Evolutionary impacts of assisted gene flow: Fitness consequences of hybridization along a geo-climatic gradient in an annual prairie legume

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For my parents

Introduction

Each of my chapters uses the same experimental design to answer key questions related to the impact of gene flow on adaptation to novel environments. I focused on *Chamaecrista fasciculata,* an annual legume of the tallgrass prairie and grasslands of North America, to conduct this work due to its tractability and rich history in the field of quantitative genetics. I chose a focal population from central Minnesota to hybridize with three other populations spanning from southern Minnesota to northern Missouri. The southern Minnesota population represented local gene flow and is permissible under current seed sourcing guidelines in the state of Minnesota (Minnesota Department of Natural Resources 2015). I used two different populations to investigate the impact of long-distance assisted gene flow. The first was from central Iowa, an area for which the historical climate closely matches the projected temperature of the Minnesota focal population site in 2080 under low emissions. The second was from northern Missouri, an area for which the historical climate closely matches predictions of Minnesota population's home site climate in 2080 given current emissions scenarios (Fitzpatrick and Dunn 2019). Each of the populations created using within and among population crossing in the greenhouse was planted into a local prairie, where a subset was exposed to increased ambient temperatures using warming structures. Using this design, I was able to address both basic evolutionary questions about gene flow and the capacity for adaptation as well as applied conservation questions about the efficacy of assisted gene flow.

In my first chapter, I investigated the immediate impact of long and short distance assisted gene flow on population fitness. Conservationists have proposed that assisted gene flow may introduce "pre-adapted" traits into a population that will be beneficial under stressful, often warmer environments associated with climate change (Aitken and Whitlock 2013a). I obtained mean lifetime fitness for two generations of each population in both ambient and warmed prairie environments. I found that local gene flow that was within the range currently allowed by the state of Minnesota had the highest fitness compared to all other populations and that the non-hybridized long-distance population from Missouri had the lowest fitness in both treatments. However, these fitness differences were not maintained in the second generation. Upon further analysis, I found that germination was a limitation to population performance in the long-distance gene flow and migration scenarios. Therefore, while assisted gene flow provided no clear benefits to population fitness over local gene flow, much of the difference between the two was due to lower germination success.

Chapter 2 examines the genetic effects of hybridization and any resulting changes to adaptive capacity. Though hybridization may increase fitness in some scenarios, its impact on a population's capacity to adapt to novel conditions remains poorly understood. This chapter used the same populations and experimental design as chapter 1, but we changed the notation due to the anticipated audience of this work (i.e. $MN_{central}$ and MN_{south} instead of GC and ML, respectively). Here, I used random effects models to estimate additive genetic variance for fitness ($V_A(W)$, the portion of variance on which natural selection acts. I found substantial $V_A(W)$ in both the focal population and all hybrid populations in the ambient environment. However, only the hybridized populations had detectable $V_A(W)$ in at least one generation of individuals exposed to the novel, warmed environment and thus harbors the capacity to adapt to these harsh conditions. The non-hybrid, long-distance population from Missouri had no detectable $V_A(W)$ in either the ambient or the warmed treatment. Thus, gene flow affords both immediate and long-term benefits to population performance and persistence given rapidly changing environmental conditions. Furthermore, the benefit of long-distance gene flow and the drawback of no gene flow was only apparent once I examined adaptive capacity in warmed conditions rather than the population size consequences.

In chapter 3, I examined how this hybridization along a geo-climatic gradient (i.e. a climatic gradient associated with geographic distance) affected flower morphology and phenology and its subsequent effects on plasticity in response to increased temperatures. I found significant plasticity in all morphological traits, but no significant difference between populations in their trait values. However, I did detect significant differences in mean population fitness with respect to the different floral traits, wherein some populations had higher fitness with larger floral traits and others had higher fitness with smaller floral traits. Even though there were population differences with respect to fitness, these were not ordered along the geo-climatic gradient and therefore these differences were likely population specific. Yet, flowering phenology was ordered along this gradient and is most likely due to the corresponding latitudinal gradient and its impact on photoperiod. Therefore, expectations that assisted gene flow will introduce warm adapted genes and that those genes are ordered along a geo-climatic gradient are not fully borne out in the floral characteristics of these populations.

The results of this work provide much needed empirical evidence of the impact of assisted gene flow on a population's capacity to persist under rapid climate change. Previous work has focused on theoretical expectations of the benefits of assisted gene flow but lacked experimental evidence for its evolutionary impacts. My first chapter demonstrates the benefit of hybridization on population fitness in both ambient and warmed conditions, especially the case of local hybridization. Long-distance hybrid populations had higher fitness only when accounting for the decreased germination success. Therefore, land managers should consider planting seeds from other nearby populations in prairies when managing for climate change. Those considering an assisted gene flow approach may not find benefits greater than the local approach but may find greater success planting seedlings instead of seeds to circumvent the lower germination success. Chapter 2 supported many of these recommendations with only hybrid populations having detectable $V_A(W)$ in the warmed environment. Therefore, hybridized populations had both in increased fitness and substantial genetic variation. My third chapter showed that while populations had substantial phenotypic plasticity in floral traits, the differences in plasticity and trait values between populations were not ordered along our geo-climatic gradient, contrary to theoretical expectations. Although I only included floral traits and there may be other unmeasured traits that have a greater impact on fitness, this finding in conjunction with those of the previous two chapter indicates that the benefits of hybridization are not necessarily linked to specific traits. Rather, assisted gene flow increases genetic variation in general leading to higher fitness and adaptive capacity. Thus, management actions that increase genetic variation within populations may have the greatest impact on population persistence during rapid climate change. Additionally, outbreeding depression may not be as big of a factor in this system and the drawbacks of long-distance hybridization may be alleviated if managers use an additional grow-out and controlled pollination step prior to planting seeds into a prairie. This work illustrates the importance of maintaining genetically variable populations through either long or short-distance assisted gene flow in promoting persistence in a rapidly changing environment.

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CHAPTER 1

Early generation consequences for assisted gene flow and population hybridization on an annual prairie legume

SYNOPSIS

Assisted gene flow (AGF) is a conservation strategy that aims to promote population fitness in light of anthropogenic warming by introducing genetic material from populations that have undergone selection in warmer environments. However, AGF could decrease population performance by disrupting local adaptation or co-adapted gene complexes. Furthermore, the timing of introgression may have profound influence on its success, as the benefits of AGF may only be realized under future warmer conditions, and empirical evidence about the direct effects of AGF on population fitness is scant. In this study, we investigated the impact of AGF on the fitness of *Chamaecrista fasciculata* in a tallgrass prairie ecosystem. We examined the immediate consequences of gene flow by crossing a focal population with three others along a geo-climatic gradient corresponding to temperatures expected under different climate warming scenarios. We assessed fitness of the first and second generation in both ambient and warmed environments to understand how the effects of gene flow depended on environmental conditions. Our results showed that the hybrid population generated from populations within the current seed transfer zones approved by the state of Minnesota had higher fitness in the first generation, while AGF from the Missouri population did not impact fitness in either the ambient or warmed conditions. Seeds sown from a distant, southern population had the lowest fitness compared to all other populations. These population differences were not maintained in the subsequent generation after recombination. Our focal population did not benefit nor was harmed from AGF; however, efforts that enhance local gene flow may provide the greatest benefit to population fitness. This study provides key insights into the immediate impacts of AGF and their implications for future seed sourcing and restoration efforts in this the prairie ecosystems in the Midwest and others.

INTRODUCTION

Human caused climate change has significantly altered both the size and location of species' ranges. While some species have or will eventually shift their ranges and track these changes, others are left vulnerable to their newly inhospitable environment (Parmesan 2006). Substantial habitat fragmentation coinciding with climate change limits successful dispersal. Separation of small habitat patches by large geographic distances (Collingham and Huntley 2000; Fahrig 2003) may be an insurmountable barrier to migration for many populations, reducing the likelihood of that species' range shifting in line with changing climate (Corlett and Westcott 2013). In these cases, species persistence will depend on either physiological tolerance or adaptation to changing environmental conditions (Davis and Shaw 2001; McLaughlin et al. 2002). Populations of small size or with limited variation in traits on which persistence depends in a changing environment may require aggressive conservation approaches to maintain populations, such as assisted gene flow.

Assisted gene flow (AGF) is a subset of climate-informed conservation practices of managed relocation or assisted migration, wherein populations and species are moved from their home sites to new areas that better match climatic conditions to which they have become adapted (Richardson et al. 2009). The premise of AGF is that it increases population fitness by increasing genetic variation and introducing trait values that pre-adapt a recipient population to the environmental conditions that are expected to arise due to climate change (Aitken and Whitlock 2013b). Specifically, populations subject to rapid climate change may benefit from gene flow from populations with historical climate conditions that match present, already changed, and future conditions (MacLachlan et al. 2018; Fitzpatrick and Reid 2019). Even in the absence of pre-adapted traits, gene flow between genetically divergent populations may increase their fitness (i.e. heterosis) (Edmands 1999), an effect that may be seen in both short and long-distance gene flow events. Therefore, the benefits of gene flow and hybridization may be realized across much shorter geographic distances (Bucharova et al. 2019) and may be unrelated to the population's climatic history (Bontrager and Angert 2019).

Early work has found promise in the use of AGF to maintain populations *in situ* (Hagedorn et al. 2021; Saenz-Romero et al. 2021; Pregler et al. 2023), but the geographic, genetic, and temporal scale at which this intervention is beneficial remain unknown (Kelly and

Phillips 2019). In the absence of climate change, there is evidence that gene flow over longer geographic or genetic distances can lead to outbreeding depression, in which offspring are less fit due to the introduction of maladaptive alleles, disruption of co-adapted gene complexes, or dilution of locally adapted traits (Edmands 1999; Fenster and Galloway 2000a). This is the justification many practitioners have for using local seedstock in restoration, as it seeks to increase variation without disrupting the degree of local adaptation (Bucharova et al. 2019). Under climate change, however, reduction in fitness due to outbreeding depression may be tempered when environmental changes are correlated with the direction of gene flow, and the advantage of local adaptation weakens (Bontrager et al. 2020). This paradox, that AGF may result in outbreeding depression under current environmental conditions but lead to increased fitness in a warmed environment suggests that the distance and time of AGF may be crucial to its utility.

How and when AGF is implemented may dramatically affect its impact on population fitness. Depending on whether climate change is rapid or slow, different management options may be more or less advantageous. If change is expected to be quite rapid, more intensive AGF measures may be more beneficial whereas slower changes may necessitate hybridization across shorter distances as populations are theoretically less maladapted to their local conditions. In either case, the method of introgression will be important to the overall success of the management strategy. Seeds sown directly into translocated sites may produce plants that are poorly adapted to the new environment or have dramatically different phenology such that they do not make a substantial contribution to the next generation and the benefits of gene flow are not realized (Griffith and Watson 2006; Wadgymar and Weis 2017; Gorton et al. 2019). Therefore, direct seed sowing may produce plants that have little chance to intermate with the resident population and thus fail to yield the desired high fitness outcome of the first generation of hybridization (Fenster and Galloway 2000a). An intermediate seed production step, wherein populations are hybridized prior to planting in the focal site may be necessary to ensure that the benefits of gene flow are realized.

Understanding the geographic scale at which AGF is beneficial and the impact of AGF on population fitness in both current and future environments is imperative to assessing its utility as a conservation strategy, particularly in fragmented ecosystems. Our study focuses on the tallgrass prairie of the Midwestern United States, now reduced to about 1% of its former extent (Sampson and Knopf 1996). The remaining prairie is heavily fragmented into many small patches, likely leading to small, isolated, and vulnerable populations where long distance gene flow is restricted (Minnesota Prairie Plan Working Group 2018). Rapid climate change impinging on the northern tallgrass prairie will likely result in 2 to 3.6ºC increase in summertime temperatures by 2080. Climate models predict that warming will be accompanied by a projected increase in overall precipitation by 6.7-8.9%, but in fewer and more sporadic precipitation events. These changes follow a general poleward shift in environmental conditions, with climate patterns potentially moving up to 1000 km from where they are currently observed over the next 60 years. These predictions are now manifest (Fitzpatrick and Dunn 2019). These dramatic changes, compounded with high fragmentation, leave populations at risk of local extinction due to a lack of preadapted alleles and absence of variation for adaptive evolution. Therefore, tools such as AGF may be implemented to attempt to ameliorate large scale ecological shifts and species loss, which are likely to have downstream effects on ecosystems and their services. However, empirical work investigating these assumptions about the utility of AGF in population conservation must be conducted in order to fully understand its impact.

In this study, we used *Chamaecrista fasciculata*, a bee-pollinated annual legume whose range extends from the northern tallgrass prairie of Minnesota to central Mexico (Irwin and Barnaby 1982) to assess the impact of gene flow on lifetime fitness. Gene flow between most prairie remnants is unlikely because pollen dispersal is limited to pollinator movement and seeds dispersal is limited to animal movement. Consequently, isolated populations are more likely to have limited standing genetic variation (Richards 2000) and may not adapt at the pace of rapid climate change. We intercrossed populations across a 1000 km range and grew the resulting offspring in a common garden with two temperature treatments to answer the following questions: 1) At what geographic scale does gene flow benefit population fitness? 2) Are the impacts of gene flow dependent on how similar the temperature of the planting site is to the historic environment of the "pre-adapted" population (i.e., the population used as a donor in AGF)? 3) Are these benefits or costs maintained in the recombined generation when the fitness benefits of heterosis are expected to decline?

MATERIALS AND METHODS

Study sites

To investigate the impact of gene flow on a population's capacity to adapt to climate change, we selected a population of *C. fasciculata* near its current northern range limit as the focal population serving as recipient of gene flow. This population, located in Grey Cloud Dunes SNA (GC, 44.799095, -92.972005), occurs on remnant sand prairie. As populations representing sources of gene flow for experimental crosses, we selected three additional remnant populations of *C. fasciculata* from southeastern Minnesota (ML, 44.263861, -91.965574), central Iowa (IA, 41.680194, -92.859765), and northern Missouri (MO, 40.526091, -94.119518). These populations span a climate gradient by latitude and hereafter are referred to as GC, ML, IA, and MO, respectively (Figure 1). Crosses between GC (central Minnesota) and ML (southern Minnesota, McCarthy Lake WMA) represent a local gene flow scenario that is currently feasible within the DNR recommendations for seed transfer within and across adjacent seed zones (Minnesota Department of Natural Resources 2015). Crosses with the populations from IA (Conard Environmental Research Area in central Iowa) and MO (Dunn Ranch Prairie in northern Missouri) are located within areas that are similar to the predicted climate of the Grey Cloud area under different emissions scenarios by 2080. Climate analog maps produced by Fitzpatrick and Dunn (2019), indicated that under a low emissions scenario, the average summer climate analog to Grey Cloud is east-central Iowa with an average summer temperature of 2 °C warmer than Grey Cloud. In contrast, under a high emissions scenario the average summer climate analog of Grey Cloud is most likely Northern Missouri/Kansas, with an average summer temperature 3.6 °C warmer than Grey Cloud. These three populations served as pollen donors to the Grey Cloud Dunes population to generate three hybrid populations. We retained a subset of the Missouri population as a pure Missouri population so that we could compare the impact of long-range gene flow to the most geographically distant parental population. We assessed all populations in ambient and warmed conditions, where a portion of the prairie was warmed to mimic projected climate change conditions using open top chamber greenhouses that elevated daytime temperature by about 2 °C, similar to the temperatures historically found in central Iowa. This comparison was made to assess context dependency of the effects of gene flow on a population's ability to persist and adapt to rapid climate change.

Greenhouse

First Generation

Live seedlings were obtained in 2019 from the IA population and the MO population. To reduce the potential for collecting closely related individuals, all seedlings were harvested at least three meters apart from one another. The GC and ML populations were grown from seed in the same greenhouse as IA and MO populations. Seeds were randomly chosen from previous experiments using pedigreed populations in 2014 (GC) and 2016 (ML) (Kulbaba et al. 2019).

In fall 2019-spring 2020, we used a nested paternal half-sibling design, wherein thirty seedlings from each population (including GC) were randomly assigned to be pollen donors (sires) and sixty GC seedlings to be pollen recipients (dams). Two randomly chosen dams were mated to each sire (one from each population), maintaining the same half sibling groups with each population cross. As such, each dam was mated to four sires, one from each population. The pedigreed population of pure MO was created with 15 sires and 30 dams. Due to limited plant numbers, each sire served as a dam in another sire group. Insect and fungal pressure in the greenhouse decreased final family numbers from the original design (Table S2).

Second Generation:

To assess the impact of gene flow on populations after the segregating generation, a second generation of each population was created using a nested paternal half-sibling pedigree design, where a subset of each first-generation population was randomly assigned to be pollen donors (sires) and the remainder were assigned as pollen recipients (dams). Two randomly chosen, unrelated dams were mated to a single sire. The plants used to create the second generation were all produced as the first generation in 2019-20 (Figure 2; i.e., full siblings of those planted in the 2020 field season). In addition, a second cohort of first-generation ML x GC seeds were produced in the greenhouse in 2021 using full siblings of the original parental generation. This population was produced to control for different greenhouse conditions in the production of the first and second generations.

To produce the second generation of each population (and the secondary ML x GC population), five seeds from each pedigreed individual (i.e., full-sibling family) were chosen at random and planted in 2.3” propagation pots using Sun Gro Professional Metro-Mix 830. A single seedling was randomly chosen from each full sibling family to use in the pedigree created for this experimental generation. Seedlings were transplanted to larger conetainers (2.5” x 10” tree pots filled with 100% sand) once they had five leaves (small differences in methods between the two experimental years for both greenhouse and the following field methods are noted in Table S1). Pest and fungal pressure were much lower for the seeds produced in 2021 than in the previous generation, due to differences in neighboring greenhouses.

Estimating mean fitness in native prairie

All seeds were planted in a natural prairie at the St. Croix Watershed Field Station, 45 km from the focal GC population. Regulations regarding introducing non-native genotypes into Scientific and Natural Areas owned by the state of Minnesota necessitated planting seeds into a prairie 43 km from the source of the original seed collection. In 2020, 20 seeds were haphazardly selected from each full-sibling family. Due to low germination in 2020, 40 seeds were haphazardly selected for planting in 2021 (Table S2). Each seed was surface sterilized using 80% ethanol to prevent bacterial or fungal contamination and hand scarified using a sterile razor blade to nick the seed coat surface. Seeds from each family were randomly assigned a block, treatment, and position, so that each full sibling family was in all five blocks and both temperature treatments. Prior to planting, aboveground biomass was removed from all plots. In the spring of 2021, significant gopher activity required us to flatten gopher mounds in blocks 2 and 4. Seeds were planted in offset rows using a hexagonal design so that each seed was equidistant from each of its six nearest neighbors. Planting positions were 8 cm apart along rows 16 planting positions long in a 1.25 m^2 plot. For each planting position, four seeds were planted with forceps less than 1 cm under the soil surface and 1 cm apart, centered on a toothpick.

Warming treatment

Warming shelters were employed to passively warm half of the plots. Shelters were modeled after the open top chambers (OTCs) used in Welshofer et al. (2018) for tallgrass prairies. To maintain stability in an open prairie, these hexagonal open top chambers used a single panel per side and were constructed using PVC and 6 mil greenhouse film. The truncated hexagonal pyramid had a top opening large enough to contain the 1.25 m^2 plot with buffers on each side (Figure S1). Temperature sensors were placed in the northwest corner of both treatment plots in a randomly selected block. OTCs increased plot daytime temperature by an average of 1.85°C and nighttime temperature by 0.4°C (Figure 3). To reduce the impact of rodents on the plants, all gaps in the bottom of the OTCs were filled with soil and all ambient plots were surrounded with 3 ft tall chicken wire fencing. In 2021, space constraints in the warming shelter limited the number of populations represented in that treatment. Therefore, IA x GC and the second generation of MO were not included in the warming shelter but were represented in the ambient treatment. Warming shelters and fencing around the ambient plots were installed immediately after planting. To ameliorate the observed water stress due to exceptionally low precipitation in 2021 (approximately 21.6 mm precipitation in June 2021 compared to 30-year mean of 125.26 mm), all plots were watered three times in late June and once again in late July after two weeks of no rainfall. All plots were watered so that they received approximately 1.5 cm of water during each watering.

<u>Plant Measurements</u>

Censuses of all planted positions were taken for each major life history stage: germination (late June-early July), flowering (early August), and fruit production (September-October). Positions were thinned to one plant during the second germination census. The seedling closest to the marker toothpick was chosen to represent the family for the entirety of the experiment, others were removed from the plots. For each census, every position was scanned for presence of a plant. When a plant was recorded present, relevant status information was also recorded such as whether it was flowering or producing fruit. Because *C. fasciculata* pods explosively dehisce, ripening pods were covered with organza bags and all ripe pods were collected on a 2–3-day basis. We used deterrents such as coyote urine and reinforced any small gaps in the structures or chicken wire to prevent loss to small mammals. Nevertheless, gopher and ground squirrel pressure were heavy throughout the season. Damage from small mammals was recorded in each census. Heavy late season ground squirrel browsing led to substantial plant and fruit loss in both treatments. When possible, plant and fruit material found on the ground was matched to the remains of plants still standing. Total final pod counts include these pods taken from the ground.

Seed counts were taken from a subset of the pods collected directly from the plant. All plant and fruit material were collected at the end of the field season. Prior to collection, final counts of pods remaining on the plant were counted and separated into four categories: ripe pods, green pods, small pods, and aborted pods. Green pods were distinguished by being fully elongated with countable seeds inside, whereas small pods are not fully elongated, with viable seeds indistinguishable inviable ones.

Statistical Analysis

Unconditional aster models for lifetime fitness (Shaw et al. 2008) were used to evaluate the mean fitness of both generations of each population, in both treatments. The graphical model (Figure 4) for fitness included the number that of seeds that germinated from those planted, whether the plant flowered, and whether the plant produced fruit as Bernoulli distributions. Because each planting position had to be thinned to one seedling and the difficulty of collecting all pods before they explosively dehisced, two additional nodes were included to account for the subsampling at each of these stages. These two subsampling nodes were modeled with the Bernoulli family of distributions. The total number of pods produced by the plant and the total number of seeds from the pods that were collected were modeled with zero-truncated Poisson distributions. Block, treatment, population, generation, and soil disturbance prior to planting in blocks 2 and 4 of the ambient treatment were used as fixed factors in the model. Likelihood ratio testing was employed to determine which predictors best fit the data. The interaction of treatment and block as well as treatment and population were both significant and were therefore included in the model. Estimates of absolute fitness for each population were quantified using the median estimate among the five blocks, as in Kulbaba et al. (2019). These estimates were then transformed from the canonical scale to the mean parameter scale following Ryskamp & Geyer (2021).

In order to evaluate the impact of the different maternal plant environments in the two greenhouse years on offspring fitness, a secondary aster analysis was conducted using germination as the root node, rather than number of seeds planted. Because maternal environments may impact offspring germination timing and success (Donohue 2009), this supplementary analysis was an effort to isolate differences in germination success from the overall population fitness.

RESULTS

Immediate consequences of gene flow for fitness in 2020

Few plants survived to reproduce in the field experiment in 2020, and no plants from the pure MO population produced seeds. We did not detect a significant difference in mean fitness among the remaining populations in either the ambient or the warming treatments. We found that populations had moderately higher mean fitness in the ambient treatment than in the warming treatment (Figure 5) and that the ML x GC hybrid population, representing a local admixture approach to seed sourcing, had a higher mean fitness than the other populations. This hybrid population was the only one to have a mean fitness greater than one, which indicates population growth. This experiment had insufficient statistical power to detect differences between populations and treatments, which limits further inference.

Model selection

Likelihood ration test for the 2021 data set showed that block, treatment, population, and ground disturbance prior to the field season were all significant main predictors of fitness. Forward inclusion steps for interaction effects indicated that the interaction of population x treatment as well as the interactions of population x generation and block x treatment were significant (Table 1).

Local gene flow increased fitness in the first generation of hybridization

In both the warming and ambient treatments, all populations of the first generation (i.e., progeny sets from a single round of intra- and inter- population crossing) had a mean fitness above one (Figure 6). In the ambient treatment, the mean fitness of the ML x GC hybrid population produced in 2020 and in 2021, were higher than that of all other populations. The pure Missouri population (MO) had significantly lower fitness than all other populations. There was no significant difference among the remaining three populations: GC, IA x GC, and MO x GC.

In the warming treatment, the MO population had a significantly lower mean fitness than all other populations. The ML x GC hybrid population had higher fitness than all other populations. Differences between means of each other population and treatment combination were slight and overshadowed by their error bars. Additionally, the difference between treatments was stark in the pure MO population.

Mean fitness of second-generation was higher than first-generation due to higher germination

The trends observed for population in the first-generation were not maintained in the second-generation of each population (Figure 6). All populations had higher mean fitness in the ambient treatment as compared with the warming treatment. All populations, except ML x GC, had higher mean fitness than the first-generation in both treatments, including the non-hybridized populations. In a subsequent analysis that equalized the number of germinants by removing germination probability from the graphical model, the estimated difference between generations in mean fitness was reduced, indicating that the differences between generations are in part due to differences in germination rather than survival or reproductive success (Figure 7). This supplementary analysis also found decreased fitness of ML x GC in the second generation and higher fitness of MO x GC and MO than the focal GC population, indicating that poor germination in these long-distance crosses would likely present a barrier to population establishment.

DISCUSSION

In a common garden experiment, we evaluated the impact of gene flow across a range of geographic distances on fitness in a population of *C. fasciculata*, a prairie annual. We imposed a warming treatment on a subset of populations to assess if introgression improved fitness in temperatures corresponding to climate warming. We estimated mean lifetime fitness for all hybrid and non-hybrid populations and found that local gene flow within current seed transfer zones (ML x GC) increased fitness in both ambient and warmed environments compared to the pure focal population (GC) and all other hybrid populations in the first generation. In addition, seeds planted directly from the Missouri (MO) population had significantly lower fitness than all other populations, even when subjected to warmer temperatures that are more in line with the

MO population's adaptive history. However, mean population fitness differences were less pronounced in the second generation. Here we discuss the implications of these immediate effects of gene flow on population fitness in ambient and warmed environments and the dissolution of population level differences following one generation of recombination. We evaluate the role of germination on the measure of population fitness and consider how our results may inform future seed sourcing and restoration projects in this species and more generally for implementation of AGF.

Local admixture increases fitness in the first generation of hybridization

In the first generation of crossing between populations along a geographic gradient, gene flow over lesser distances (100 km) resulted in significantly higher fitness than all other populations in the ambient treatment. Gene flow over longer distances (340-480 km) did not, however, confer higher mean fitness than the pure focal population. Additionally, seeds directly sown from MO, the southernmost population, had significantly lower fitness than all other populations. The limited reproductive fitness of MO implies that direct seeding of southern populations, typically considered "pre-adapted", for AGF would not enhance the fitness of the focal population under the condition applied. Omnibus tests indicated that temperature treatment had a detrimental effect on fitness. Plants in the warming treatment had lower fitness than those in the ambient, though the difference was not substantial in all cases. The pilot experiment in 2020 yielded trends in population mean fitness, albeit not significant, that matched those seen in 2021. This consistency of fitness expression between years demonstrates persistent expression of the genetic differences among populations, despite inter-annual environmental variation.

Under warming conditions, population origin was a significant predictor of mean fitness for the first generation. As in the ambient conditions, ML x GC had significantly higher fitness than the focal population or long-distance gene flow and direct seeding from MO had significantly lower fitness. Gene flow on the local level likely increased heterozygosity, while maintaining adaptation to local conditions (Bucharova et al. 2019). Contrary to expectations of preadaptation to warmer environments, the MO population had significantly lower fitness in the warming treatment than all other populations. This suggests mismatch of both abiotic and biotic factors between Minnesota and Missouri. While temperature may mimic that of its home site, this study did not manipulate precipitation, soil type, or any biotic factors. Therefore, while the MO population may have a higher tolerance for warmer temperatures, it is possible that the totality of the environmental conditions present in this native prairie were unlike the conditions to which it has adapted in its home site over many generations.

The observed differences in mean fitness among populations were more modest in the second generation of crosses. Specifically, the second generation had higher mean fitness than the first generation. This is in contrast to both theoretical expectations that hybrid fitness should decrease in the recombining generation (Falconer and Mackay 1996) and empirical work in this species (Erickson and Fenster 2006). Therefore, we expected to find a similar pattern of population fitness for the hybrids in the two generations. Our findings suggest that the fitness benefits of local gene flow are most apparent in the first generation of hybridization. Further studies using additional generations must be conducted to understand the full long-term effects of hybridization on population performance.

Germination limits fitness of long-distance pairings

Each population had significantly higher mean fitness in the second generation. This increase does not reflect selection in the first generation as the second generation was produced using pedigreed crosses in the greenhouse. Because the fitness increase also occurred in non-hybrid populations and in the first generation of hybrids of ML x GC produced alongside the second-generation crosses in the greenhouse in 2021, it is likely that this increase reflects differences in the environmental conditions in the greenhouse during the crossing and seed maturation phases, i.e., a difference due to an environmental maternal effect. Maternal plants in the greenhouse in 2021 had far less pest pressure compared to those in 2020. Therefore, seeds produced in 2021 may have been better quality than those produced in 2020. These maternal effects could have downstream effects on offspring germination success, and therefore fitness (Platenkamp and Shaw 1993; Donohue 2009). Analysis without germination in the graphical model yielded smaller differences in F1 and F2 mean fitness, though it remained significant. It also resulted in the ML x GC population fitness decreasing in the second generation, as heterosis theory predicts. Further research producing first and second generations simultaneously would be needed to remove the differential maternal environment from the evaluation of fitness in two generations.

Our analysis excluding probability of germinating also led to an increase in fitness of our populations approximating an AGF scenario so that they were greater than the native Minnesota population and closer in fitness to, but did not surpass, the local admixture scenario that had the highest fitness. This indicates that germination may be the limiting life history stage for populations undergoing AGF from distant sources and that once plants are established, we may see some benefit to population size.

Implications for Assisted Gene Flow

Our results indicate that local and short distance gene flow provides the greatest benefit to population fitness in ambient and warmed conditions compared to longer distance AGF population pairings and populations not undergoing gene flow. Therefore, reinforcing local gene flow through conservation corridors and intentional hybridization would likely provide a benefit to fitness, and population growth, even in extreme thermal conditions. Our work contradicts the assumption that conservation practitioners will be able to fully take advantage of adaptation to local climates via introducing "pre-adapted" alleles from warmer areas that will promote fitness (Aitken and Whitlock 2013b). While we did not find AGF to be more beneficial than local gene flow under our experimental conditions, we also did not find that it was harmful as we saw no indication of outbreeding depression. Instead, we find that our results align with recent work on assisted migration that found that plants from warmer environments often did not have higher fitness in a warmer environment compared to the local population (Bucharova et al. 2019; Rushing et al. 2021; Gorton et al. 2022; Rushing 2022) and that concerns over outbreeding depression may not be warranted in this system (Frankham et al. 2011).

Introducing new genotypes into a focal population via pollen transfer may lead to faster changes in population fitness, as it eliminates the possibility that the new genotypes do not hybridize with the local population and ensures substantial numbers of hybrid offspring in the next generation. We found that the hybridized MO x GC population performed better or equal to the population of non-hybridized MO and that the MO population was more limited by germination success. Therefore, it may be beneficial to introduce new genotypes via pollen transfer rather than via directly seeded individuals. The latter approach entails delay of at least one season before crossing with the resident population. Removing this intermediate step would increase the likelihood of hybridization. Previous work has found that populations across a latitudinal gradient may have different phenology, such that natural reproduction may be limited (Wadgymar and Weis 2017; Rushing 2022). Additionally, the benefits of hybridization would occur earlier as practitioners would use individuals that are already flowering to create hybrid offspring, rather than waiting for planted individuals to reach maturity, which may take several years in more long-lived perennials.

Although the benefit of local gene flow was not maintained in the second generation of our crosses, the significant increase in fitness in the first generation may result in substantial increase in population size compared to other approaches. Larger populations likely harbor significant variation that may buffer them from the extreme climatic conditions we expect to encounter with climate change (Ellstrand and Elam 1993). Additionally, we saw no indication of outbreeding depression or other drawbacks of AGF other than a potential limitation to germination. Therefore, concerns that AGF may cause population decline may not apply in this system. Further work looking at natural reproduction in these populations is needed to make further inference on the long-term efficacy of AGF.

Conclusions and recommendations

This field-based study evaluating mean lifetime fitness of a focal Minnesota population undergoing local and assisted gene flow found that gene flow within existing seed transfer zones increased fitness in both current and predicted future temperature treatments. In second generation crosses, we found no effect of gene flow, but the potential increase in population size due to effects of local gene flow in the first generation provides evidence of its utility in restoration and conservation efforts. We found that germination limited fitness of AGF populations, while their fitness after establishment closely matches the local crosses. Thus, AGF may be a useful tool in situations once concerns about reduced germination can be addressed. There were no apparent drawbacks to AGF, but the limited germination in populations from further away and no measured benefits beyond the local crosses suggests that AGF may not be appropriate for this system, especially given the costs and time associated with such efforts.

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ILLUSTRATIONS

Table 1. Summary of comparisons of fixed effect aster models for 2021 experiment using likelihood ratio tests. The basic model included Block as a predictor (resp ~ varb + fit:Block). Each row corresponds to a model that includes all terms in the basic model in addition to the term presented and every term in the preceding rows. *P*-values of <0.05 are considered significant.

Models compared	**Test Df**	**Deviance**	***P*-Value**
resp~varb+fit:Block			
fit:population	5	39.41	1.97E-07
fit:treatment	1	12.09	0.0005
fit:generation	1	45.62	1.44E-11
fit:disturbed	1	350.2	2.20E-16
fit:population:treatment	3	13.16	0.004
fit:Block:treatment	4	182.6	2.20E-16
fit:population:generation	4	16.14	0.003
fit:treatment:generation	1	0.196	0.658

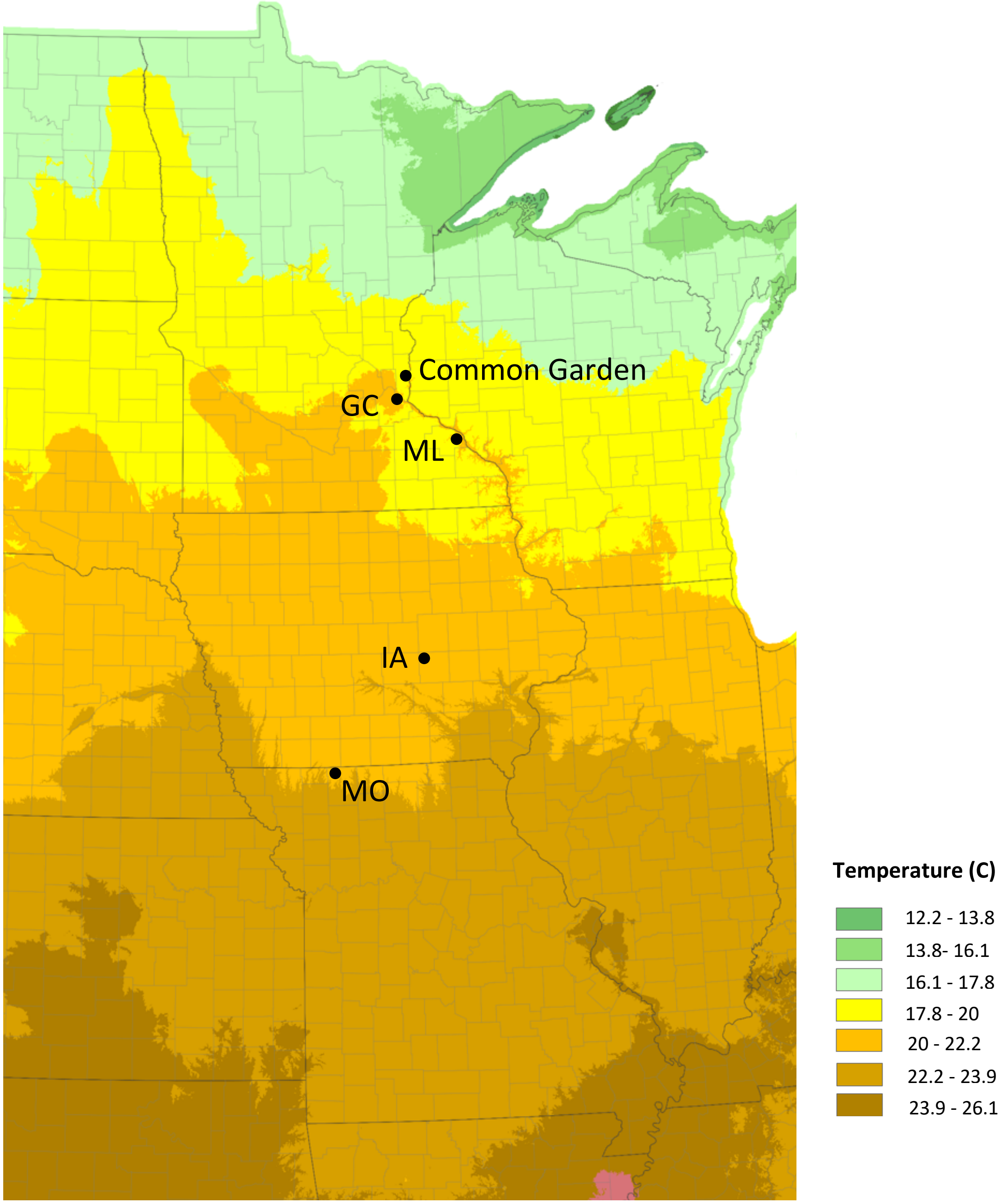

Figure 1. Map of the four populations and site of the common garden. GC is the focal population receiving pollen from the nearby ML population (McCarthy Lake) and from two populations with historically warmer climate conditions IA (Grinnell, Iowa) and MO (Dunn Ranch Prairie, Missouri). Field sites are overlayed on top of a temperature gradient map of 30-year normal mean temperatures for June (normal from https://prism.oregonstate.edu/)

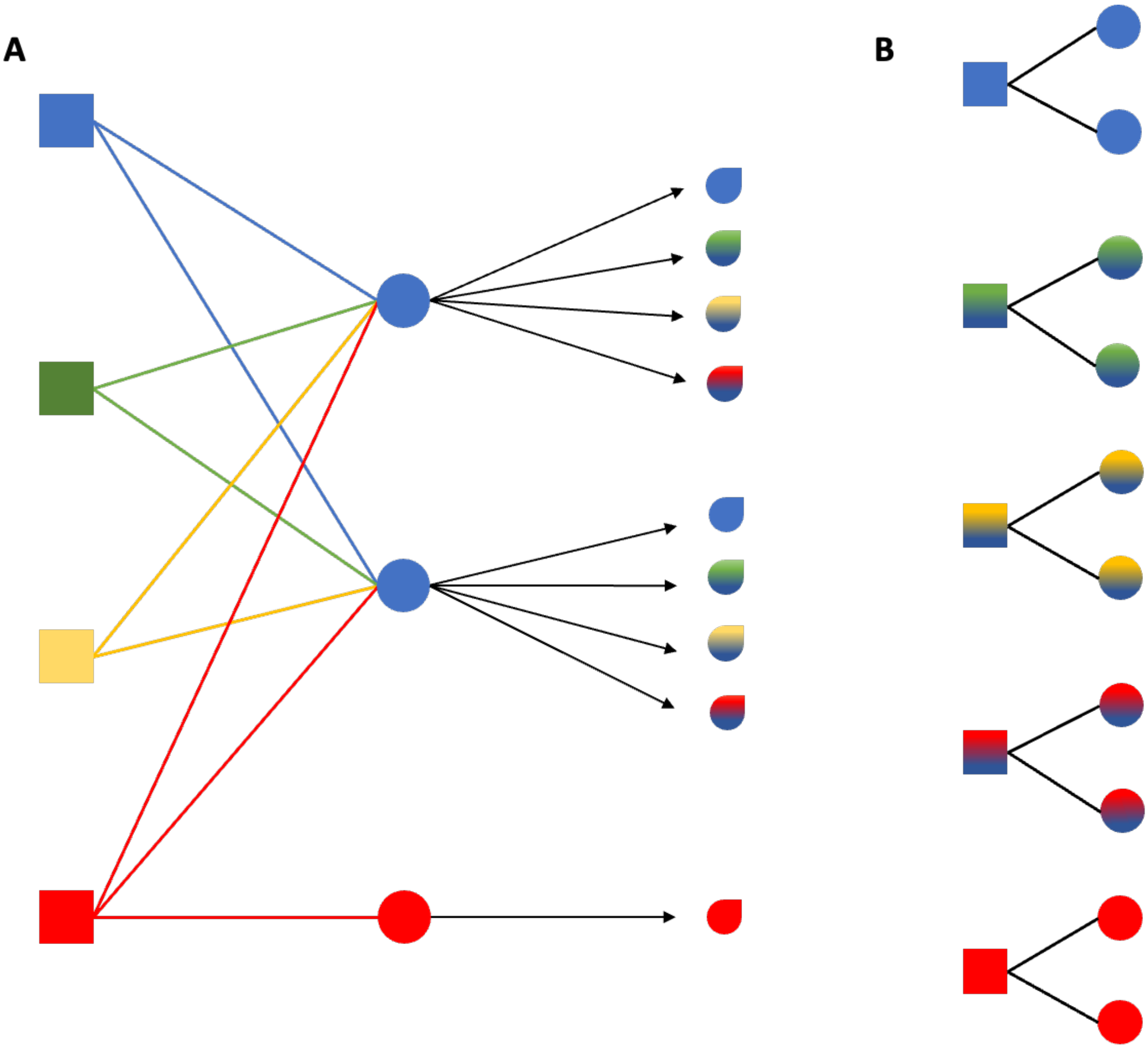

Figure 2. A) Diagram of the pedigree crossing structure for the first generation of hybridization. Squares represent pollen donors (sires) and circles represent pollen recipients (dams). Blue indicates individuals from the focal Grey Cloud population (GC), green represents the McCarthy Lake population (ML), yellow represents the Iowa population (IA), and red represents the Missouri population (MO). Hybrid offspring are represented as the shaded seed shape on the right of the black arrows. The seed shade colors indicate the parental origin of that seed. B) Diagram of crossing structure for the second generation. All crosses were conducted within the newly created populations using seeds produced in the first generation of crosses.

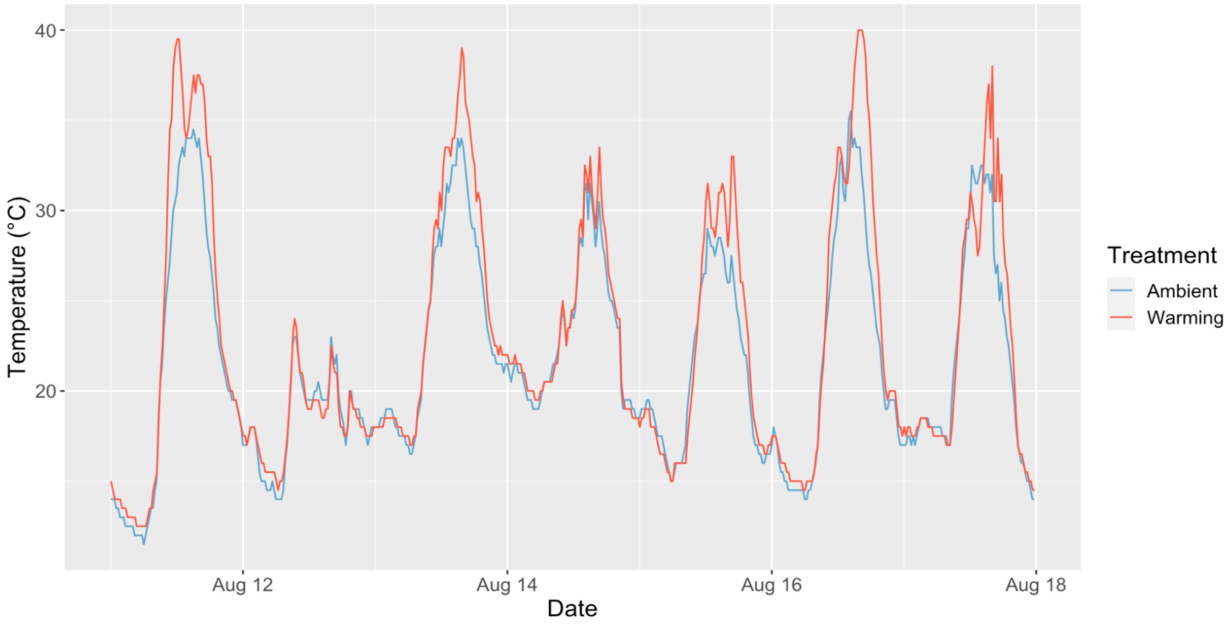

Figure 3. Temperature in Celsius of ambient and warming treatment plots over four days in August. The red line represents temperature in the warming treatment and the blue line represents temperature in the ambient treatment

$$\text{Seeds planted (1-4)} \xrightarrow{\text{Ber}} \text{germ}_{\text{total}} \xrightarrow{\text{samp}} \text{germ}_{\text{sub}} \xrightarrow{\text{Ber}} \text{surv}_{\text{flowers}} \xrightarrow{\text{Ber}} \text{surv}_{\text{pods}} \xrightarrow{\text{0-Poi}} \text{pods}_{\text{total}} \xrightarrow{\text{samp}} \text{pods}_{\text{sub}} \xrightarrow{\text{0-Poi}} \text{seeds}_{\text{total}}$$

Figure 4. Graphical model of lifetime fitness used to estimate mean fitness and standard error of all populations, treatments, and generation. Life history stage is indicated between the arrows as germ, surv, pods, and seeds meaning germination, survival, fruit production, and seeds produced respectively. Statistical distributions and subsampling are noted above the arrows with Ber, 0-Poi, and samp as Bernoulli, 0-truncated Poisson, and subsampling respectively.

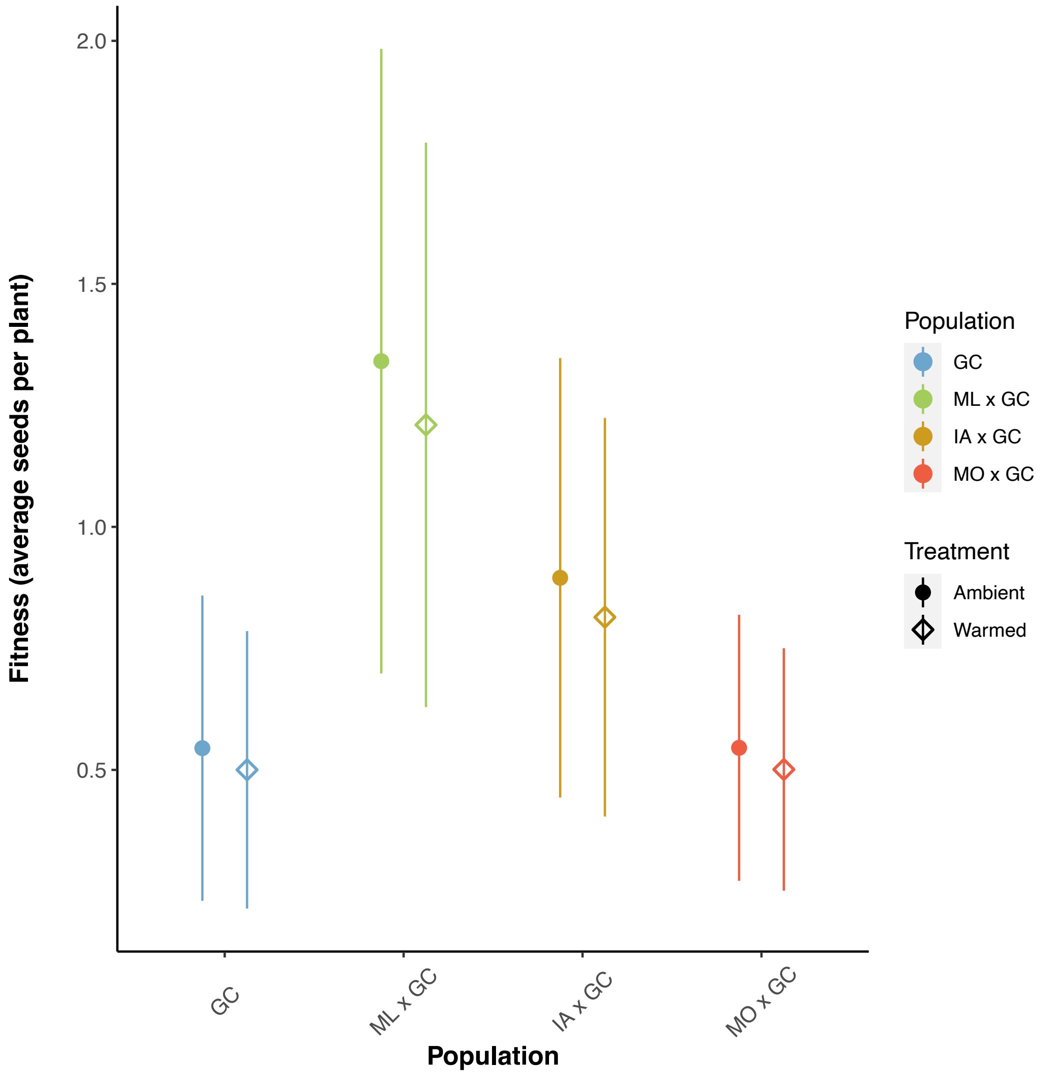

Figure 5. Mean population fitness from fixed effects aster models of all populations in both treatments planted in 2020. Color represents population and shape of data point represents treatment. Error bars represent standard error. Results from the pure Missouri population were omitted as it had a fitness of zero.

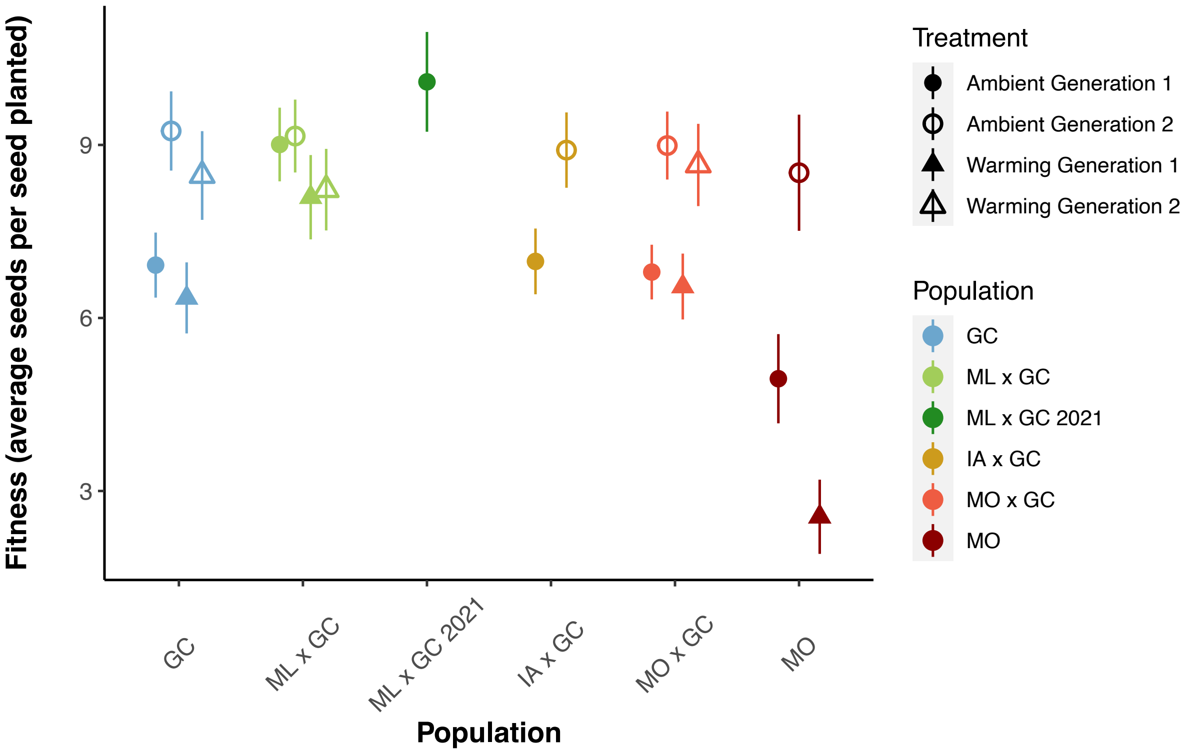

Figure 6. Mean fitness produced from fixed effects aster models for both generations and treatments. The shape represents treatment and generation, with circles representing ambient and triangles representing warming. Filled shapes are the first generation of a population and open shapes are the second generation. Error bars represent standard error.

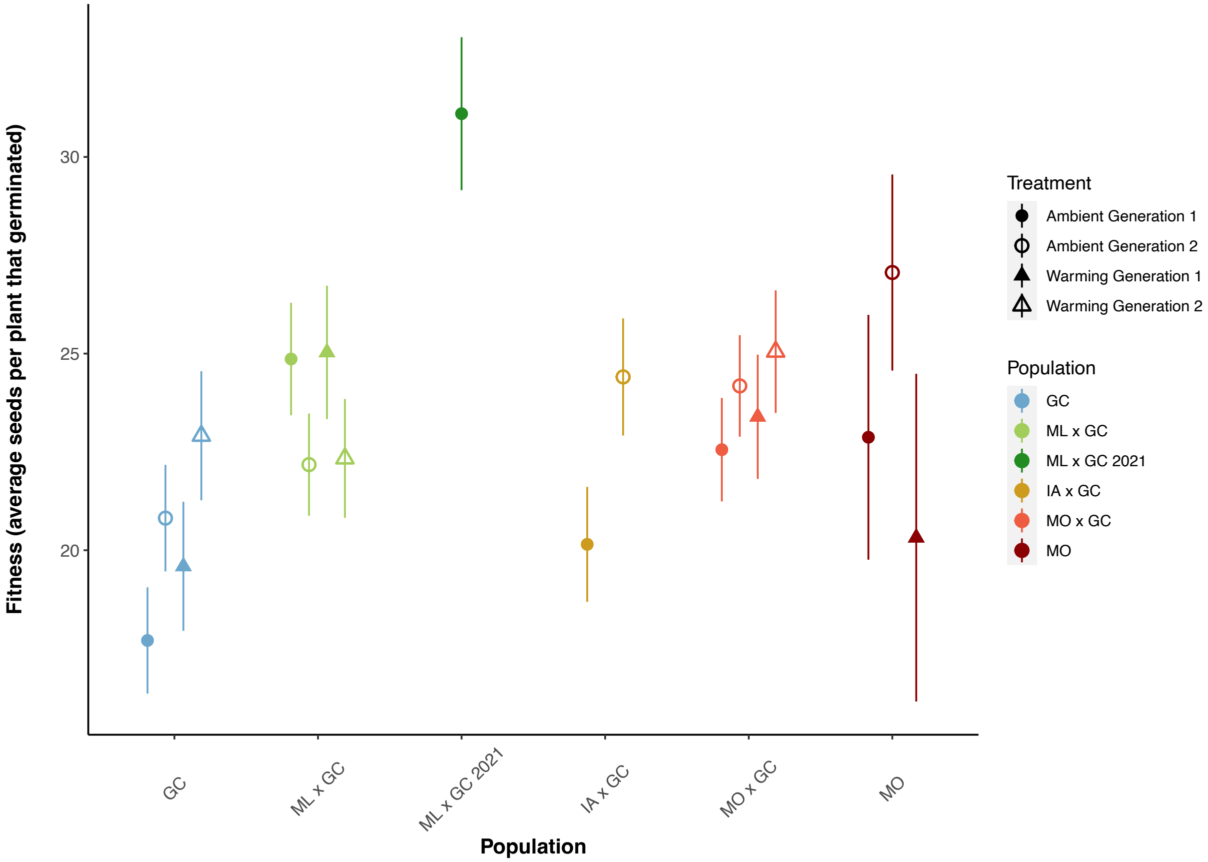

Figure 7. Mean fitness produced from fixed effects aster models for both generations and treatments accounting for differences in germination by beginning aster model with root=germination. The shape represents treatment and generation, with circles representing ambient and triangles representing warming. Filled shapes are the first generation of a population and open shapes are the second generation. Error bars represent standard error.

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CHAPTER 2

Evolutionary consequences of population hybridization along a geo-climatic gradient

SYNOPSIS

Climate change poses a significant risk to species' persistence and, in turn, ecosystem stability. Adaptation *in situ* will be crucial for population survival. Populations with greater genetic diversity likely have greater capacity to adapt to rapid environmental change. Hybridization between populations may increase diversity and adaptive capacity. If hybridizations occurs with a population that has undergone selection in environments similar to those experienced in climate change, hybridization may have the added benefit of introducing beneficial alleles that enhance fitness under those conditions. However, crossing over larger geographic and genetic distances may cause populations to decline due to outbreeding depression. This study investigates the spatial scale at which hybridization increases fitness and adaptive capacity as well as the context dependency of the environment on these effects in the widespread species *Chamaecrista fasciculata* under simulated warming. I estimated mean fitness ($\bar{W}$) and additive genetic variance for fitness ($V_A(W)$) of hybrid populations along a geo-climatic gradient in the first and second generation after hybridization and in both ambient and warmed conditions. My results indicate that hybridization increased mean fitness and $V_A(W)$ compared to the non-hybridized population, with the hybridization between the populations that are geographically nearby showing greatest benefits. Seeds sown from the longest distance source population had the lowest fitness, while both this long-distance population and the non-hybridized local population had non-detectable $V_A(W)$. These results suggest that although hybridization increases adaptive capacity under warming its effects on fitness are distance dependent. This study demonstrates the importance of investigating the effects of hybridization beyond fitness consequences in the first generation and more fully understanding the impacts of introgression on adaptation to changing conditions.

INTRODUCTION

The persistence of many species through rapid climate change may depend on their dispersal to more hospitable environments and their capacity to adapt to ongoing change, if they are unable to tolerate the increasing climate severity. While there is evidence that some species ranges have shifted, tracking their climatic envelope (Parmesan 2006; Chen et al. 2011; Freeman et al. 2018), dispersal may be unlikely for many species as their movement and migration is dependent on environmental factors or animal movement (Marsico and Hellmann 2009). Therefore, their capacity to adapt *in situ* will be the most important factor to their long-term persistence, and it will certainly be necessary for population persistence (i.e. particular, local forms of a species). Although some populations may harbor substantial additive genetic variation for fitness (i.e. the genetic variation within a population available for selection to act on (Conner and Hartl 2004; Sheth et al. 2018; Kulbaba et al. 2019), others may be more limited due to long term isolation, genetic drift, and inbreeding (Ellstrand and Elam 1993). Restoration of gene flow between populations will likely be highly important for these populations, as well as the injection of specific genes that benefit fitness under changing conditions. However, while natural hybridization among local or nearby populations will relieve many negative effects of inbreeding, it may be inadequate in maintaining populations under large-scale, rapid climate change and other novel environmental conditions (Aitken and Bemmels 2016). Instead, human mediated hybridization across long distances and with populations that have historically lived in warmer conditions, known as assisted gene flow (AGF), may introduce adaptive alleles that are necessary for survival in the new conditions (Aitken and Whitlock 2013b). This assisted gene flow may increase the capacity for adaptation by introducing specific warmth-adapted genes, general variation from a warmth-adapted population, or by simply increasing genetic variation in general.

There are several conservation strategies that attempt to harness the benefits of gene flow and hybridization to increase population performance and evolutionary potential, including regional admixture and assisted gene flow. In both cases, expectations of population persistence center on the benefits of hybridization, both in the immediate term with genetic rescue resulting from heterosis and the long-term benefits of evolutionary rescue resulting from higher standing genetic variation. Both strategies introduce allelic diversity and increase heterozygosity, thereby decreasing inbreeding depression and increasing population size, genetic diversity, and integrity (Hoffmann et al. 2021). However, the two differ in the source and supposed context of the genetic diversity they introduce. Regional admixture approaches combine multiple local populations to effectively reduce inbreeding depression and increase population size, with the expectation of maintaining adaptation to local conditions (Bucharova et al. 2019; Nolan et al. 2023). Assisted gene flow (AGF) also aims to facilitate adaptation but presupposes that local gene flow would provide inadequate genetic diversity and specific beneficial traits that are pre-adapted to warmer conditions, therefore providing fitness benefits under climate change. AGF theoretically capitalizes on alleles from populations that have undergone selection in environmental conditions most similar to those that receiving populations will experience due to climate change (Aitken and Whitlock 2013b). As such, AGF would introduce a suite of genes that are different from the target population and would provide a wide range of genetic variation and potential for adaptation to novel conditions. Empirical studies have found evidence that moving populations northward or to higher elevations increased fitness when climate change disrupts a population's local adaptation to its environmental conditions (Chen et al. 2011; Freeman et al. 2018; Gorton et al. 2022). Thus, even genetically diverse populations may be vulnerable to climate change and may have higher fitness if interventions like AGF track their climatic envelope.

Geographic and genetic origin of hybridized populations can have large effects on the fitness of offspring and long-term population success. On the one hand, hybridization of populations across large geographic areas may produce offspring that are less fit than their parents due to outbreeding depression. On the other hand, this new genetic variation may be beneficial if conditions become markedly different from the historic climate conditions. Geographically distant populations are likely to be genetically distant, such that hybridization may disrupt co-adapted gene complexes, introduce maladapted alleles, and interrupt local adaptation (Edmands 1999; Fenster and Galloway 2000b; Bridle and Vines 2007). The negative effects of hybridization theoretically decrease as populations are closer together, with closer pairings having increased heterozygosity but also minimal negative side effects from outbreeding (Lynch 1991). This increase in fitness when compared with the parental populations is likely maintained even in the subsequent recombining generations, when fitness is expected to decrease from the initial generation due to increased homozygosity (Falconer and Mackay 1996). The effects of hybridization across long distances may not be realized for many generations because the impact of hybridization is dependent on the number of loci involved, the magnitude of their effect, their environmental conditions, and the number of hybrid individuals introduced to a native population, among other specifics (Grummer et al. 2022). As such, while the general expectation that geographically distant populations are also likely genetically distant and therefore hybridization may result in lower fitness, the relative impact of hybridization is likely population specific and temporally variable. Consequently, it is imperative that work assessing hybridization investigates its impact beyond the fitness of the initial population and examines subsequent generations and their capacity to adapt to particular environmental conditions.

Theory suggests that populations in their native habitat undergo consistent natural selection and therefore should have little to no additive genetic variation for fitness ($V_A(W)$) (Fisher 1930; Bradshaw 1991) but empirical work has shown that even populations in their source sites have significant $V_A(W)$ (Fowler et al. 1997; Kulbaba et al. 2019). This detectable and significant $V_A(W)$may be attributable to many phenomena, including changing trait optima due to interannual environmental variation (Zhang 2012). Furthermore, there is increasing evidence that standing genetic variation is still detectable when populations are exposed to novel environments (Hoffmann and Merilä 1999; Etterson 2004a; Zhang 2012; Sheth et al. 2018; Torres-Martínez et al. 2019; Peschel et al. 2021).

Yet, there is ongoing debate as to whether that V_A for fitness and other traits will persist in a given population considering the high stochasticity and extreme conditions expected under current climate change predictions (Hoffmann and Merilä 1999; Pain, 2024; Chapter 1). Some studies have found that $V_A(W)$ was maintained even under rapid change (Bradshaw and Holzapfel 2001; Franks et al. 2007; Sheth et al. 2018; Walter et al. 2023), while others indicate a reduction in $V_A(W)$ due to novel environmental conditions limiting growth rates (Gebhardt-Henrich and Van Noordwijk 1991). Based on this empirical work, $V_A(W)$ is unlikely to be zero, but populations may still benefit from hybridization as it may increase $V_A(W)$ via introduced genetic variation. Higher genetic variation could lead to increased fitness benefits many generations later and under different and stressful environmental conditions, such as increased temperature and drought. Therefore, we must consider the impact of hybridization beyond fitness and focus our attention on its consequences for the capacity for to adapt to novel environments.

To examine the effect of hybridization across a thermal gradient and fitness and $V_A(W)$ and its relationship to the environmental conditions, I used a common garden approach in the tallgrass prairie system of the central United States of America, where a subset of plants were exposed to a warming treatment. Although many prairie plant species are wind pollinated and dispersed, a large subset are insect pollinated and pollen movement beyond their immediate population area is restricted to chance animal encounters. Therefore, insect-pollinated plant species' reproductive pools are generally restricted to a smaller area than wind pollinated plants, and we expect gene flow and migration to be limited. These factors are especially limited in highly fragmented landscapes such as the tallgrass prairie of the United States, where the geographic area has been restricted to less than 1% of its original extent (Sampson and Knopf 1996). Previous discussions of the adaptive capacity of species in this system have suggested that increasing gene flow and using assisted gene flow may be necessary given these constraints on natural movement and gene flow (as in McKone and Hernández 2021). As such, I used the annual legume *Chamaecrista fasciculata,* to examine the environmental and geo-climatic context (i.e. climate factors that vary along a geographic gradient) of gene flow under climate change. I worked with a focal population in central Minnesota and three other isolated populations to create hybrid populations along a geo-climatic gradient (populations varying in both distance and climate) and exposed them to ambient and warmed conditions to answer the following questions: 1) At what geo-climatic scale does hybridization increase a population's fitness and adaptive capacity? And to what extent do those two measures of performance coincide under ambient and altered environments? 2) To what extent are the impacts of hybridization environmentally dependent when conditions are more similar to the donor parental population's mean thermal conditions?

MATERIALS AND METHODS

Study system

I estimated mean fitness and $V_A(W)$ of *Chamaecrista fasciculata* populations hybridized across a geographic gradient and planted into a restored prairie. *C. fasciculata* is a hermaphroditic, bee-pollinated annual legume with geographic range that spans from Central Mexico in the south to the northern great plains in Minnesota and from the central prairie regions to the eastern seaboard of North America (Irwin and Barnaby 1982). Because it is an annual, I am able to estimate lifetime fitness, unlike most prairie plant species which are perennial. Its experimental tractability and long history as a study system for work in quantitative genetics (Kelly 1993; Etterson 2004b; Kulbaba et al. 2019), gene flow (Fenster and Galloway 2000b; Erickson and Fenster 2006; Stanton-Geddes et al. 2012, 2013; Wadgymar and Weis 2017), adaptation to environmental change (Etterson and Shaw 2001; Sheth et al. 2018; Peschel et al. 2021), and mutualisms (Stanton-Geddes and Anderson 2011; Keller 2014; Pain et al. 2018) make it an ideal study system to evaluate the effects of gene flow on population performance and capacity for adaption to future climate conditions.

I conducted an experiment to elucidate the impact of past selection to warmer temperatures on a hybrid population's performance under climate warming. I used a population from Grey Cloud Dunes SNA in central Minnesota ($\mathrm{MN_{central}}$) as the focal population for this study (Figure 1). I used a second population from southern Minnesota (100 km south of $\mathrm{MN_{central}}$) as a pollen donor to generate a hybrid population representing gene flow from relatively nearby location, but one at sufficient distance to preclude recent interbreeding between the populations. In addition, I used populations from two other sites that are within the regions that are most like the climate conditions expected for south-central Minnesota. I selected a population from the Conard Environmental Research Area in Kellogg, Iowa to reflect a lower emissions scenario with a temperature increase of 2 °C, i.e., similar to historical conditions of central Iowa (Figure S1). I selected a population from Dunn Ranch Prairie in northern Missouri as the longest distance gene flow population, and this distance reflects a higher emissions scenario with a temperature increase of 3.6 °C (Fitzpatrick and Dunn 2019). I used the same protocols and experimental designs as described in greater detail in Pain (Chapter 1, 2024).

Greenhouse

For both Minnesota populations, I used pedigreed seed produced in 2014 for experiments reported in (Sheth et al. 2018; Kulbaba et al. 2019).To sample the populations at the Conard Experimental Research Area and Dunn Ranch Prairie, I collected live seedlings. I created a first generation of pedigreed hybrid and pure populations in 2019 and a second generation for each of those populations in 2020. The second generation was produced using full siblings of the individuals representing the first generation of the experiment. Both generations of all populations and hybrid populations were produced in the greenhouse under 16:8 light exposure. Each population and generation were manually crossed using a nested half-sibling design, with two dams per sire. To reduce confounding maternal genetic effects, each population in the first generation was used as sires to pollinate the same nested dams of the $MN_{central}$ population. To examine the effects of hybridization in a recombinant generation (second generation), I crossed within populations rather than between populations. This work produced two generations of each of the five populations. The populations are as follows: non-hybridized focal population ($MN_{central,}$), a local hybrid population (MN_{south} x $MN_{central}$), mid-distance hybrid population (IA x $MN_{central}$), long-distance hybrid population (MO x $MN_{central}$), and a long-distance pure population (MO).

Mean fitness and $V_A(W)$ in natural prairie

Field installation

Two generations of each population were planted into an existing prairie matrix vin both ambient and warming treatments in five blocks, apart from the Iowa population and second generation of the pure Missouri which were only planted in the ambient environment due to space constraints inside the warming treatment. All families of each of the populations represented in the experimental plots were randomized across all planting positions. I trimmed all aboveground biomass prior to planting to ensure that all seeds were planted in the ground and to ease the installation of the warming treatment. I planted up to four seeds per planting position along 1.25 m rows, such that ambient plots had up to 426 planting positions and warming plots had up to 272 planting positions. The ambient plots in blocks 2-4 had substantial ground disturbance from gopher activity prior to planting, leaving those areas devoid of vegetation. Positions affected by this disturbance were noted prior to planting.

For the warming treatment, I designed open top chambers based on Welshofer et al. (2018). However, I replaced the double panel sides with a single panel to make it more stable in high winds and adjusted the side lengths to accommodate a 1.25 m^2 plot (Pain, 2024; Chapter 1).

Warming treatments were installed immediately after planting. To account for the reduction in rodent and other small animal movement in the warming treatment, I installed plastic lined chicken wire fencing around ambient plots at the same time as the warming treatments. Temperature sensors were placed in the northwest corner of both ambient and warming plots of block three. The warming treatment increased daytime temperatures by an average of 1.9 °C and daily average temperatures by 1.2 °C.

Due to an unprecedented early drought, in which the precipitation levels in June at the experimental site were 17.2% of the average rainfall amounts, each plot was watered four times during June and July. I watered plots when they had not received any rainfall within a 10-day period as this coincided with observed plant water stress and death. Each plot received approximately 1.5 cm of water during each water addition.

Fitness component data collection

Each planting position was censused five times throughout the growing season. I conducted two additional censuses only on positions where plants were marked present in previous censuses. During the first census, I thinned each position from the original 4 seeds planted to the seedling that was closest to the planting marker. Each census assessed plant germination or survival, as well as later fitness traits such as flowering and fruit production. *C. fasciculata* explosively dehisces. For this reason, pods were collected from plants every 2-3 days so that I could obtain accurate fitness estimates of each individual plant.

Data Analysis

Data was analyzed using aster analysis for lifetime fitness (Shaw et al. 2008) with the “aster” package (Geyer 2021) in the R 4.2.1 (R Core Team 2023). Aster analysis accounts for the non-normal distribution of lifetime fitness measures through joint analysis of individual fitness components, each modeled according to its own distribution and accounting for its dependence on prior fitness components. Germination, flowering, and presence of fruit were modeled with Bernoulli distributions, whereas number of pods produced, and number of seeds produced were modeled with zero-truncated Poisson distributions. Because I thinned my initial planting to one seedling and was unable to capture all fruit produced, estimates of mean fitness account for subsampling at both of those nodes in the model with Bernoulli distributions (Figure 2). I used mixed effects models to estimate mean fitness and additive genetic variance for fitness with block as a fixed effect and parental identity as a random effect with both generations of each population in both treatments analyzed separately. I included soil disturbance as fixed effect in analysis of populations in the ambient treatment because gopher soil disturbance was limited to this treatment.

To evaluate genotype by environment interaction, I conducted an initial analysis within a single generation of each population. I used likelihood ratio tests to determine the significance of fixed effects and included parent identity and the interaction of treatment and parent identity as random effects. I quantified the effect of genotype and the interaction of genotype and environment, as well as their statistical significance. For each generation of each population in both treatments, I used the median block to estimate mean population fitness as in Sheth et al. (2018). Because I thinned germinants from the planting positions and was unable to collect all pods for final seed counts, I accounted for this subsampling by multiplying together the conditional estimates for all non-subsampled nodes of my graphical model and thus obtained an unconditional estimate of mean fitness for each population as in Ryskamp & Geyer (Ryskamp and Geyer 2021). To obtain estimates of mean fitness and $V_A(W)$ and standard error on the mean parameter scale, I used a mapping function and the delta method (Kulbaba et al. 2019; Ryskamp and Geyer 2021). Estimates of $V_A(W)$ greater than zero is evidence that a population has the capacity for ongoing adaptation, whereas a non-detectable $V_A(W)$ indicates insufficient evidence of adaptive capacity. I assessed differences in mean fitness between populations, generations, and treatments using maximum likelihood ratio testing of a fixed effects model that included all factors in chapter 1 and visually assessed those differences using standard error bars.

RESULTS

Genotype by treatment interaction

There was little evidence of genotype by treatment interaction in the populations represented in this study. Genotype was significant for both generations within each hybrid population, except for MN_{south} x $MN_{central}$ and MO x $MN_{central}$ in the warming treatment. Genotype was either non-detectable or estimates were not significant each generation and treatment for the

$MN_{central}$ and the MO populations (Table 1). Significant genotype interaction indicates a consistent rank and difference in families between treatments, whereas non-significance indicates that all genotypes have essentially the same trait value. The only population that had detectable genotype by treatment interaction effects was the second generation of MN_{south} x $MN_{central}$ (0.003, p=0.1982). As such, this population is the only one in which families have differential fitness in each treatment.

Fitness impacted by gene flow

Fitness estimates from aster models that account for parental effects indicated that the first generation of the local hybrid scenario (MN_{south} x $MN_{central}$) had higher fitness than all other populations. For example, MN_{south} x $MN_{central}$ had a $\overline{W}$ of 9.90 ± 1.96 in the ambient compared to the next highest fitness of 6.68 ± 1.29 in the IA x $MN_{central}$ population (Table 1). However, there were no apparent differences in fitness among populations in the second generation. Thus, while the local hybrid pairing increased fitness in the first generation, its benefits were not realized in the recombining generation. While fitness estimates were similar or slightly lower in the warming treatment compared to the ambient treatment in the first generation, they were slightly higher in the second generation for the $MN_{central}$ and MN_{south} x $MN_{central}$ populations (Figure 3a), increasing from 5.98 ± 1.44 to 12.2 ± 2.90 and 7.90 ± 1.44 to 11.0 ± 1.92, respectively. Therefore, the impact of the treatment on fitness was inconsistent between generations.

Capacity for adaptation

Apart from the pure MO population, additive genetic variance for fitness $V_A(W)$ was detectable (i.e. estimated as greater than 0) and significant for all populations in the ambient treatment. The greatest $V_A(W)$ was in the first generation of MN_{south} x $MN_{central}$ population (91.2 ± 39.4), with both other hybrids and generations having $V_A(W)$ between 5 and 20. Non-hybridized populations have $V_A(W)$ below 5 (Table 1). In contrast, in the warming treatment, $V_A(W)$ was only detectable (yet not significant) for two of the populations: the first generation of MN_{south} x $MN_{central}$ and the second generation of MO x $MN_{central}$ (Figure 3b, Table 1). Therefore, the thermal treatment effectively reduced variation in each non-hybrid population, while the hybrid populations harbored measurable $V_A(W)$ depending on the genetic mixture of the given generation.

DISCUSSION

In the present study, hybridization increased the capacity for adaptation in a warmed environment compared to the non-hybridized focal population. Hybridization via assisted gene flow may theoretically increase fitness via increased variation, introduction of specific pre-adapted traits, or increase variation in the direction of selection. Here I discuss the impacts of gene flow on a population's capacity to adapt to changing climate conditions, especially focusing on the practice of human- assisted gene flow and evaluate the context dependence of these impacts.

Hybridization of local populations (up to 100km between populations) that is consistent with current seed sourcing standards in Minnesota increased mean fitness compared to the pure focal population (Minnesota Department of Natural Resources 2015). This finding is supported by the outcome of the fixed effects analysis of these data in Pain (2024, Chapter 1), in which the local hybrid population had the highest fitness in both treatments. This fitness benefit of interpopulation crossing, i.e. heterosis, implies parental populations were diverged such that hybridization increased heterozygosity and led to higher mean fitness in these hybridized populations. These genetic consequences of crossing evidently override any (possibly slight) fitness deficit that could result from introducing the alleles from the foreign population to the experimental site (i.e., local adaptation).

Based on AGF expectations, the benefit of gene flow from populations in warmer locations should be realized in the warmed environment, which was more similar to the past selective environment of that long-distance source population. However, their fitness was greater only in the second generation of the ambient population compared to other populations. Therefore, the benefit expressed in fitness is likely due to genetic rescue (Richards 2000) and may not be attributed to the introduction of "pre-adapted" alleles. Whereas direct transplants are expected to perform well in both the ambient and warmed environment when faced with the extreme thermal conditions in this field experiment, individuals from the most distant, non-hybridized MO population had lower fitness compared to other populations. This was especially true in the warming treatment where temperatures exceeded the 30-year means for the site where MO was collected by over 2.5°C, but also in the ambient treatment where temperatures exceeded the 30-year means by over 1.5 °C. Contrary to expectations of increased tolerance and adaptation to warmer environments (McKone and Hernández 2021), these findings indicate that long-distance hybridization did not produce individuals better adapted to warmer environments. Thus, although hybrid populations had generally had higher fitness than the focal population, this is likely attributed to increasing heterozygosity rather than introducing alleles that enhance fitness specifically with respect to warming.

Contrary to expectations of heterosis, the generation after hybridization (i.e. my second generation) had higher fitness than the initial hybrid generation for all populations other than the MN_{south} x $MN_{central}$ population. Under a simple dominance model, we may expect the F_2 generation to have lower fitness than its parents (i.e. the F_1), decreasing to halfway between the fitness of the first generation and the mid-parent value (Falconer and Mackay 1996). Instead, my results paint a more complicated picture as they suggest one or more of several phenomena may be at play. The first possible explanation for higher fitness of the F_2 is epistatic effects, wherein interactions among genotypes at different loci may lead to higher fitness than expectations under models that allow for only additive and dominance effects at individual loci. However, this does not account for the parallel increase in fitness in the two non-hybrid populations in the second generation. The second, and potentially more likely cause, is that maternal environments differing between the generations contribute to the differences in mean fitness. The two generations of seeds were produced in two separate years. Although every effort was made to ensure that the conditions were the same over the two separate years, it is impossible to account and correct for all environmental differences. For example, the second generation faced much lower insect pressure because of the difference in the plants in neighboring greenhouses. It is possible that the second generation of seeds was produced under more favorable conditions that resulted in better provisioning of seeds and greater seed viability. Previous studies have found that seeds from larger maternal plants and those with fewer competitors and less stress have higher germination success and fitness (Roach and Wulff 1987; Platenkamp and Shaw 1993; Donohue 2009). The third is that the two generations were produced in separate years and the first generation may have simply exhibited signs of age. Although I stored seeds in low humidity and in refrigeration, older seeds can exhibit decreased germination and vigor (De Vitis et al. 2020). Both maternal environmental effects and seed age may be especially likely given that analyses in Chapter 1 indicated that failure to germinate was a major contributor to lower fitness in the first generation. These potential explanations make interpretation of the effects of hybridization between generations challenging, but they should have little bearing on interpretation within a single generation of population performance and variation.

Gene flow increased $V_A(W)$ compared to the $MN_{central}$ population for all hybrid combinations in the ambient conditions, with estimates 2-50x those of the pure focal ($MN_{central}$) population. While $MN_{central}$ had the lowest detectable $V_A(W)$, the pure MO population had no detectable $V_A(W)$. Thus, whereas the mean fitness of the pure MO population is above replacement, our evidence raises doubt that it has the capacity to adapt to the conditions in Minnesota. Higher $V_A(W)$ in hybrid populations is a manifestation of genetic selection in that population and implies an increase in the capacity to adapt to the present conditions and an increase in fitness in the next generation. Using Fisher's Fundamental Theorem of Natural Selection (Fisher 1930), we predict that progeny of the hybrid generation in the ambient environment should produce on average 0.8-9 more seeds than their parents. However, the pure $MN_{central}$ population in the ambient and the two populations with detectable $V_A(W)$ in the warming treatment are predicted to increase by less than 0.5 seeds (Table 1). This difference in predicted mean fitness of the progeny generation exemplifies the importance of interrogating the effects of gene flow beyond the immediate measurement of mean absolute fitness and work to understand its impact genetic variation and the potential genetic change in mean fitness. Populations whose mean fitness appears to suffice for their persistence may have low to no variation and are therefore vulnerable to changing environments.

Our results show that $V_A(W)$ decreased in a stressful environment. $V_A(W)$ in all populations declined in warmed environments relative to the $V_A(W)$ in the ambient conditions, with most having non-detectable $V_A(W)$. Only a single generation of the short (MN_{south} x $MN_{central}$) and long-distance (MO x $MN_{central}$) gene flow scenarios had detectable, yet not significant $V_A(W)$. This finding is contrary to many studies which have found that novel or stressful environments increase $V_A(W)$ (Hoffmann and Merilä 1999; Sheth et al. 2018; So et al. 2022). It might be that these conditions were so unfavorable that the full growth potential of an individual was not realized, subsequently decreasing variation (Hoffmann and Merilä 1999). This may be likely as the mean temperature of the ambient conditions in the common garden was about 1.5-3 °C warmer in June than any of the home sites, with maximum temperatures exceeding 3-4.3 °C warmer than home sites (Figure S2). These high temperatures, compounded with the warming treatment temperature increase of about 1.9 °C, made for extreme environmental conditions that may have led to a severe reduction in $V_A(W)$ of all populations subjected to the warming treatment. Because the majority of the populations had no discernable difference in the response of genotypes to the imposed condition (i.e. genotype by treatment interaction), and no $V_A(W)$, the response to this novel environment is plastic rather than selective. Therefore, these populations are illustrating a tolerance to the environment rather than an adaptation to the thermal conditions.

Although temperature was the only environmental attribute explicitly manipulated for this experiment, warming may have impacted other unmeasured factors that could have contributed to my findings. For example, studies have found that warming treatments increased aboveground productivity of some legume and C4 grass species (Cowles et al. 2016), which could have increased crowding by heterospecifics such that plants were competing for light or the limited water available in the soil. Previous work with this type of open top chamber found few impacts on other environmental conditions such as humidity and soil moisture, but that these findings may be context specific (Welshofer et al. 2018). Nevertheless, the effect of extreme temperature and potentially other correlated environmental conditions dramatically reduced $V_A(W)$ in all populations. Nevertheless, in some instances, hybridized populations had detectable $V_A(W)$ in the warming treatment, an indication that increasing genetic diversity could improve a population's capacity to adapt to harsh environmental conditions.

When considering whether a population can withstand the effects of climate change, it is important to look beyond the immediate impact on mean fitness. The capacity for ongoing adaptation, $V_A(W)$, also varies with environmental stress, yet the full consequence of the environmental conditions may not be detectable in simple population counts. Upon further examination, under stressful environments only some generations of hybridized populations had detectable $V_A(W)$. This is likely due to the specific combination of alleles and environmental conditions in the natural prairie impinging on these individuals. These results indicate that while we may detect capacity for adaptation, these estimates are heavily influenced by the environmental and genetic conditions of the experiment. The vagaries of field-based estimations of $V_A(W)$ are born out in the debate over the effect of gene flow and environmental stress on $V_A(W)$. The opposing findings of increased vs decreased $V_A(W)$ under environmental stress may be due to a variety of contextual differences, but is also likely due to differences in the direction and magnitude of stress (Hoffmann and Merilä 1999; Torres-Martínez et al. 2019; Walter et al. 2023). Consequently, considerations of the effect of dramatic environmental change on population performance should include this uncertainty.

The year this study was completed was one of the hottest summers on record (NOAA National Centers for Environmental Information 2023), and so the conditions during the experiment were outside the historic averages for all populations (Figure S1). These extreme conditions complicate inference of the impact of gene flow on population mean fitness and $V_A(W)$, given the context dependence of the expression of individual fitness and, hence, its mean fitness and genetic variance (Hoffmann and Merilä 1999). Therefore, future work should include a gradient of environmental conditions so that we may expand the body of experimental studies and gain better understanding of the generality of the impact of environmental stress on the capacity for adaptation. Additionally, the effects of hybridization change over generations of recombination (Erickson and Fenster 2006) so further work would benefit from expanding the number of recombining generations to understand the interaction of recombination and gene flow, the relative impact of phenotypic plasticity and genetic adaptation, and how well predictions of adaptive capacity are realized in natural populations.

Conclusions

Our results provide evidence that gene flow affords immediate and long-term benefits to population performance in *Chamaecrista fasciculata*. Despite the possibility of outbreeding depression due to crossing two long separated populations, this study did not detect a reduction in mean fitness of hybrids relative to pure populations. However, it also found that there was little fitness benefit of interpopulation crosses between the focal population and one whose historical selective environment was on average 1.3°C warmer and whose maximum temperature was on average 1.8 °C warmer, even when subjected to a temperature increase of 1.9°C. The benefit of this pairing was only evident in expression of $V_A(W)$ but did not exceed the benefit of crossing to nearer populations. Therefore, hybridization via assisted gene flow did not increase fitness via pre-adapted traits or variation in the direction of selection, rather the general increase in both variation in general increased fitness and maintained $V_A(W)$ under warming conditions. As environments change, considerations of performance of populations must go beyond the immediate impacts on individual fitness and population size and instead include a thorough examination of the evolutionary consequences of these treatments over multiple generations.

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ILLUSTRATIONS

Table 1. Significance of genotype and estimates of mean fitness ($\overline{W}$), additive genetic variance for fitness V_A(W), the predicted change in fitness given through Fisher's Fundamental Theorem of Natural Selection $V_A(W)/\overline{W}$, and the predicted fitness of the progeny for both generations of each population, grown in each treatment. Standard error is provided in parentheses.

Population	**Treatment**	**Generation**	**Genotype Significance**	**$\overline{W}$ (se)**	**V_A(W) (se)**	**$V_A(W)/\overline{W}$ (se)**	**Predicted Fitness of Progeny**
$MN_{central}$	Ambient	1	0.177	6.31 (1.39)	1.70 (3.66)	0.269 (0.585)	6.58
$MN_{central}$	Ambient	2	0.0691	8.22 (1.69)	4.07 (5.50)	0.490 (0.670)	8.71
$MN_{central}$	Warming	1	NA	5.98 (1.44)	0	0	5.98
$MN_{central}$	Warming	2	NA	12.2 (2.90)	0	0	12.3
MN_{south} x $MN_{central}$	Ambient	1	1.87E-06	9.90 (1.96)	91.2 (39.4)	9.21 (4.36)	19.1
MN_{south} x $MN_{central}$	Ambient	2	0.000559	9.46 (1.74)	14.9 (9.16)	1.58 (1.03)	11.0
MN_{south} x $MN_{central}$	Warming	1	0.217	7.90 (1.44)	2.60 (6.66)	0.330 (0.840)	8.23
MN_{south} x $MN_{central}$	Warming	2	NA	11.0 (1.92)	0	0	11.0
MN_{south} x $MN_{central}$	Ambient	1 (2021)	0.00227	5.55 (1.58)	7.71 (5.57)	1.39 (1.12)	6.94
IA x $MN_{central}$	Ambient	1	0.0148	6.68 (1.29)	5.83 (5.36)	0.873 (0.836)	7.55
IA x $MN_{central}$	Ambient	2	7.19E-06	9.41 (1.51)	19.9 (9.19)	2.12 (1.09)	11.5
MO x $MN_{central}$	Ambient	1	1.37E-06	4.60 (0.926)	7.66 (3.26)	1.67 (0.821)	6.27
MO x $MN_{central}$	Ambient	2	0.00916	11.9 (1.63)	12.2 (10.4)	1.03 (0.890)	13.0
MO x $MN_{central}$	Warming	1	NA	6.30 (1.47)	0	0	6.30
MO x $MN_{central}$	Warming	2	0.183	8.80 (1.32)	1.55 (3.43)	0.176 (0.391)	8.98
MO	Ambient	1	NA	4.60 (3.62)	0	0	4.60
MO	Ambient	2	NA	9.72 (11.2)	0	0	9.72
MO	Warming	1	NA	1.47 (0.825)	0	0	1.47

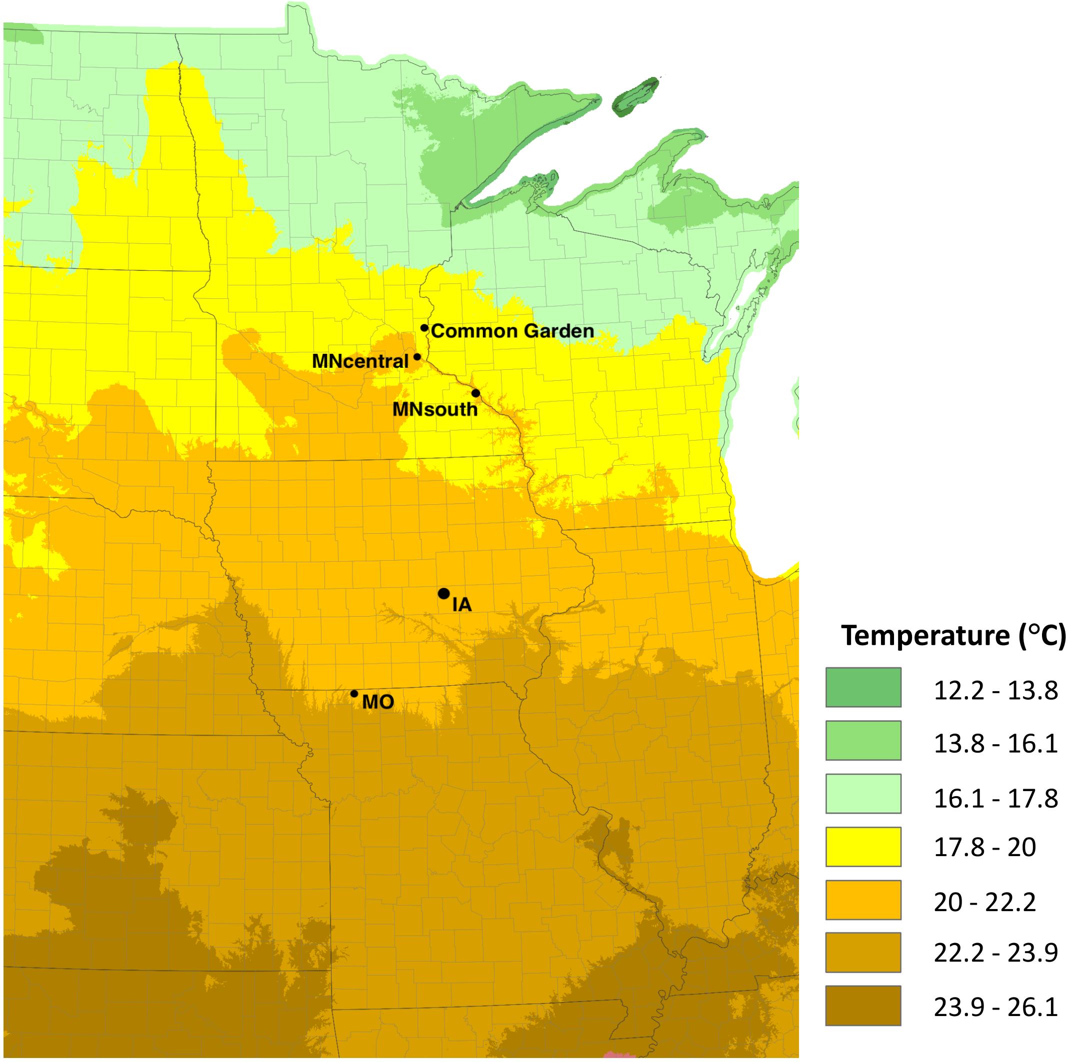

Figure 1. Map of populations and common garden site in Minnesota, United States. Color on the map corresponds to mean temperature in June in degrees Celsius from 1991-2020. Temperature increases from green to brown. Climate data is from PRISM (prism.oregonstate.edu/normals).

$$\text{Seeds planted (1-4)} \xrightarrow{\text{Ber}} \text{germ}_{\text{total}} \xrightarrow{\text{samp}} \text{germ}_{\text{sub}} \xrightarrow{\text{Ber}} \text{surv}_{\text{flowers}} \xrightarrow{\text{Ber}} \text{surv}_{\text{pods}} \xrightarrow{\text{0-Poi}} \text{pods}_{\text{total}} \xrightarrow{\text{samp}} \text{pods}_{\text{sub}} \xrightarrow{\text{0-Poi}} \text{seeds}_{\text{total}}$$

Figure 2. Graphical aster model used in random effects analysis for lifetime fitness and additive genetic variance for fitness. Each node in the model corresponds to a different life history fitness component. Two nodes where we subsampled are noted with the subscript “sub”, the subscript for other nodes describes the data type. Above each arrow is the statistical distribution associated with the transition between nodes: Ber=Bernoulli, samp=subsample Bernoulli, and 0-Poi= zero-truncated Poisson distribution.

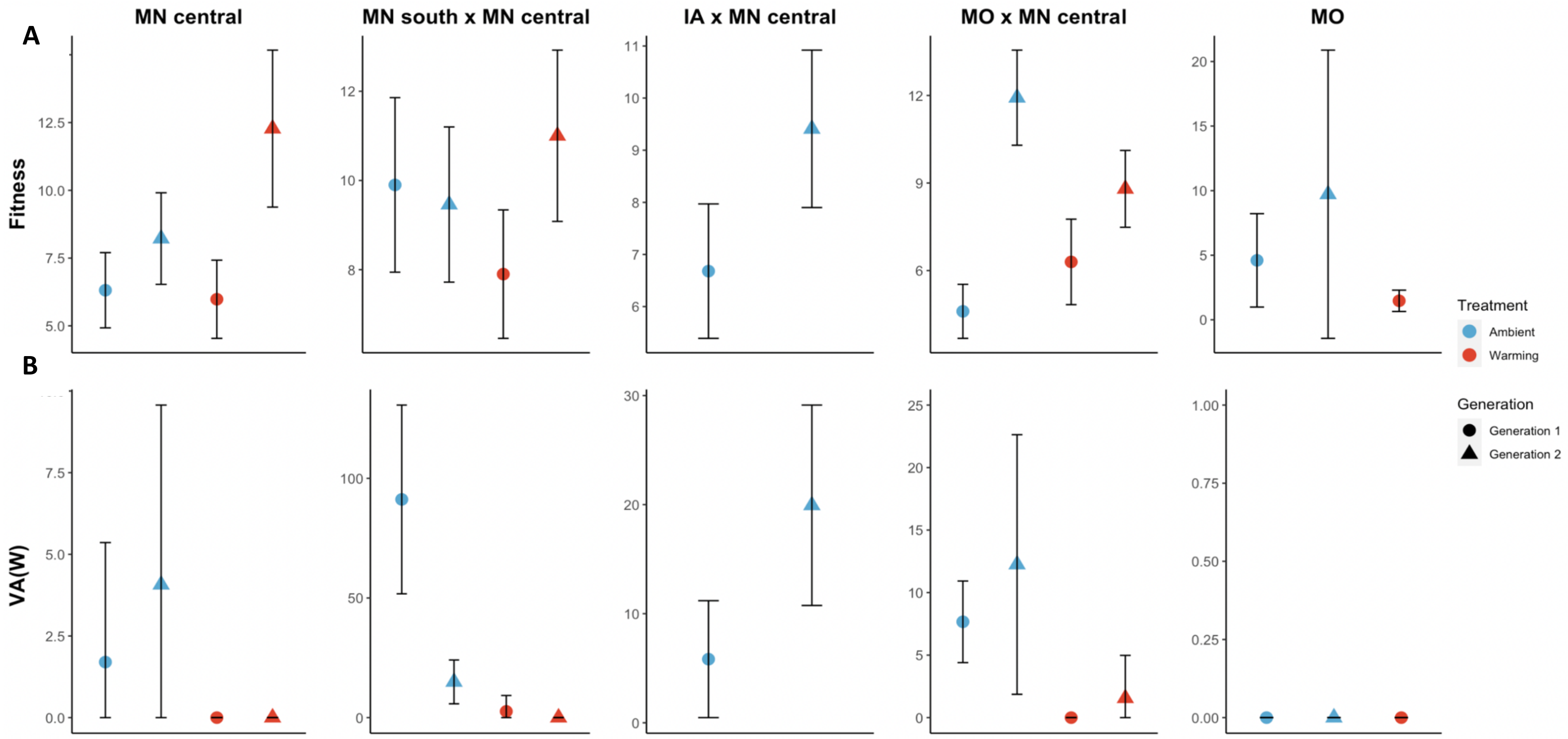

Figure 3. A) Fitness and B) $V_A(W)$ for each population from random effects aster models. Populations are separated by column and each column is labeled with the corresponding population. The ambient treatment is shown in blue and the warming treatment is shown in red. Circles represent first generation and triangles represent the second generation. For simplicity, the second production of the first generation of the MN_{south} x $MN_{central}$ population is not shown above.

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CHAPTER 3

The effect of hybridization along an environmental gradient on phenotypic plasticity in floral traits of an annual prairie legume

SYNOPSIS

Climate change has increased temperature and environmental stochasticity, such that plant populations may no longer be well adapted to their home environment and floral traits associated with warmer conditions may be less desirable to pollinators. Adaptive phenotypic plasticity in floral traits could buffer some of these effects but may be insufficient to maintain pollinator attraction given the rapidity of the change in climate. Therefore, hybridization with other, more southerly, populations that have undergone selection in thermal conditions similar to those impinging on the focal population (i.e. assisted gene flow) has been considered as a management option to introduce genetic diversity as a basis for evolutionary response to these extreme conditions. Through this, additional adaptive plasticity and traits may be introduced that increase tolerance and pollinator attraction. However, in natural populations empirical evidence of increased tolerance to warmer environments is lacking. I examined the effect of increased temperature on four morphological traits, flowering phenology, and population fitness in a focal population of the buzz-pollinated, *Chamaecrista fasciculata* and also in its hybrids with other populations. I hybridized my focal population with three others along a geo-climatic gradient (i.e. a climatic gradient associated with geographic distance) to investigate how a population origin impacts offspring response to environmental warming. I found significant phenotypic plasticity of floral morphological traits in each of five populations, but that those trait means were not ordered along a geo-climatic gradient. Flowering phenology was ordered along a geographic gradient but showed no evidence of plasticity. I found evidence of adaptive, maladaptive, and neutral plasticity in these floral traits and significant population differences in the relationship between trait values and fitness. The impacts of hybridization were population specific; the trait responses expressed and their associations with fitness were specific to the population, not ordered along a geographic gradient. Therefore, the impact of hybridization on floral traits found in this study does not support the theoretical underpinnings of assisted gene

flow as a conservation measure meant to enhance population fitness via introgression of genes and plasticity from more southerly locations.

INTRODUCTION

Climate warming is expected to profoundly affect the physical characteristics and fitness of plants. Plasticity of traits in response to novel and stressful environments may buffer the effects of the environment on overall plant fitness. There is substantial evidence of plasticity and genetic adaptation in many traits including floral characteristics that likely influence pollinator attraction and reproductive success (Galen 1999; Anderson et al. 2012; Lambrecht et al. 2017). Individuals in a population may exhibit plasticity in a trait in response to environmental change. For example, a population may have smaller flowers in response to higher temperature due to differences in resource acquisition. Other populations may exhibit this same trait state in their home environment under typical conditions (ex. flowers are smaller on average). Or may respond to environmental change with a variety of other trait states depending on the selective environment and their reproductive isolation from other populations (ex. even smaller flowers, or longer anthers) (Bradshaw 1965). Therefore, in addition to phenotypic plasticity within one population, there may also be genetic divergence in flower size across species' ranges. Genetic divergence in traits and their responses to environmental change is the basis of conservation approaches involving hybridization between two or more populations, such as assisted gene flow. These approaches have the goal of introducing variants that enhance fitness, such as decreased flower size, into populations and, thus, population persistence under a rapidly changing climate.

Evidence of plasticity in plant populations is substantial, including with respect to climate change (Nicotra et al. 2010; Merilä and Hendry 2014; Stotz et al. 2021). Much of this work has focused on traits expected to have substantial impact on plant performance (i.e. leaf, root, and growth-related traits) and has found several instances of adaptive plastic responses to environmental change (Royer et al. 2009; Gratani 2014; Henn et al. 2018). Plasticity in these vegetative traits may support continued growth and nutrient acquisition in harsh environments, buffering their effects on plant fitness. Research examining plasticity in floral traits indicates that while there are notable reductions in size, nectar quality, etc. in warmer and drier conditions (van der Kooi et al. 2019; Descamps et al. 2021), this response is more limited compared to the vegetative response (Lambrecht et al. 2017). Many of these phenotypic responses can be attributed to limits on acquisition of resources to support large, attractive flowers and often coincide with changes in flowering phenology such that plants flower earlier in warmer conditions (Lesica and Kittelson 2010; Li et al. 2019; Descamps et al. 2021; Wiszniewski et al. 2022). While this plasticity may allow plants to flower and reproduce under warmer conditions, there may be additional consequences of this resource limitation, such as changes in pollinator interactions.

Flower size and quality may have substantial impacts on an individual's reproductive output via their ability to attract pollinators. Within a species, there is often a positive correlation between flower size and pollinator attraction, with patterns of higher pollinator rewards in larger flowers (Zhao et al. 2016; Ortiz et al. 2021). When plants are grown in warmer or drier conditions, floral display size (i.e. petal, anther, and stigma size) decreases and can lead to decreased visits from pollinators such as bumble bees (Descamps et al. 2021; Wiszniewski et al. 2022; de Manincor et al. 2023). Bumble bees may recognize that smaller flowers yield fewer rewards (e.g. quantity and quality of pollen and nectar) and could therefore be passed over in favor of a higher reward flower and greater foraging efficiency (Descamps et al. 2018). As such, these smaller flowered individuals may have lower reproductive fitness compared to their larger counterparts. In addition to flower size, flowering phenology is highly dependent on environmental conditions, with individuals in stressful conditions often flowering earlier than those in more hospitable environments (Anderson et al. 2012; de Manincor et al. 2023). If changes in pollinator phenology do not mirror those of the plants, shifts in plant reproductive phenology away from the historic timing may lead to mismatches with pollinator timing and increased interspecific competition for pollinator visits, both of which contribute to lower plant fitness (Rafferty and Ives 2012; Mohan et al. 2019). Given that warmer temperatures may lead to smaller flowers and earlier flowering onset across an entire population, reduced attractiveness and availability to pollinators may lead to profound population level consequences (de Manincor et al. 2023). This may be especially important in smaller flowered species whose reduction in flower size compared to larger flowered species may be associated with greater declines in pollinator visitation.

Plastic responses to environmental change may buffer a population from variable environmental conditions, whereas genetic adaptation may provide long term stability in response to ongoing conditions (Bradshaw 1965). Divergence in mean flower size between populations may be attributed to either adaptation and/or drift, as a result of reproductive isolation. In populations consistently exposed to conditions that are warmer and/or drier than their home environment, performance in those environmental conditions likely includes both genetic adaptation and plasticity (Etterson 2000; Peschel et al. 2021). Plasticity implies sensitivity of trait expression to environmental conditions, whereas genetic adaptation necessitates natural selection on genetic variation. Plasticity and genetic adaptation can and often do occur simultaneously. For example, populations exposed to warmer conditions over many generations may have different trait values compared to populations exposed to milder conditions. However, their trait expression may be different when individuals from those populations are exposed to harsher or milder environmental conditions (Elle 2004). Since each population has different environmental stressors, plasticity and genetic adaptation may differ among populations (Bucharova et al. 2017; Ehrlén et al. 2023).

Genetic differentiation in adaptive and beneficial plastic responses may be especially important in the context of conservation efforts that utilize population hybridization. These approaches rely on the hypothesis that hybridization will introduce adaptive variation into a population, while maintaining some degree of local adaptation (Aitken and Whitlock 2013a). Some populations may be selected for their higher plasticity, especially if environmental change is expected to be somewhat stochastic. For quantitative traits, hybridization typically introduces new alleles into a population that may alter the expressed traits such that the average phenotypes of the hybrid offspring are between the two means values of the parental populations (Falconer and Mackay 1996). The degree to which the new generation's phenotypes differ from this expectation may be due to the effect sizes of their alleles, their genetic dominance, and epistasis.

There may be a large degree of variation in the impact of hybridization on different traits and between different populations. Conservation approaches aiming to capitalize on "pre-adapted" alleles may also benefit from the plasticity of those same populations to the extent that it is adaptive. For example, flowers in hybrid populations may show increased tolerance for warmer conditions through the addition of warm-adapted alleles, meaning that their size and/or phenology is maintained in both warm and cool environments (Takatsu et al. 2001; Brennan et al. 2021). Alternatively, they may demonstrate a change in plasticity such that the hybrid population has a wider difference in trait values over different thermal conditions. However, the extent to which these differences are clinal along an environmental gradient is poorly understood. Furthermore, the detection of plastic responses does not imply that there is a relationship between that plasticity and fitness or that that relationship is positive. Observed plasticity may be adaptive, but it may also be neutral or maladaptive, as in cases where flower size directly inhibits pollinator activity (Noble et al. 2019; Gomulkiewicz and Stinchcombe 2022). Conservation decision-making can benefit from an understanding of whether plasticity and hybridization are beneficial to populations in response to climate change.

This study investigates hybridization along a geo-climatic gradient and its impact on plasticity floral characteristics of *Chamaecrista fasciculata*. Previous research with populations of this species provided evidence that both local adaptation and selection for phenotypic plasticity in response to drought were clinally arranged along a large climate gradient (Etterson 2000). I used populations along this same gradient to hybridize and expose to a warming treatment to ask the following questions: 1) To what extent do hybrid population exhibit different floral characteristics and phenology than the focal population? 2) How do differences in floral traits impact population mean fitness? 3) How do these differences correlate with geographic/environmental distance from the focal population? 4) Does hybridization provide a benefit to the focal population in their response to warming conditions via phenotypic plasticity?

MATERIALS AND METHODS

Study system

Chamaecrista fasciculata is a buzz pollinated prairie annual whose range extends from Mexico to Minnesota and from the eastern seaboard up to the foothills of the Rocky Mountains (Irwin and Barnaby 1982). Therefore, it spans a large range of environmental conditions, with populations genetically isolated because gene flow via pollen is restricted by the foraging distance of resident bumble bees. *C. fasciculata* exhibits indeterminate growth and has showy, nectarless yellow flowers that are ephemeral. Each flower has a one large, showy petal and four smaller petals. This larger petal may serve as a flight guide or signal to pollinators (Thorp and

Estes 1975), but information about its impact on fitness is lacking. Flowers are hermaphroditic, with a cluster of small anthers and a single longer deflexed anther that comes from the base of the stigma. When bumble bees visit the flower their vibration releases pollen from the apical pore of the anthers (Wolfe and Estes 1992). Anther length is linearly related to the quantity of pollen in a flower and is therefore an important component of male fitness (Frazee and Marquis 1994). Several studies have found that environmental factors influence floral traits and phenology in *C. fasciculata* (Frazee and Marquis 1994; Etterson 2000; Wadgymar and Weis 2017; Peschel 2021; Rushing 2022).

Focal populations

I used four populations along a latitudinal and temperature gradient from central Minnesota to northern Missouri. Southern populations represented in this study inhabit geographic areas whose 30-year climate history most closely aligns with 60-year climate predictions for the location of the focal population in central Minnesota. The thermal averages of the source sites are as follows: 1) $MN_{central}$ (44.79, -92.96) averaging 20.26° C and an average maximum temperature of 25.40 °C in June, 2) MN_{south} (44.25, -91.96) averaging 20.54° and an average maximum temperature of 26.07 °C in June 3) IA (41.68, -92.86) averaging 21.14° C and an average maximum temperature of 26.89 °C in June, and 4) MO (40.53, -94.12) averaging 21.6° C and an average maximum temperature of 27.12 °C in June.

Seed production in greenhouse

Pedigreed seed was produced following the procedure outlined in Chapter 2, described briefly here. In 2019-2020, I grew a sample of each population in the greenhouse and conducted hand-pollinations to generate sets of paternal half-siblings. Crosses generating hybrid populations were structured such that all maternal plants were from the $MN_{central}$ population, and each maternal plant was mated to one paternal plant from each population (i.e. a single plant produced seed from four paternal sources). Half sibling groups were maintained between hybrid pedigrees so that maternal half-sibling groups were consistent between hybrid populations. In addition, I conducted similar crosses within the MO population as a basis for comparison with long-distance hybrid $MN_{central}$ x MO population. In 2021, I produced a second generation of each population using a similar paternal half sibling pedigree structure (Suppl. Fig. 1).

Field experimental plots

All seeds were planted into a restored prairie at the St. Croix Watershed Research Station approximately 43 km from the source of the $MN_{central}$ population origin. Prior to planting, I removed all aboveground biomass from the areas my experiment occupied. Substantial gopher activity made it impossible to avoid gopher mounds. Consequently, I flattened gopher mounds in the ambient plots for blocks 2 and 4. Preliminary data from 2020 suggested little difference in the mean fitness of IA x GC and MO x GC. Accordingly, due to space limitations, I did not include the IA x GC population and the second generation of the MO population in the warming treatment. All other populations had each full sibling family represented in both treatments. Individuals from all populations and generations were randomized across planting positions within plots. I planted up to four seeds per full sibling family at each planting position using forceps and marked with a toothpick. Each planting position was 8 cm from the next in rows of 16 positions. Rows were offset to maintain 8 cm between nearest neighbors.

I installed open top chambers (OTCs) to warm each of the warming treatment plots. I modeled my OTC after those used in (Welshofer et al. 2018) . I constructed the hexagonal structure using PVC and 6 mil greenhouse film. Each side consisted of a single panel that was larger on the bottom and smaller on the top so that the walls were angled, optimizing solar radiation for warming the inside of the structure. The truncated hexagonal pyramid was large enough to contain the 1.25 m^2 plot with a buffer on each side of the plot (see chapter 1, Figure S1). I placed temperature sensors in the northeast corner of both treatment plots in a randomly selected block so that I could monitor the increase in temperature. OTCs increased daytime temperature by an average of 2°C and maintained an increase of 0.5°C overnight. To limit potential differences in animal herbivory between the two treatments, I deployed chicken wire fencing around all ambient plots. An unprecedented early season drought at my field site resulted from extremely low precipitation: 21.6 mm in June 2021 compared to the 30-year mean of 125.26 mm, I watered all plots with approximately 1.5 cm water four times over a 1-month period spanning from late June to late July.

Plant measurements for lifetime fitness

I conducted censuses of all planting positions for each major life history stage (germination, flowering, fruit production, and seed production. During the first census I recorded the number of seeds that germinated at each planting position and then thinned to one seedling per position. I selected the seedling closest to the toothpick marker to represent the family for the experiment, while all others were removed. I recorded plant presence and life history stage each census (i.e. flowering, fruiting, etc.). Because fruits of *C. fasciculata* explosively dehisce, I covered ripening pods with organza bags and collected ripe pods every 2-3 days. Late season herbivory by small mammals led to loss of plants and fruits in both treatments, despite the use of coyote urine deterrents and reinforcing small gaps in the structures and fencing. I noted herbivory in the census in which it was detected. When possible, I matched plant and fruit material found on the ground to the remains of plants that were still standing. Total pod counts include these ripe pods taken from the ground. Final fruit counts included four categories: ripe pods, green pods, small pods, and aborted pods. Green pods were fully elongated with countable seeds inside that would have likely fully ripened had I allowed the experiment to run until a killing frost, whereas small pods were not fully elongated, and viable seeds were indistinguishable from non-viable ones.

Flower measurements

All plants were assessed for flowering every 2-3 days from the onset of flowering, totaling 6 flowering censuses. In late August, up to two flowers were measured for each plant flowering on the day of assessment. For consistency, I only collected flower measurements on flowers at the top of the plant. For each flower I measured size of the showy, lowest petal, deflexed anther, and non-extended stigma in a non-destructive manner, using a caliper (Fowler Electronic Caliper). The deflexed anther is the longest anther in the flower and set apart from the grouped anthers. This anther offers most pollen for transport (Wolfe and Estes 1992; Shaw et al. 2008). Additionally, the lowest petal on *C. fasciculata* is the largest and is considered important in attracting pollinators as well as a good indicator of total flower size (Etterson 2000). Therefore, length and width measurements were also collected.

Statistical analyses

For individuals that had two floral measurements, I took the mean of the two measurements. I used linear models and likelihood ratio tests to assess the impact of treatment, population, generation, soil disturbance, and block on floral characteristics and time of first flower.

I used unconditional aster models for lifetime fitness (Shaw et al. 2008) to evaluate the mean fitness of each population in both treatments. I used the same graphical model as in chapter 1 and 2 (chapter 1, Figure 3), which included the number of seeds that germinated, subsampling to one seedling, whether the plant flowered, whether the plant produced fruit, and the number of pods that were subsampled for harvest as Bernoulli distributions. I did not include paternal and maternal families as predictors due to the limited sample size of some of the populations. The total number of pods produced by the plant and the number of seeds produced from the pods that I collected were modeled with zero-truncated Poisson distributions. Maximum likelihood testing conducted in Chapter 1 produced a model with block, treatment, population, soil disturbance prior to planting (gopher mounds), the interaction of population and treatment, and the interaction of block and treatment as fixed effects. I used this model as a base model for maximum likelihood testing of flower measurements as predictors of fitness. I conducted separate analyses for each flower measurement and its interactions so that I could assess the individual effect of those floral characteristics on mean fitness. I conducted additional analyses for the interaction of flower length and width and the interaction between date of onset of flowering and each of the floral measurements. Estimates of absolute fitness for each population were transformed from the canonical scale to mean parameter scale so that estimates are biologically meaningful (Kulbaba et al. 2019; Ryskamp and Geyer 2021).

RESULTS

I found substantial evidence of phenotypic plasticity in the populations included in this study, with populations having statistically significant differences in petal length, width, and anther length between ambient and warming conditions, However, there was no detectable effect on stigma length. Plants had longer and wider petals in the warming treatment in addition to longer anthers, with some exceptions (Figure 1). However, there was no significant effect of population identity on the traits measured and there was no interaction between population and treatment (Table 1). Thus, populations did not differ in their plastic response to the warming treatment. This result is in contrast with flowering phenology, where I found a significant difference between temperature treatments and the effect of population origin (Figure 2A). All populations flowered earlier in the warming treatment. Phenology of flowering was ordered along the geographic gradient with $\text{MN}_{\text{central}}$ flowering earliest and the MO population flowering latest. All of the populations had a mean flowering time between the 4th and 5th flowering census, except MO which had a mean flowering time between the 5th and 6th census.

Previous analysis of the effect of treatment on fitness indicated that the warming treatment significantly reduced fitness (Pain, 2024; Chapter 1). I found that fitness of individuals varied significantly in relation to each of the floral and floral phenology traits in this study, and these relationships differed significantly among populations, with the sole exception of petal length (Table 2). The relationships between fitness and traits did not differ significantly between treatments, except for petal width ($P = 0.053$). The interaction between petal width and generation was a significant predictor of fitness (Table 2) where larger petals were associated with higher fitness in the second generation compared to the first generation (Figure 3 A,B in the ambient and Figure 3 D,E in the warming treatment). Individuals with the smallest petal size (both length and width) had the lowest fitness, and individuals with long, but less wide petals had the greatest fitness (Figure3, Suppl. Fig. 2). However, I found the opposite pattern for petal width in the warming treatment with shorter petals having higher fitness in the $\text{MN}_{\text{central}}$ and MO x $\text{MN}_{\text{central}}$ populations (Figure 3D & E). The relationship of fitness with both anther and stigma length had significant curvature and had a similar pattern of higher fitness with larger stigma and anthers in $\text{MN}_{\text{central,}}$ MO x $\text{MN}_{\text{central,}}$ and the MN_{south} x $\text{MN}_{\text{central}}$ population, whereas the other two populations had highest fitness associated with low to mid anther lengths (Figure 4). Finally, the interaction of flowering time and population identity affected lifetime fitness outcomes (Table 2, Figure 2B). Fitness for all populations declined with increasing time to first flower (Fig. 2B).

DISCUSSION

This experiment investigated the impact of hybridization of focal population with others along a thermal gradient on flowering trait expression and its subsequent effect on fitness. Under quasi-natural conditions, I subjected a subset of individuals to a warming treatment to determine whether populations differed in their response to warming and examine phenotypic plasticity and the dependence of that plasticity on the origin of the parents. I detected plasticity in response to warming for all floral morphology traits except stigma length, but not in phenology. Fitness of individuals varied significantly in relation to all traits, but the relationship between trait and fitness did not vary significantly with temperature. Additionally, differences between trait means and fitness means of populations were not ordered along an environmental gradient. Thus, hybridization along a geo-climatic gradient did not confer a predictable change in floral morphology or plasticity in response to warmer temperatures, contrary to theoretical expectations from assisted gene flow.

The focal $MN_{central}$ population had longer anthers and wider petals in warmer conditions. Many of the hybrid populations similarly had similar plasticity, but MO had shorter anthers and petals in the warming treatment. Some populations appear to have more limited plasticity in individual floral traits, such as showy petal width in the MN_{south} x $MN_{central}$ population or anther length in $MN_{central}$. However, the hybrid populations and long-distance population did not significantly differ from the focal population in their response to warmer temperatures. The plastic response of larger flowers and anthers is contrary to the frequent finding of smaller flowers due to warmer temperatures. Previous studies of other species have found that flower size tends to decrease with increased temperature, much like the response of the MO population (Descamps et al. 2021; Wiszniewski et al. 2022; de Manincor et al. 2023). This may be due to several physiological stressors associated with increased temperatures, including its effect on evapotranspiration. Plastic responses are typically loosely aligned with general responses to water stress, tending to follow the pattern of smaller overall size and leaf area in water deficient environments (Etterson 2000; Jagadish et al. 2021). However, the trait values associated with highest fitness are largely consistent between treatments (Table 2, interaction between trait and temperature treatment with respect to fitness, ns; Suppl. Fig. 3). Therefore, while the observed patterns of plasticity are counter to expectations, their alignment with high fitness suggests that these larger floral structures are beneficial in this scenario.

Hybridization did not have a significant effect on floral morphology traits compared to the focal population. While most of the populations showed a plastic response to the thermal conditions, they do not differ significantly in their trait means nor in their responses to warming. Further, there was no geographic association with any morphological traits. Thus, geographic origin is a poor predictor of how hybridization impacts these traits. A result that would support assisted gene flow would have been that plant populations from warmer climates, being better adapted to those conditions, would have traits and plasticity that align with the selective pressures of warmer temperatures. However, the absence of population effects on trait means suggests that variation in individuals not captured by population differences may make greater contributions to differences in population mean fitness as expressed in the experimental site.

Trait values may not significantly differ between populations, but their effect on fitness may be substantially different depending on population origin. My results indicate that the relationship between fitness and stigma length, anther length, and petal width, respectively, differed among my populations (Table 2). In general, the focal $MN_{central}$ population, as well as MO x $MN_{central}$ and MN_{south} x $MN_{central}$ populations had higher fitness at greater trait values, while the other populations had higher fitness at lower values. As such, the relationship between those traits and fitness are specific to the genetic context of the populations in question. This counters the idea that these traits may linearly align along a geographic and environmental gradient in these hybrid populations. While several studies have found clinal patterns in plant phenotypes, including floral traits (Newman et al. 2015), variation in floral traits across a species' range is likely due to a variety of environmental pressures and therefore may not neatly align along a geographic gradient. These environmental differences are eliminated in my fully randomized common garden experimental design, and the resulting differences in trait values are expressions of genetically based differences between populations in that common environment. My results therefore directly question the assumption of a geographically correlated response to high temperatures and highlight the importance of experimental evaluation of the effect of long-distance gene flow.

The plastic response in an individual trait to temperature may correspond to a difference in fitness. Yet, the individual impact of a trait may be small and not accurately represent a population's overall response to that change in temperature. I found evidence of phenotypic plasticity, such that warming of many populations resulted in longer anthers, and longer and wider showy petals. While I did not find a significant relationship between anther length and treatment on fitness, the plasticity in petal size was counter to the direction of selection, suggesting that this plasticity may be maladaptive. Adaptive phenotypic plasticity may contribute greatly to a population's capacity to persist in a changing and warmer environment by buffering the impact of this change and maintaining fitness (Merilä and Hendry 2014; Ghalambor et al. 2015; Chevin and Hoffamann 2017). Conversely, maladaptive plasticity may contribute to a population decline because the response to warmer temperatures corresponds with a decrease in fitness. Fitness estimates for each population suggest differing responses to the warming treatment. Most populations had lower fitness in the warming treatment, while mean fitness of MO x $\mathrm{MN}_{\mathrm{central}}$ differed little in fitness between treatments. Despite the fitness decline associated the warming treatment, all populations still show evidence of population growth, given that mean fitness of individuals is greater than one, suggesting that they all have a degree of tolerance for these warming conditions.

Floral phenology was the only trait measured that was significantly impacted by population and was ordered along the geographic gradient with the $\mathrm{MN}_{\mathrm{central}}$ population flowering the earliest and the MO population flowering the latest. Additionally, plants in all populations flowered earlier in the warming treatment compared to plants in ambient temperatures, but population differences were maintained across treatments. It is well established that warming can impact plant phenology, with plants often flowering earlier under warmer conditions (Anderson et al. 2012; Richardson et al. 2017; Prevéy et al. 2019). These trends are species and population specific. In previous studies using *C. fasciculata*, warming and drought reduced time to flowering; population specific photoperiod requirements likely influence to this trait (Etterson 2000; Wadgymar and Weis 2017; Rushing 2022). Because I found a significant impact of population and not treatment on flowering phenology, my results may best align with these population specific explanations for phenology. My analyses suggest that selection in the common garden favors individuals that flower earlier, even in the case of populations that flowered later in the season. Therefore, any variation in the direction of earlier flowering would enhance fitness of populations experiencing warmer temperatures.

Implications

Climate change is and will continue to increase temperatures, abruptly disrupting local adaptation and the relationships between pollinators and plants. Logic underlying the conservation practice of assisted gene flow is to introduce traits from populations that are already adapted to warmer environments and to rely on expression of those traits in the subsequent generations. Here, I found no geographic pattern of floral traits in hybrid populations exposed to ambient and warmed conditions. Many hybrid and non-hybrid populations had significant, consistent plastic responses to temperature treatments. Furthermore, my analyses suggest that some of these plastic responses may be maladaptive, but that the overall fitness effects of the measured traits were population specific and that the relationship between traits and fitness did not differ between thermal treatments. My results provide insight into the realized effects of hybridization-based conservation strategies and do not fully align with theoretical expectations of assisted gene flow. Theoretically, hybridizing with a population that has undergone selection in warmer conditions could introduce pre-adapted traits or increased adaptive plasticity in response to warmer environments. Results presented here indicate, however, that the benefit of hybridization may be more context specific and not fully aligned with any climatic patterns that may occur across geographic space.

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ILLUSTRATIONS

Table 1. Summary of comparisons of linear models using likelihood ratio testing. The basic model included Block as a predictor (trait ~ Block). Each column corresponds to the trait which is being modeled. Each row corresponds to a model that includes all terms in the basic model in addition to the term presented and every term in the preceding rows. Values depicted are F values, with * denoting significant p-values ($p < 0.05$). For all models, population has 5 degrees of freedom (df); treatment, generation, and disturbed each have 1 df; and the interaction of population and treatment has 3 df.

	Petal Length	Petal Width	Anther Length	Stigma Length	Time to First Flower
Population	0.577	1.96	1.76	0.354	28.4*
Treatment	8.81*	8.21 *	6.67*	2.80E-03	0.899
Generation	0.718	1.95	1.15E-02	1.28	55.4*
Disturbed	2.03	3.86	3.8	0.991	206*
Population:Treatment	0.773	0.84	2.8	1.2	0.896

Table 2. Model selection results for aster models of each floral trait using likelihood ratio testing. The base model was derived from a fixed effects analysis for lifetime fitness in these populations (resp ~ varb + fit:Block.x + fit:Population + fit:Treatment + fit:disturbed +fit:generation+fit:Population:Treatment). Each row corresponds to a model that includes all terms in the basic model in addition to the term in the current and preceding rows. Each section is a separate analysis that does not include the predictors in the preceding subsections. *P*-values of <0.05 are considered significant.

Model Predictor	**DF**	**Deviance**	**p-value**
Petal Length	1	54.8	1.30E-13
Petal Length : Population	5	6.68	0.246
Petal Length : Treatment	1	1.1	0.295
Petal Length : Generation	1	0	0.989
Petal Width	1	39.2	3.92E-10
Petal Width : Population	5	12.3	0.0313
Petal Width : Treatment	1	3.73	0.0534
Petal Width : Generation	1	4.88	0.0272
Petal Length: Petal Width: Population	5	8.53	0.129
Petal Length: Petal Width: Treatment	1	0.327	0.567
Anther Length	1	55.6	8.94E-14
Anther Length : Population	5	32.001	5.94E-06
Anther Length : Treatment	1	0.191	0.662
Anther Length : Generation	1	0.372	0.542
Stigma Length	1	49.2	2.34E-12
Stigma Length : Population	5	20.6	9.85E-04
Stigma Length : Treatment	1	0.292	0.589
Stigma Length : Generation	1	0.599	0.439
Time to Flower	1	294	2.00E+16
Time to Flower : Population	5	9.79	8.13E-02
Time to Flower : Treatment	1	2.764	0.096
Time to Flower : Generation	1	1.014	0.314

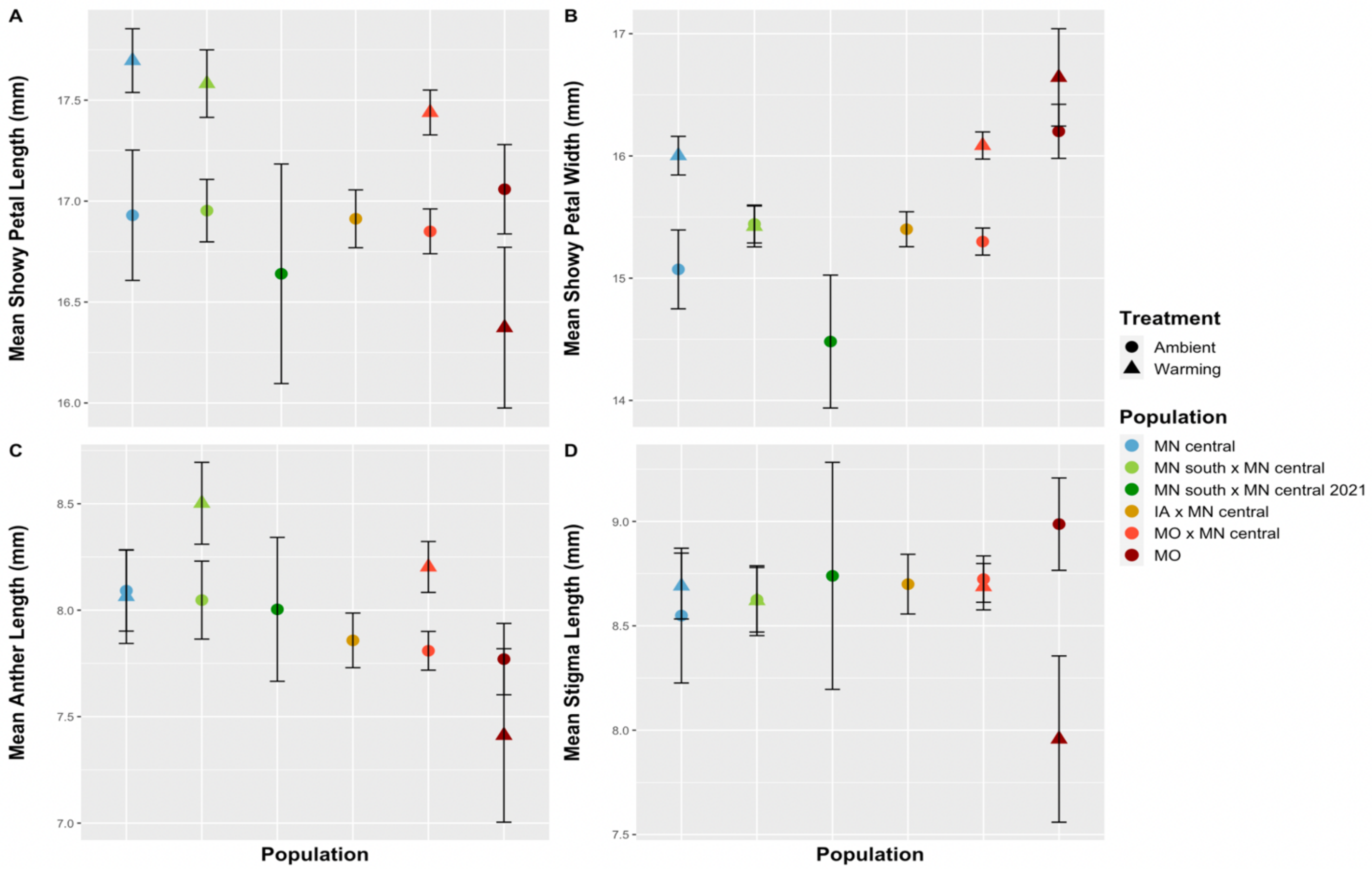

Figure 1. Mean trait values fin millimeters for a) Showy petal length, B) Showy petal width, C) Anther length, and D) Stigma Length. Each population is represented by a different color and is organized from furthest north on the left side of the graph in the coolest color and furthest south on the right in the warmest color. Individuals in the ambient treatment are represented by circles and those in the warming treatment are represented by triangles. Error bars represent standard error.

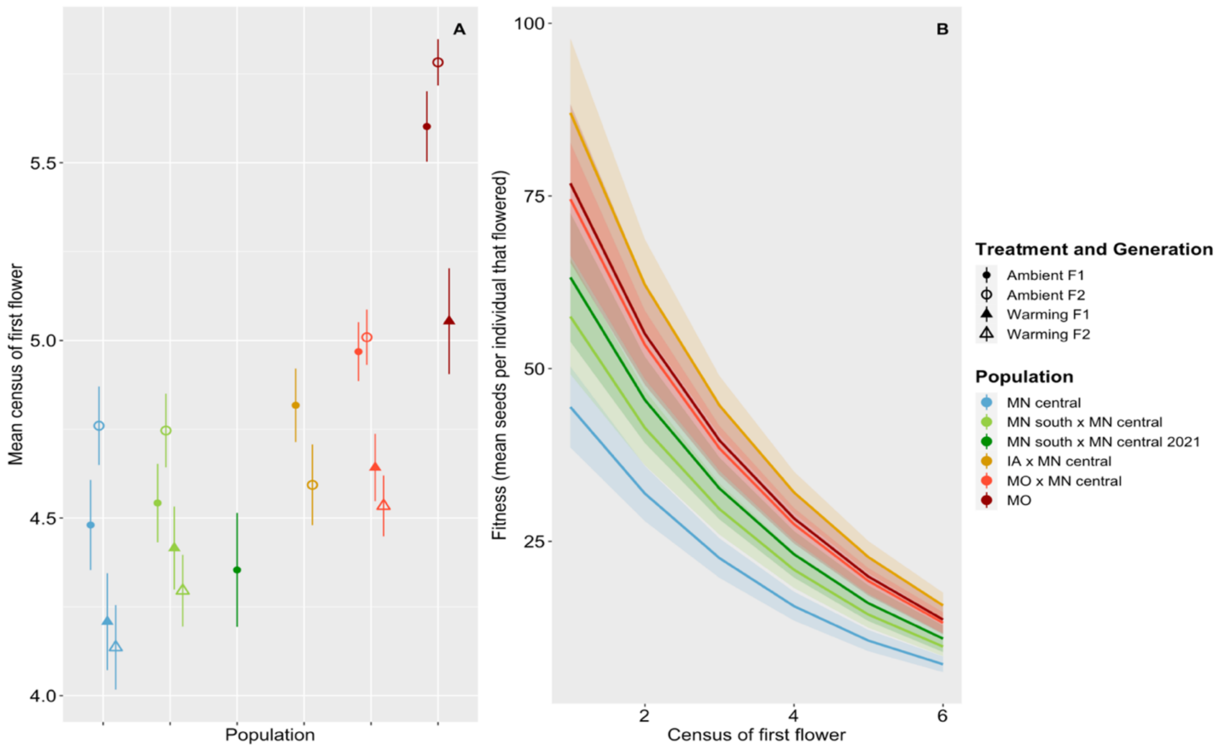

Figure 2. A) Mean census at which populations first flowered. Circles denote ambient treatment and triangles denote warming treatment. Filles shapes are the first generation, whereas open shapes denote the second generation. Populations are ordered with the northernmost population to the left in the coolest colors and the southernmost population on the right in the warmest colors. B) Mean fitness estimates using aster models for lifetime fitness in relation to the census of first flower for each population. Because the interaction of flowering time with generation and treatment were not significant, this graph is a subset of data and represents only the first generation of each population in the ambient environment. Colors correspond to those found in panel A with the coolest colors being the northernmost population and the warmest colors being the southernmost population.

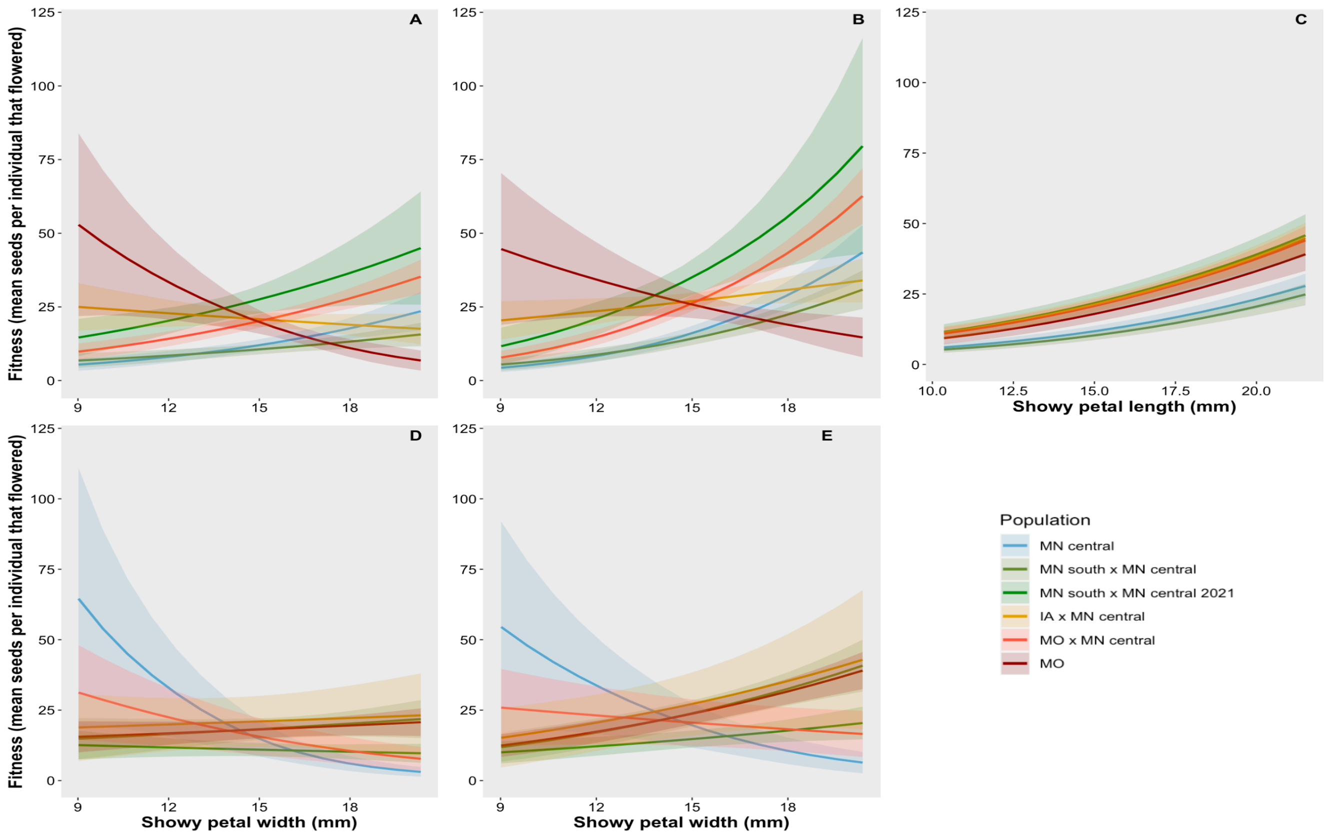

Figure 3. Mean fitness per individual that flowered in each population using aster models for lifetime fitness in relation to petal size measurements. In the ambient treatment: A) Showy petal width in the first generation of each population, B) Showy petal width in the second generation. C) The relationship between showy petal length and mean fitness in each population. The warming treatment was omitted due to no significant interaction. In the warming treatment: D) Showy petal width in the first generation of each population and E) Showy petal width in the second generation of each population. Colors correspond to population, with the northernmost populations in the coolest colors and the southernmost population in the warmest color.

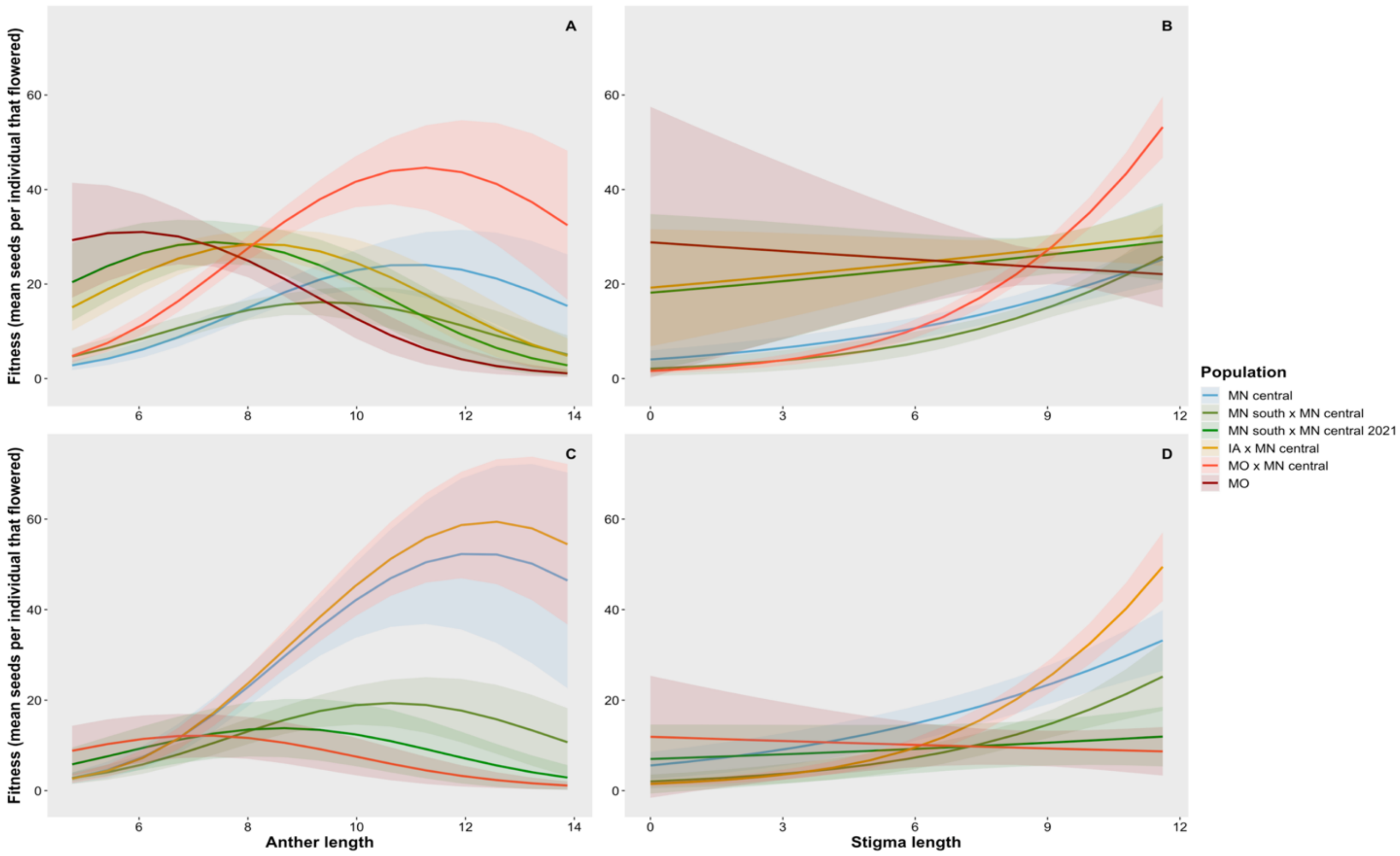

Figure 4. Mean fitness per individual that flowered in each population using aster models for lifetime fitness in relation to A) Anther length in ambient conditions; B) Stigma length in ambient conditions; C) Anther length in warming conditions; D) Stigma length in warming conditions. Colors correspond to population, with the northernmost populations in the coolest colors and the southernmost population in the warmest color. IA x MN$_{\text{central}}$ was not included in the warming treatment

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Appendix A: Chapter 1 Supplemental Tables and Figures.

Table S1. Summary of the differences in experimental design in for the 2020 and 2021 greenhouse work and field seasons

	2020	**2021**
Greenhouse	Seedlings from each population were transplanted to 4" x 14" tree pots filled with 100% sand and stabilized in 9" nursery containers filled halfway with sand. This secondary pot used for stabilization was also necessary to protect the plants from drying out too quickly in the greenhouse during the summer. All seedlings were randomized across the greenhouse to eliminate biases due to local environmental effects in the greenhouse. Plants were grown at the University of Minnesota Plant growth facilities greenhouse under a 16:8-h photoperiod.	Seedlings were transplanted to larger cone-tainers once they had five leaves. Previous work with this species has had issues with decreased growth rates and increased potential for root rot in the pots used in the first generation, therefore smaller pots were chosen to allow for better drainage and air flow. Seedlings from each population were transplanted to 2.5" x 10" tree pots filled with 100% sand.
Field Design	Seeds were planted in mid-June 2020 (June 11-14). While this was later than C. fasciculata seeds typically start germinating in this region, civil unrest in early June made it impossible for researchers to reach the field site. However, previous work with C. fasciculata has noted germination until	Up to four seeds were haphazardly chosen for each envelope to be planted at each position. Each was sterilized, packaged, and planted in the same manner as the 2020 field season. However, planting positions were 8 cm apart along rows 16 planting positions long in a 1.25 m²

	late June. Two seeds were planted at each position for each population with the intention of thinning each position to one individual after germination. Planting rows had 15 positions.	plot. All seeds were planted in mid-late May 2021. Aboveground biomass was removed from all plots prior to planting.
Treatment installation	Due to global goods production and shipping delays during covid-19, the warming treatment was installed over the designated warming plot in each block 14 days after planting (June 29th) and after the first census. Temperature sensors were placed in the northwest corner of each treatment plot in a randomly selected block. Chicken wire fencing was installed mid-July after rodent damage was noticed.	Treatment and fencing were installed upon planting.

Table S2. Number of families represented from each population and generation in both years of the experiment. Additional cohort of first-generation seeds produced in 2021 alongside the second generation of each population had 16 sires and 23 dams.

	2020 Field Season	**2021 Field Season**	
Population	*1st Generation*	*1st Generation*	*2nd Generation*
GC	26 sires & 39 dams	25 sires & 37 dams	17 sires & 34 dams
ML x GC	27 sires & 50 dams	27 sires & 50 dams	22 sires & 44 dams
IA x GC	25 sires & 48 dams	25 sires & 46 dams	21 sires & 42 dams
MO x GC	29 sires & 57 dams	29 sires & 51 dams	22 sires & 55 dams
MO	13 sires & 22 dams	13 sires & 20 dams	7 sires & 15 dams

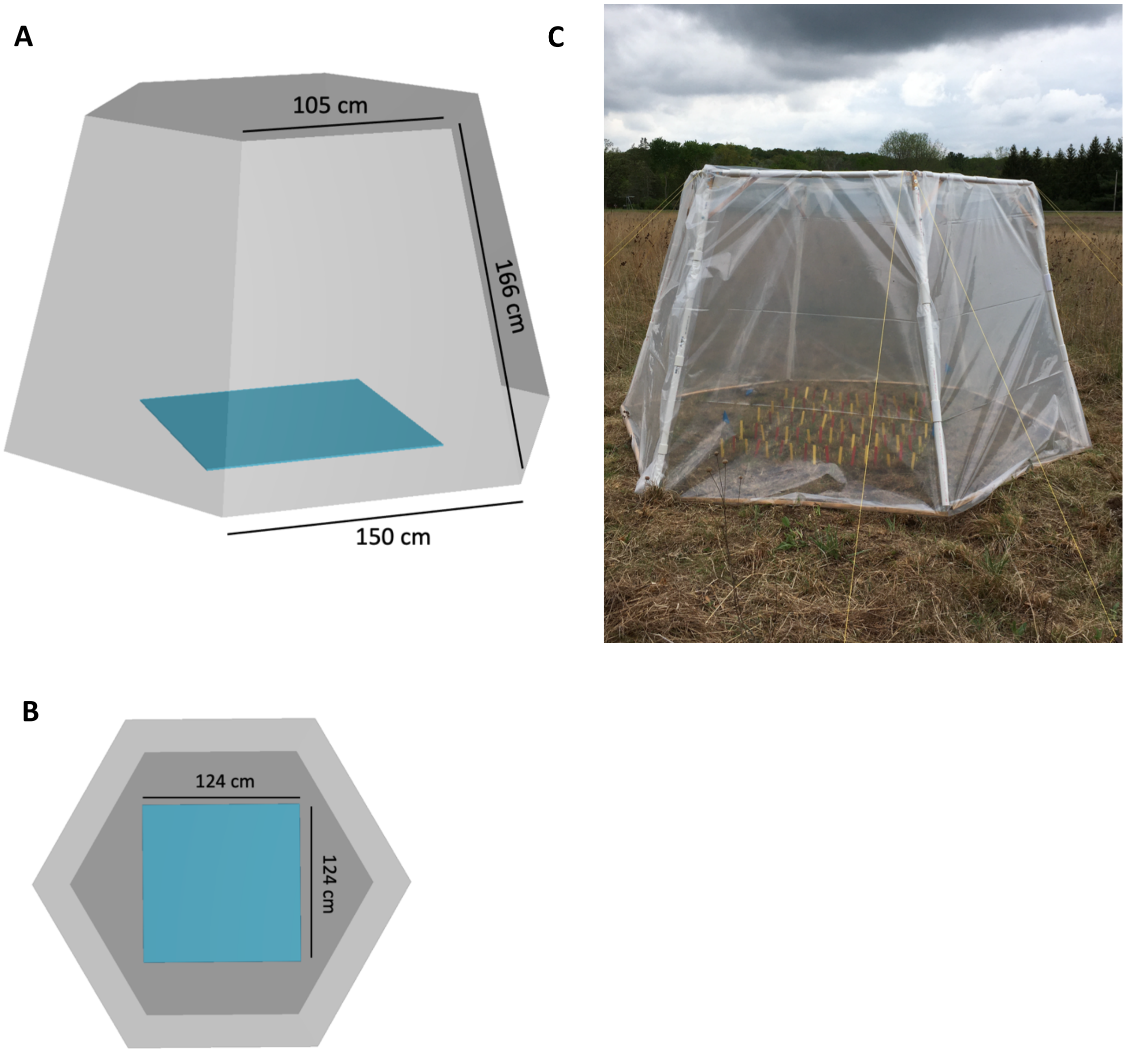

Figure S1. Design for the open top chamber (OTC) warming treatment. Each chamber accommodated a 1.25m plot and warmed the chamber 2-2.5 °C. A) LOTC blueprint with measurements of sides. Blue square in middle represents plots. B) Overhead drawing of experimental plot (with measurements) inside OTC. Dark hexagon represents the hexagonal opening of the OTC, whereas the light gray hexagon represents the lower hexagon of the OTC that touches the ground. C. Photo of OTC during the experiment.

Appendix B: Chapter 2 Supplemental Figures.

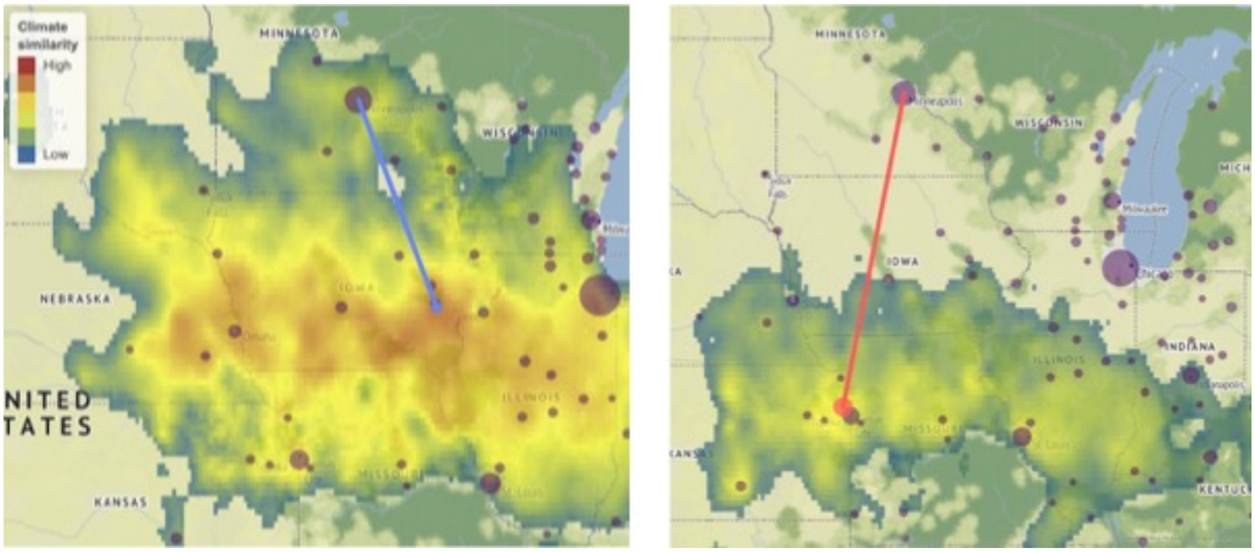

Figure S1: A) Map of climate analogs for the MN Central focal population given a 2°C increase under a lower emissions scenario. B) Map of climate analogs for the MN Central focal population given a 3.6 °C increase under the current level of emissions. Red tones indicate high similarity, while blue indicate low levels of similarity.

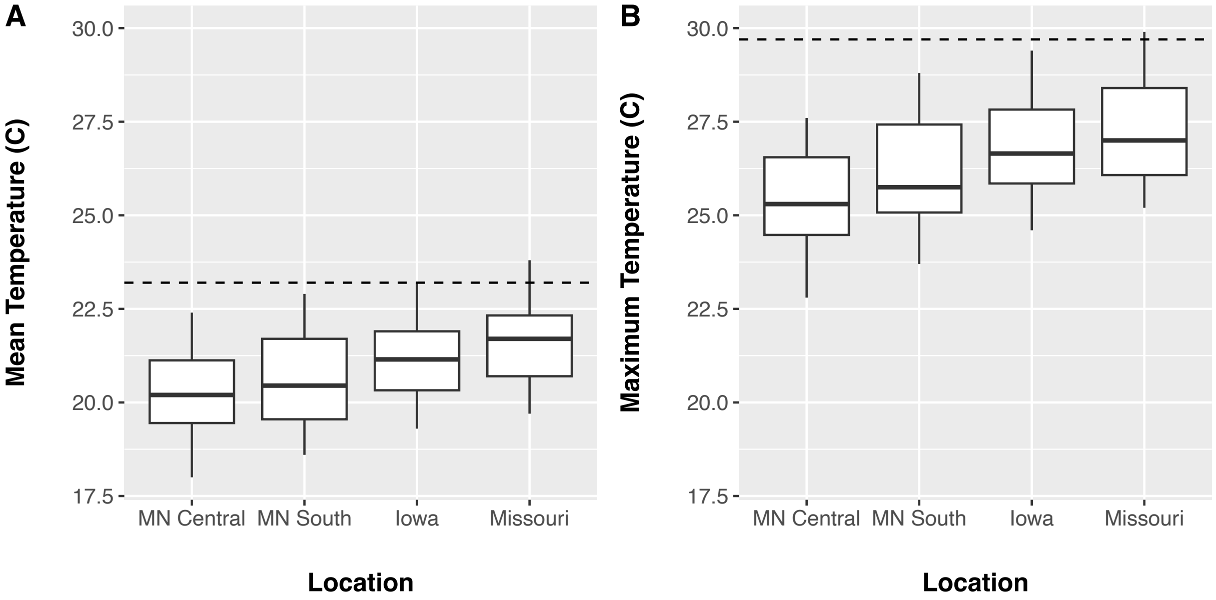

Figure S2: Box plots of A) mean temperature (°C) and B) maximum temperature (°C) over 30 years in each population origin for the month of June. The dashed line above the box plots represents the mean temperature of the ambient treatment in June of the experimental year.

Appendix C: Chapter 3 Supplemental Figures

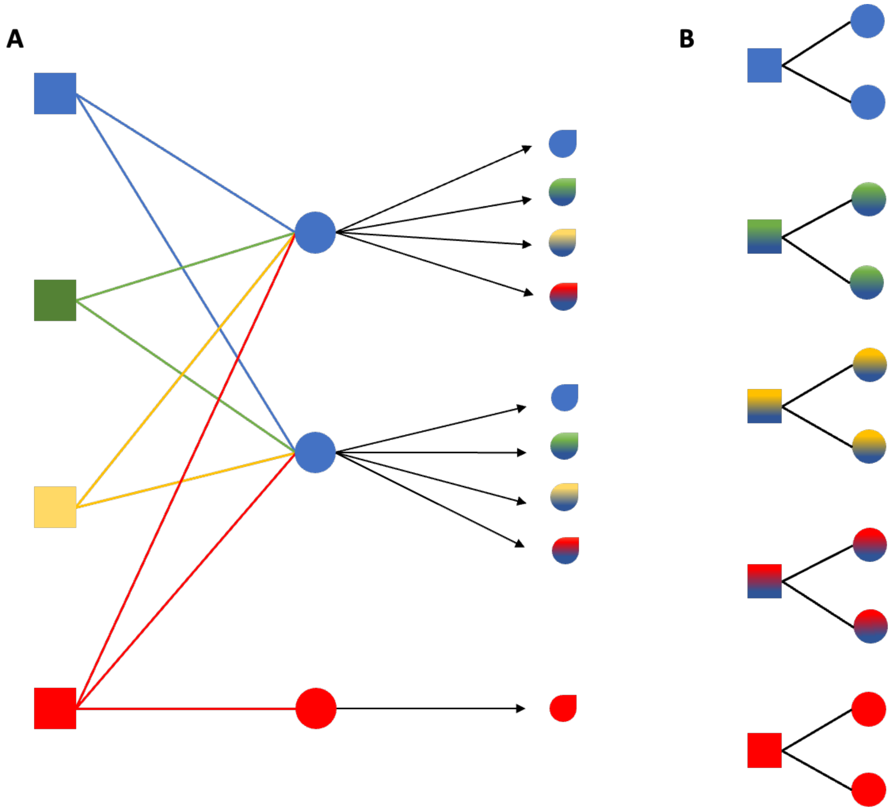

Figure S1. A) Pedigree design for the first generation of hybridization. Squares represent pollen donors (sires) and circles represent pollen recipients (dams). Blue individuals from the focal $MN_{central}$ population, green represents the MN_{south} population, yellow represents the IA population, and red represents the MO population. Hybrid offspring are represented as the shaded seed shape on the right of the black arrows with seed colors indicating the parental origin of that seed. B) Diagram of crossing structure for the second generation. Crosses were conducted within the newly created populations using seeds produced in the first generation.

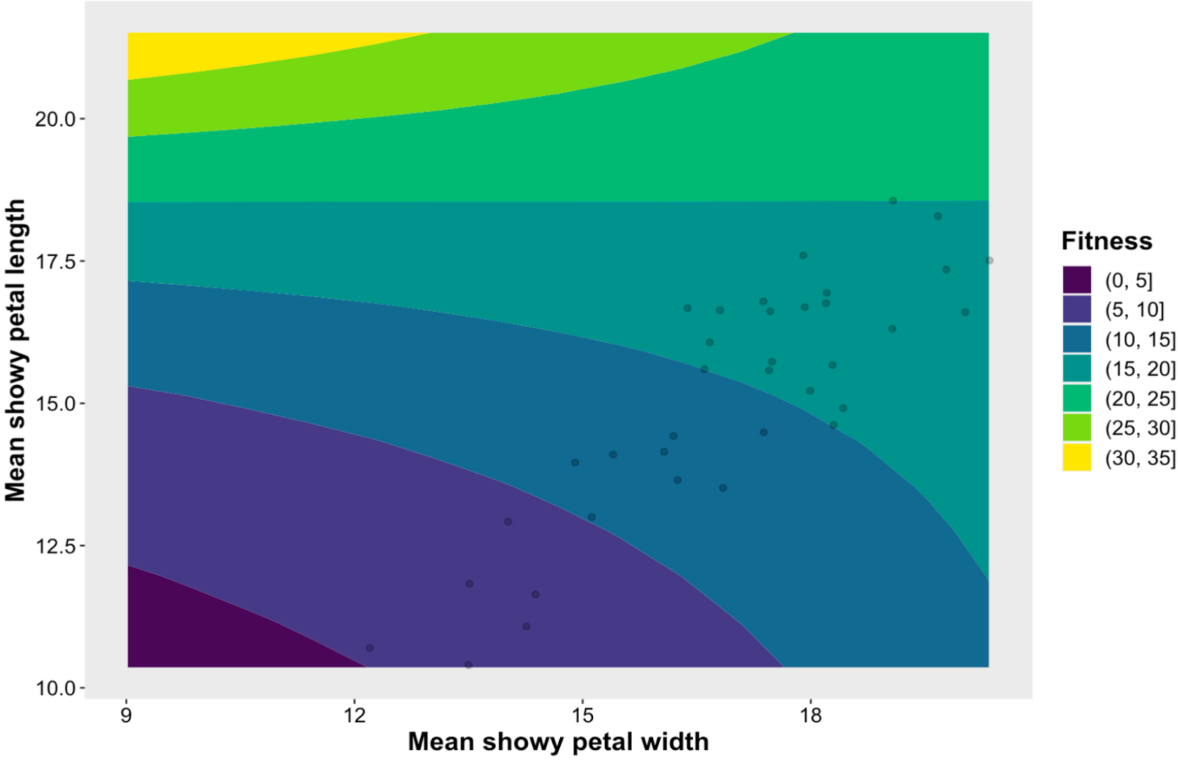

Figure S2. The interaction of showy petal width and length as it relates to fitness. Because there no interaction between the petal size measures and population and treatment meaning that all populations and treatments have similar slopes, this graph is a representation for all populations. However, only the MN$_{central}$ in the ambient treatment is depicted in this graph. Dark blue represents the lowest fitness and yellow represents the highest fitness. Data points shown in gray are included to illustrate the scatter in the data.

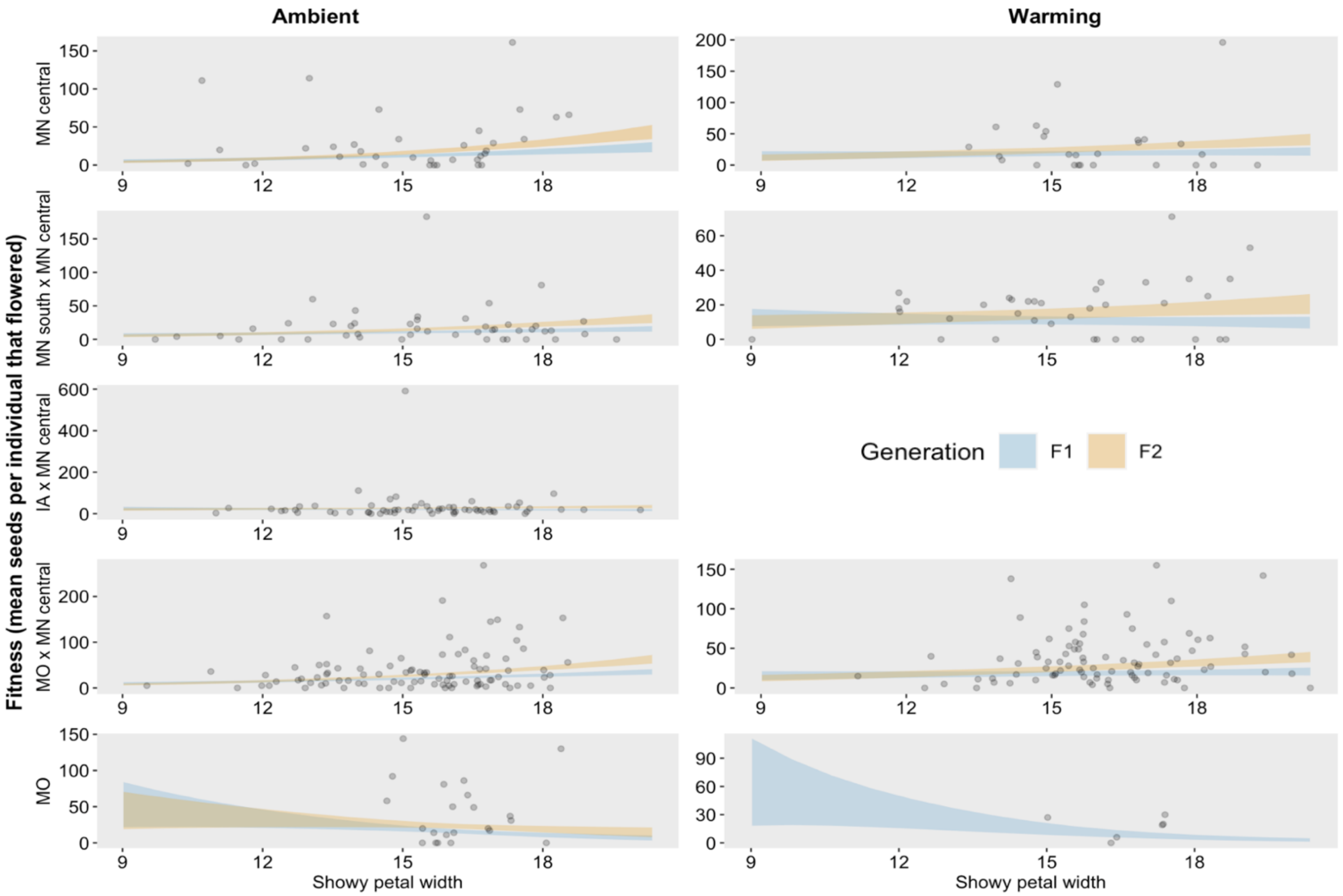

Figure S3. Fitness of individuals that flowered in each population and generation. Each row corresponds to a different population as noted on the left. Each column is a different temperature treatment. Red corresponds to the first generation, noted as F1. Blue corresponds to the second generation, noted as F2. IA x $MN_{central}$ and the second generation of MO were not included in the warming treatment due to space constraints. Data points are shown in gray and