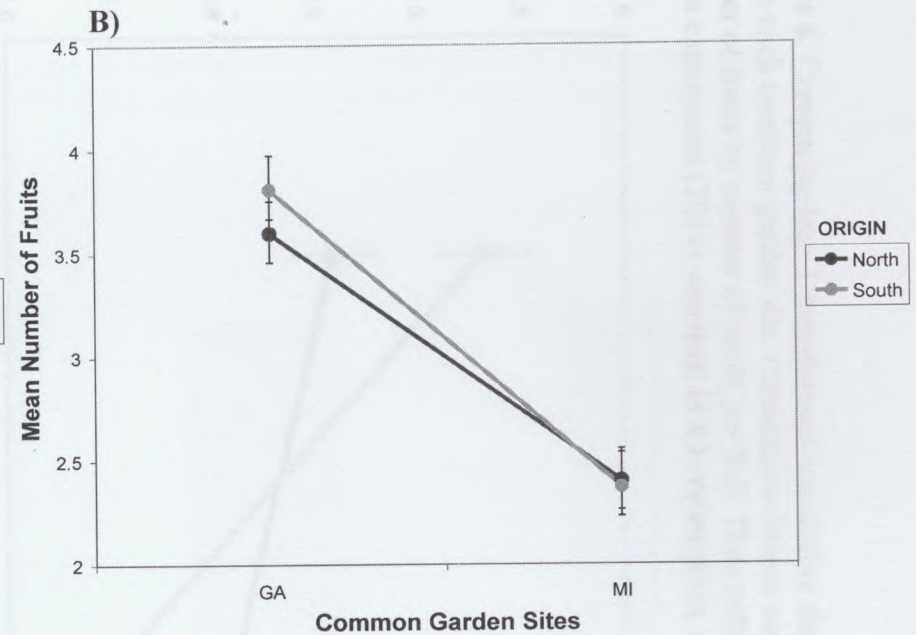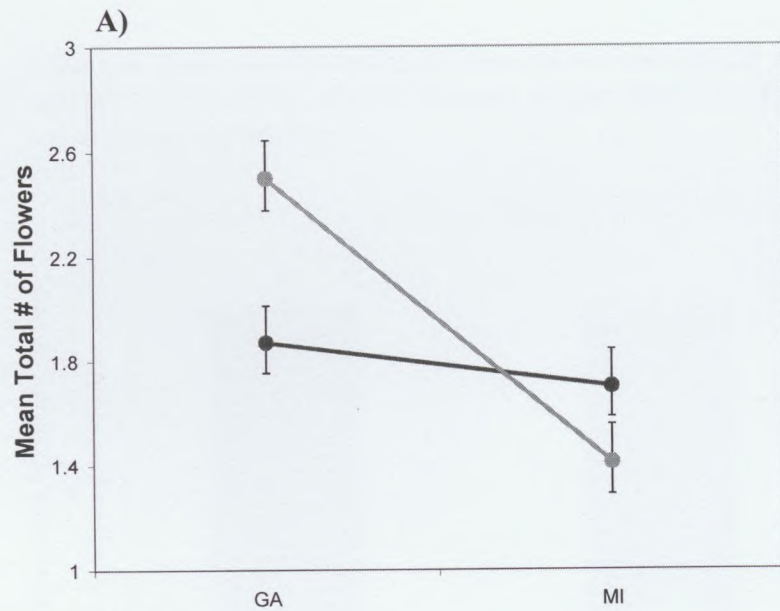
Figure 5. Assessing variation in reproductive traits. Comparing log+1-transformed mean (+/-S.E.) A) total number of flowers, B) total number of fruits, C) seeds per fruit, and across northern and southern *C. americanum* populations at Georgia and Michigan common garden sites (2014).

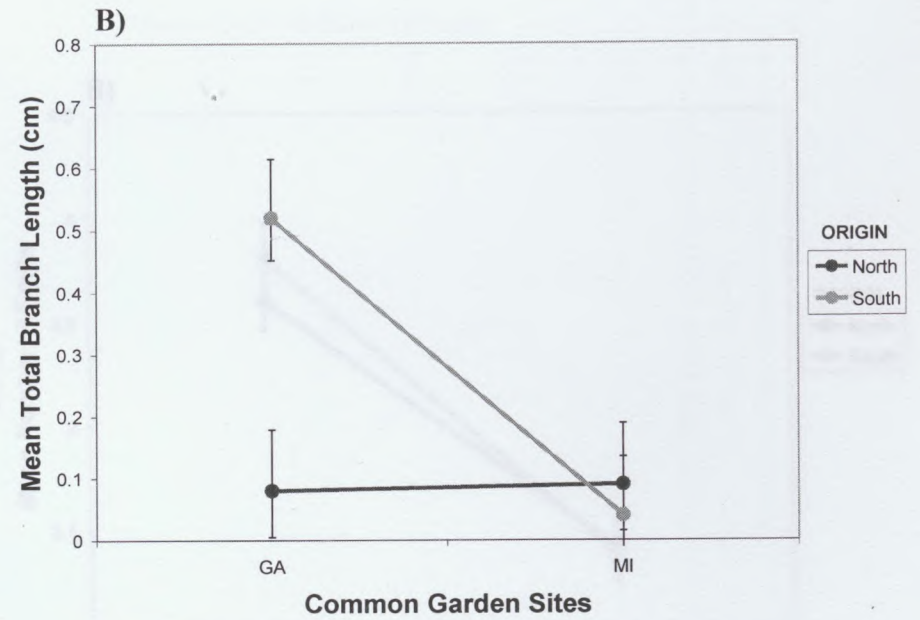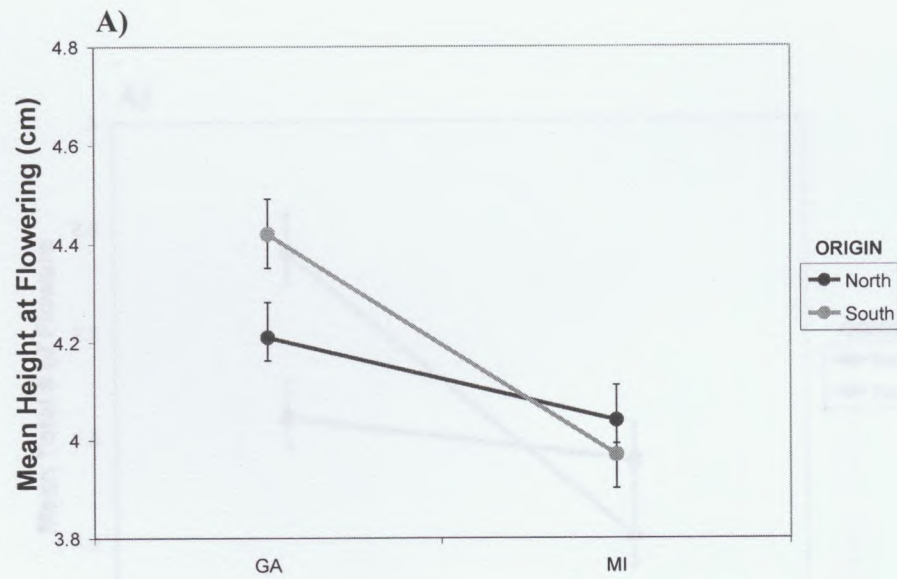**Figure 6.** Comparing log+1-transformed cumulative fitness means of source origins within each common garden site. Cumulative fitness was calculated by multiplying total number of fruits by number of seeds per fruit. Data collected from the rosette common garden experiment (2014) was used in this calculation.
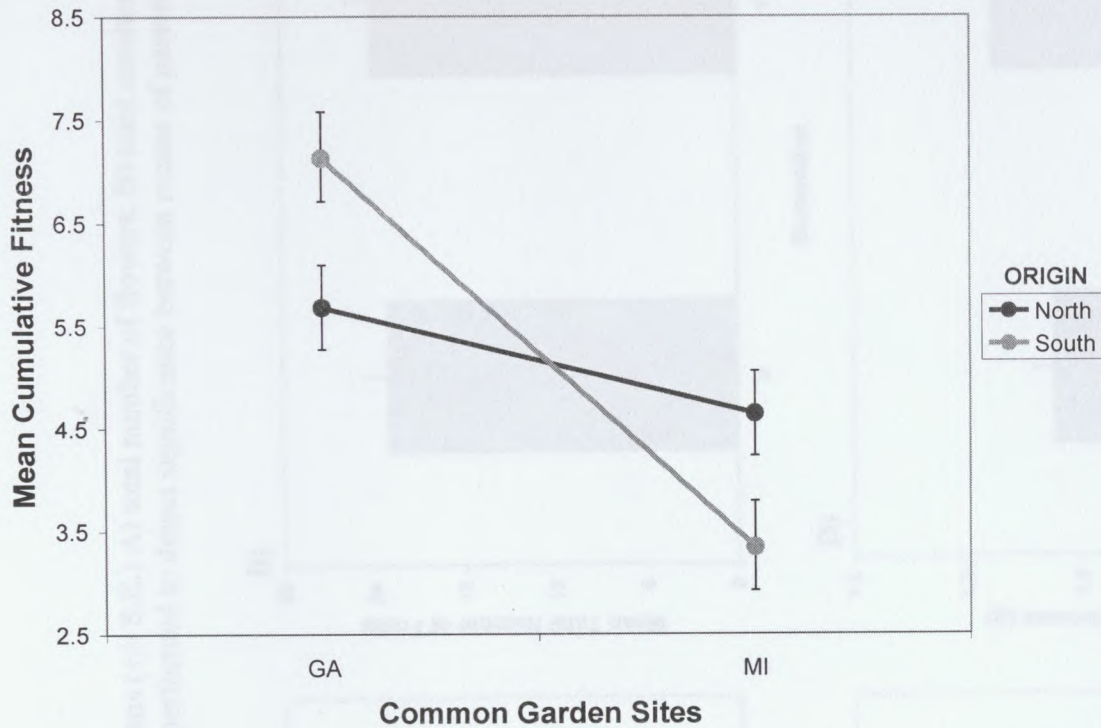
Figure 7. Assessing hybrid breakdown by comparing L.S. means (+/- S.E.) A) total number of flowers, B) total number of fruits, C) number of seeds/fruit, and D) biomass. Linear contrasts were performed to detect significance between means of parents and F2s of *C. americanum* populations.

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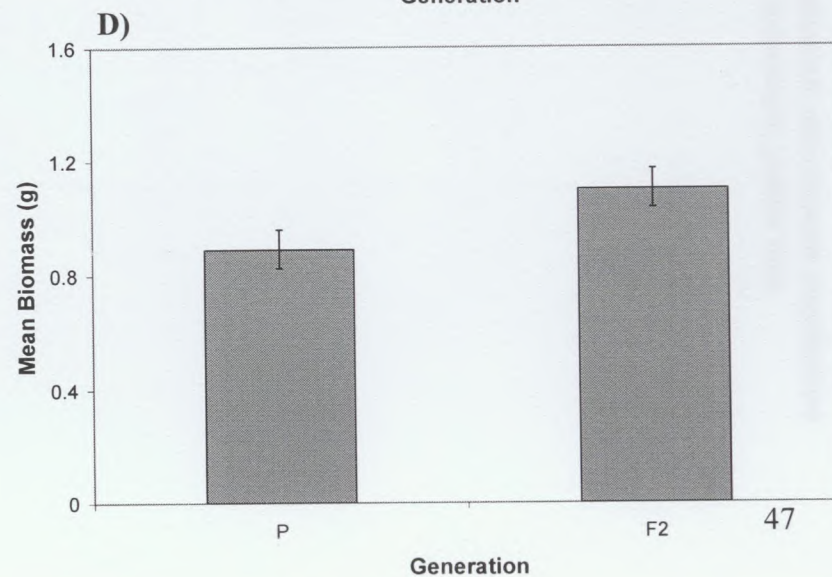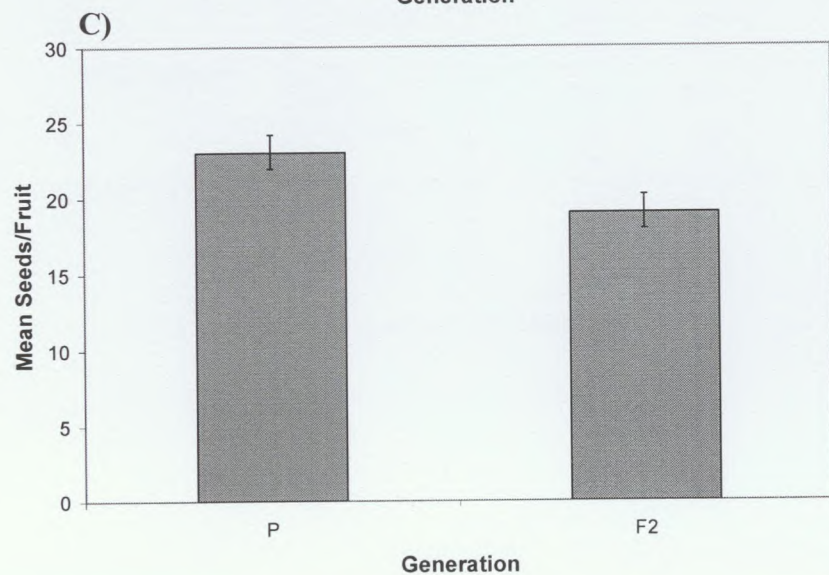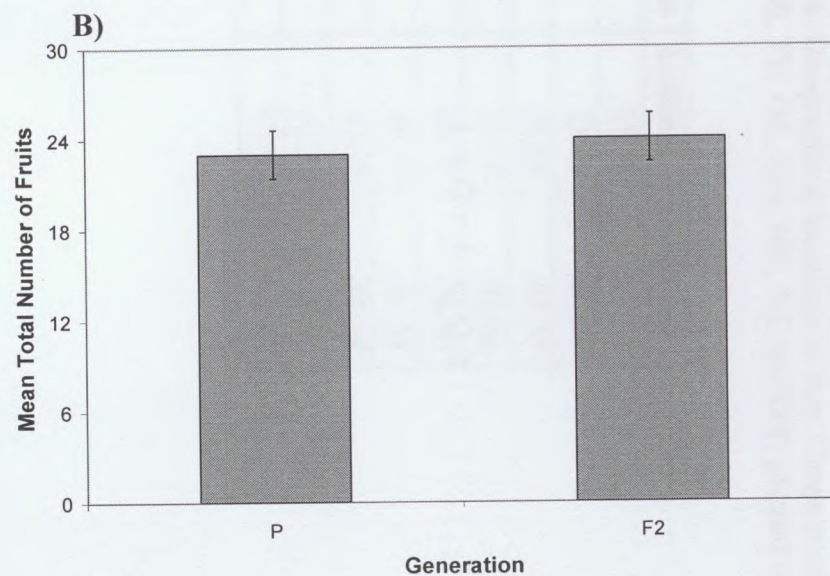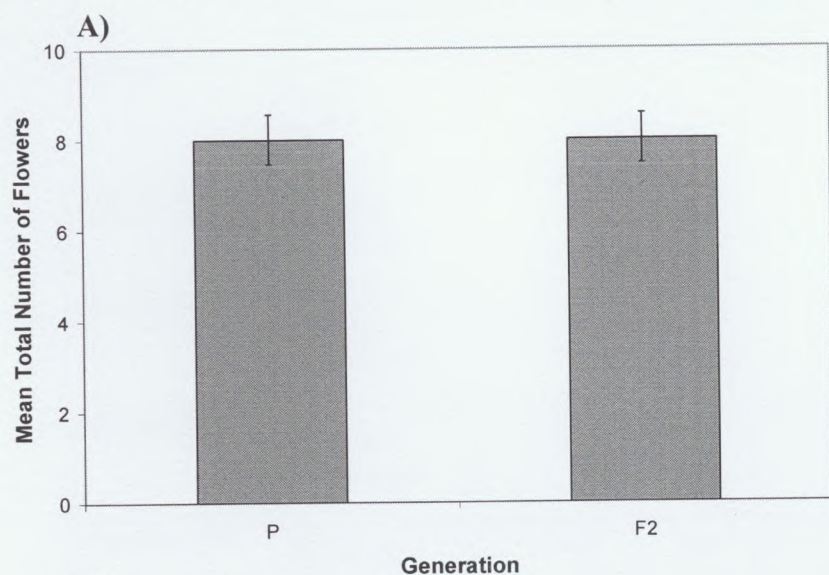
Appendix A: Geographical locations of nine *Campanualastrum americanum* populations (AL, NE, MI, TN, OK, MN, MS, WI, and OH) planted in common garden sites.

Populations	Latitude	Longitude
MS	31.74	-88.52
AL	31.92	-86.69
OK	33.95	-94.57
TN	35.77	-88.06
NE	40.75	-96.72
OH	41.12	-81.52
MI	42.30	-85.36
WI	43.35	-89.95
MN	44.82	-93.31

Appendix B1: Pearson product-moment correlation coefficients for reproductive and phenological traits of southern and northern *C. americanum* populations planted in the Georgia common garden site (2014).

		Number of Days	HAF (cm)	Total Branch Length (cm)	Seeds per fruit	Biomass (g)	Number of fruits	TOTAL FLOWERS	Ratio
Number of Days	Pearson Correlation	1	-.245**	-.153	.081	-.247**	-.267**	-.060	.150
	Sig. (2-tailed)		.007	.090	.444	.007	.004	.507	.125
	N	123	122	123	92	119	112	123	106
HAF (cm)	Pearson Correlation	-.245**	1	.263**	.271**	.467**	.526**	.534**	.013
	Sig. (2-tailed)	.007		.003	.009	.000	.000	.000	.898
	N	122	122	122	91	118	111	122	106
Total Branch Length (cm)	Pearson Correlation	-.153	.263**	1	.072	.086	.455**	.406**	-.144
	Sig. (2-tailed)	.090	.003		.470	.334	.000	.000	.142
	N	123	122	155	104	127	113	155	106
Seeds per fruit	Pearson Correlation	.081	.271**	.072	1	.268**	.308**	.300**	-.135
	Sig. (2-tailed)	.444	.009	.470		.009	.004	.002	.233
	N	92	91	104	104	95	88	104	80
Biomass (g)	Pearson Correlation	-.247**	.467**	.086	.268**	1	.685**	.488**	.043
	Sig. (2-tailed)	.007	.000	.334	.009		.000	.000	.664
	N	119	118	127	95	127	112	127	103
Number of fruits	Pearson Correlation	-.267**	.526**	.455**	.308**	.685**	1	.812**	-.138
	Sig. (2-tailed)	.004	.000	.000	.004	.000		.000	.172
	N	112	111	113	88	112	113	113	99
TOTAL FLOWERS	Pearson Correlation	-.060	.534**	.406**	.300**	.488**	.812**	1	-.108
	Sig. (2-tailed)	.507	.000	.000	.002	.000	.000		.270
	N	123	122	155	104	127	113	155	106
Ratio	Pearson Correlation	.150	.013	-.144	-.135	.043	-.138	-.108	1
	Sig. (2-tailed)	.125	.898	.142	.233	.664	.172	.270	
	N	106	106	106	80	103	99	106	106

**. Correlation is significant at the 0.01 level (2-tailed).

Appendix B2: Pearson product-moment correlation coefficients for reproductive and phenological traits of southern and northern *C. americanum* populations planted in the Michigan common garden site (2014).

		Number of Days	HAF (cm)	Total Branch Length (cm)	Seeds per fruit	Biomass (g)	Number of fruits	TOTAL FLOWERS	Ratio
Number of Days	Pearson Correlation	1	-.323**	-.024	-.079	-.172	-.265**	-.155	.390**
	Sig. (2-tailed)		.000	.789	.375	.052	.003	.080	.000
	N	128	128	128	128	128	127	128	128
HAF (cm)	Pearson Correlation	-.323**	1	.112	.298**	.814**	.867**	.381**	-.078
	Sig. (2-tailed)	.000		.205	.001	.000	.000	.000	.381
	N	128	129	129	129	129	128	129	129
Total Branch Length (cm)	Pearson Correlation	-.024	.112	1	.071	.155*	.130	-.040	.049
	Sig. (2-tailed)	.789	.205		.324	.041	.099	.573	.579
	N	128	129	203	195	174	162	203	129
Seeds per fruit	Pearson Correlation	-.079	.298**	.071	1	.194*	.228**	.218**	.124
	Sig. (2-tailed)	.375	.001	.324		.011	.004	.002	.161
	N	128	129	195	195	171	161	195	129
Biomass (g)	Pearson Correlation	-.172	.814**	.155*	.194*	1	.751**	.418**	-.014
	Sig. (2-tailed)	.052	.000	.041	.011		.000	.000	.875
	N	128	129	174	171	174	162	174	129
Number of fruits	Pearson Correlation	-.265**	.867**	.130	.228**	.751**	1	.416**	-.048
	Sig. (2-tailed)	.003	.000	.099	.004	.000		.000	.592
	N	127	128	162	161	162	162	162	128
TOTAL FLOWERS	Pearson Correlation	-.155	.381**	-.040	.218**	.418**	.416**	1	.018
	Sig. (2-tailed)	.080	.000	.573	.002	.000	.000		.840
	N	128	129	203	195	174	162	203	129
Ratio	Pearson Correlation	.390**	-.078	.049	.124	-.014	-.048	.018	1
	Sig. (2-tailed)	.000	.381	.579	.161	.875	.592	.840	
	N	128	129	129	129	129	128	129	129

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Appendix C: Detecting hybrid breakdown by analyzing whether a linear relationship $((P1+P2)/2=(F2+rF2)/2)$ exists across two generations of *C. americanum* (P, F2/RF2) for each population cross-type. Generations associated with reciprocal crosses were noted by placing R infront of the generation catoegory. Contrast linear analyzes were performed on a selected group of reproductive traits that assess the level of reproductive fitness exhibited by each generation at the Georgia common garden site. The numerator d.f. is one. Arrows in the mean comparison column indicates if F2s had means lower or higher than the parents.

| Population cross-type	Trait	d.f Error	F-value	p-value	Mean comparison
AL69 x NE59	Total number of seeds	31	11.24	0.0021	
	Biomass	43	3.67	0.0624	=
	Total number of fruits	50	2.20	0.1440	=
	Total number of flowers	45	1.80	0.1870	=
MI44 x MS55	Total number of seeds	39	2.21	0.1232	=
	Biomass	51	8.75	0.0047	
	Total number of fruits	45	1.12	0.2948	=
	Total number of flowers	43	0.33	0.5655	=
OK61 x MN38	Total number of seeds	26	1.37	0.1395	=
	Biomass	34	2.11	0.0652	=
	Total number of fruits	29	0.68	0.4097	=
	Total number of flowers	34	2.13	0.1536	=
MS55 X WI14	Total number of seeds	43	7.47	0.0091	
	Biomass	58	2.01	0.1617	=
	Total number of fruits	45	1.58	0.2154	=
	Total number of flowers	46	1.40	0.2424	=
OH64 X OK61	Total number of seeds	36	0.00	0.9941	=
	Biomass	43	0.02	0.8573	=
	Total number of fruits	37	0.00	0.9590	=
	Total number of flowers	42	1.61	0.2113	=
MN38 X TN19	Total number of seeds	22	3.89	0.0613	=
	Biomass	30	0.33	0.5707	=
	Total number of fruits	23	0.45	0.5097	=
	Total number of flowers	29	0.02	0.8990	=

Appendix D: Detecting hybrid breakdown by analyzing whether a linear relationship $((P1+P2)/2=(F2+rF2)/2)$ exists across two generations of *C. americanum* (P, F2/RF2) for each population cross-type. Generations associated with reciprocal crosses were noted by placing R infront of the generation category. Contrast linear analyzes were performed on a selected group of reproductive traits that assess the level of reproductive fitness exhibited by each generation at the Michigan common garden site. The numerator d.f. is one. Arrows in the mean comparison column indicates if F2s had means lower or higher than the parents.

Population cross-type	Trait	d.f Error	F-value	p-value	Mean Comparison
AL69 x NE59	Total number of seeds	36	0.04	0.8401	=
	Biomass	54	0.06	0.8034	=
	Total number of fruits	50	2.48	0.1215	=
	Total number of flowers	59	4.75	0.0332	
MI44 x MS55	Total number of seeds	66	0.00	0.9888	=
	Biomass	82	2.49	0.1187	=
	Total number of fruits	72	5.39	0.0231	
	Total number of flowers	73	0.24	0.6260	=
OK61 x MN38	Total number of seeds	39	0.07	0.8001	=
	Biomass	44	0.17	0.6808	=
	Total number of fruits	39	0.29	0.5939	=
	Total number of flowers	42	4.70	0.0359	
MS55 X WI14	Total number of seeds	53	1.47	0.3155	=
	Biomass	70	3.15	0.0805	=
	Total number of fruits	61	3.20	0.0787	=
	Total number of flowers	64	0.13	0.7178	=
OH64 X OK61	Total number of seeds	59	0.15	0.6977	=
	Biomass	58	0.04	0.8334	=
	Total number of fruits	77	0.33	0.5660	=
	Total number of flowers	63	0.39	0.5371	=
MN38 X TN19	Total number of seeds	54	0.17	0.6788	=
	Biomass	58	0.64	0.4283	=
	Total number of fruits	52	0.20	0.6562	=
	Total number of flowers	54	0.01	0.9259	=

Appendix E: Assessing variations in phenology across *C. americanum* populations. Comparing L.S. means A) number of days to germination, B) bolting rate, C) number of days to first flower, and D) number of days to fruit maturation across northern and southern populations at Georgia and Michigan common garden sites (2014).
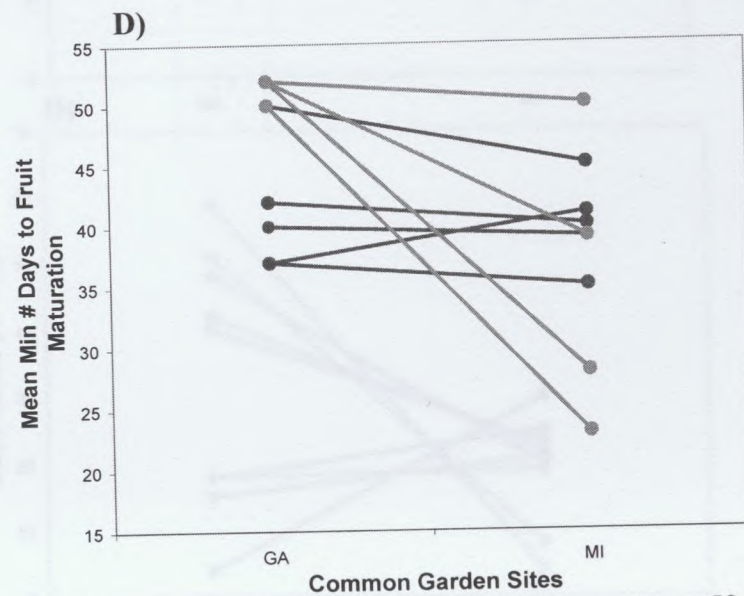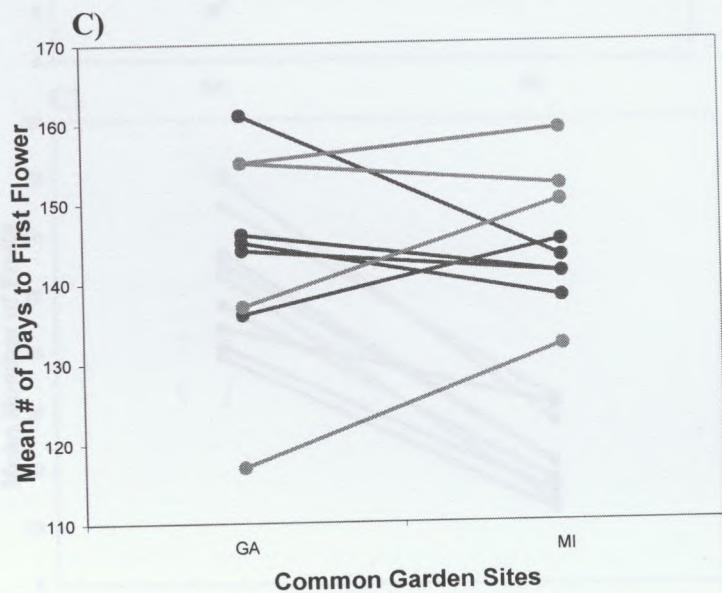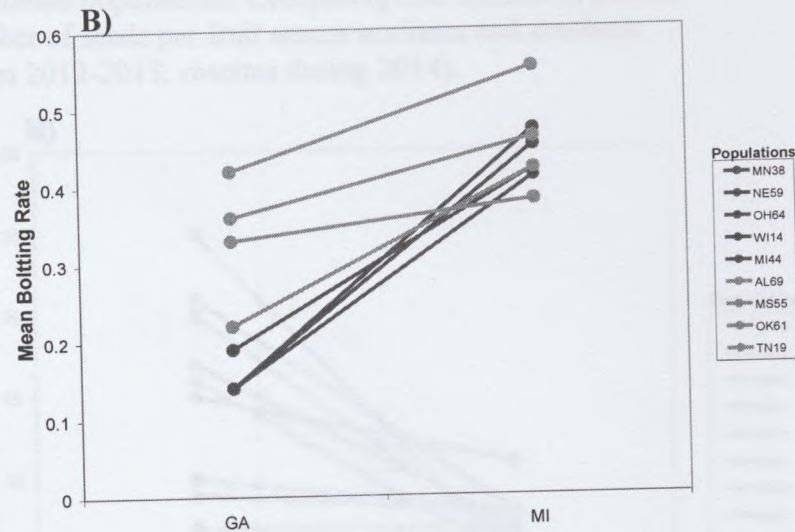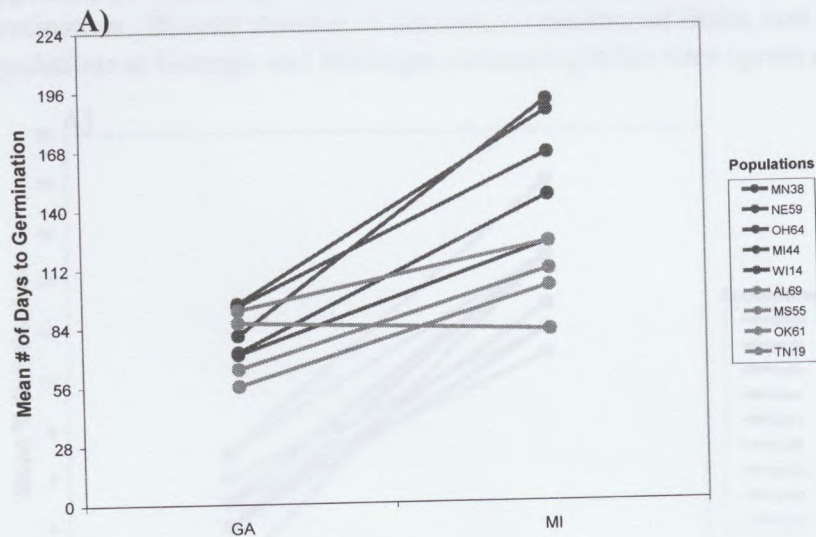
Appendix F: Assessing variations in reproductive fitness across *C. americanum* populations. Comparing L.S. means A) percent germination , B) total number of flowers, C) number of fruits, and D) number of seeds per fruit across northern and southern populations at Georgia and Michigan common garden sites (germ data from 2012-2013; rosettes during 2014).

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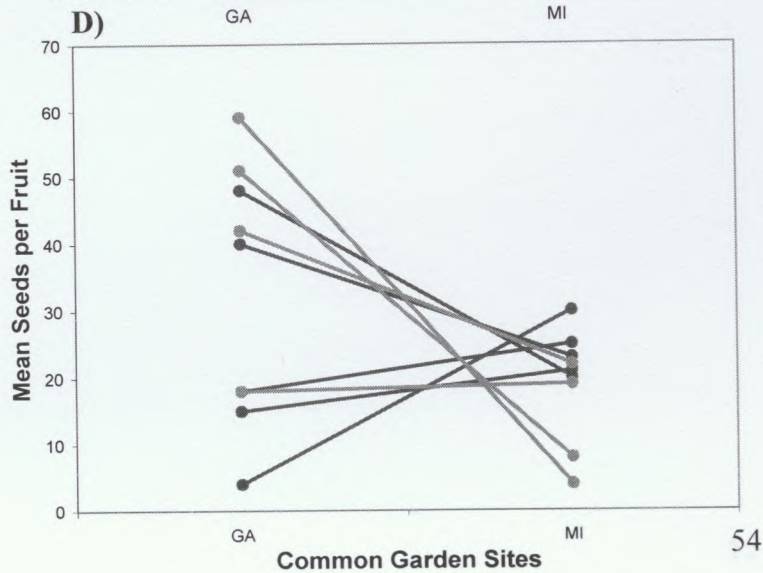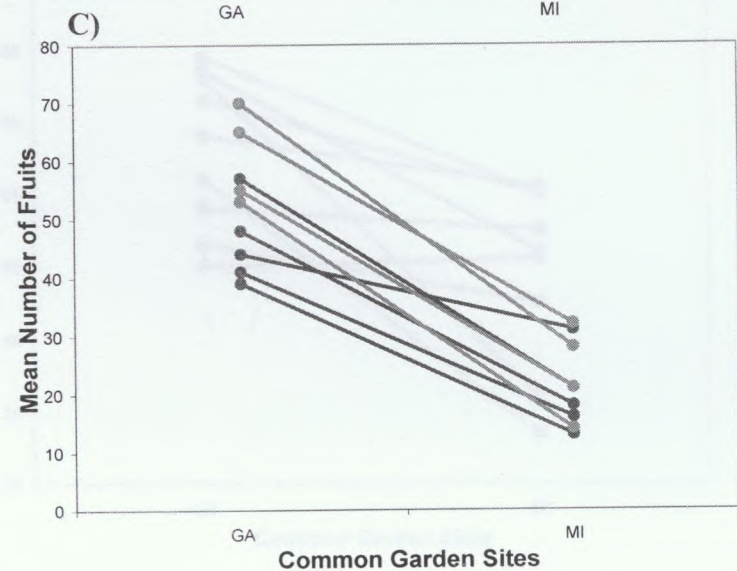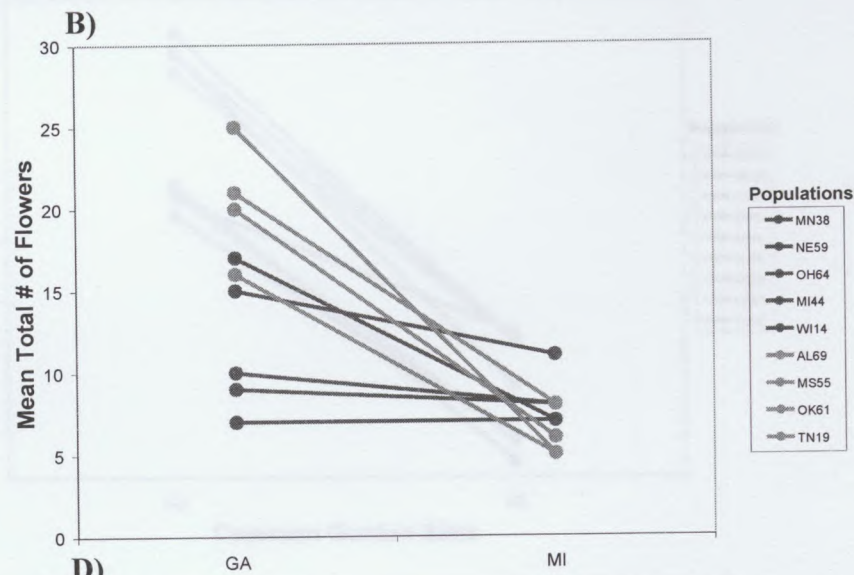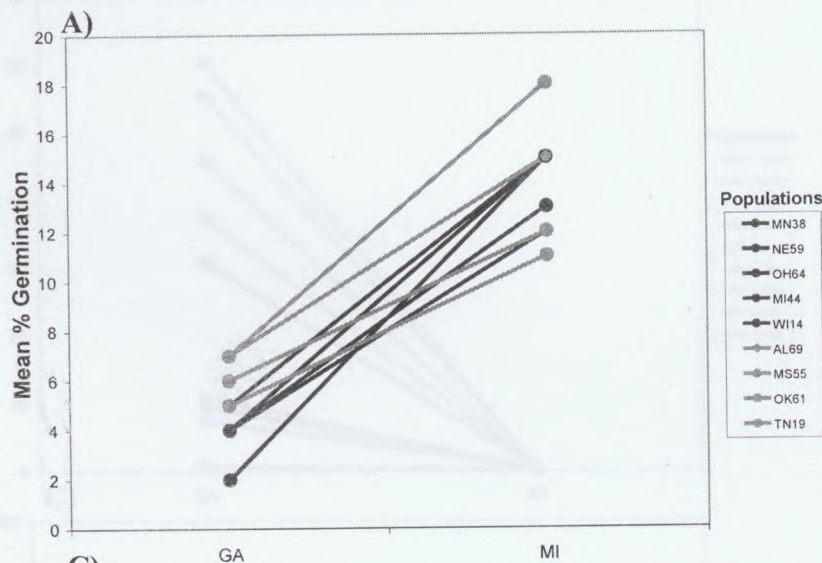
Appendix G: Assessing variations in size across *C. americanum* populations. Comparing L.S. means A) total branch length, B) biomass, and C) height at flowering across northern and southern populations at Georgia and Michigan common garden sites (2014).