

# Evidence of climate-driven selection on both tree traits and trait plasticity across the climatic range of a riparian foundation species

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## Abstract

Selection on quantitative traits by divergent climatic conditions can lead to substantial trait variation across a species range. In the context of rapidly changing environments, however, it is equally important to understand selection on trait plasticity. To evaluate the role of selection in driving divergences in traits and their associated plasticity within a widespread species, we compared molecular and quantitative trait variation in *Populus fremontii* (Fremont cottonwood) populations throughout Arizona. Using SNP data and genotypes from 16 populations reciprocally planted in three common gardens, we first performed QST-FST analyses to detect selection on traits and trait plasticity. We then explored the mechanistic basis of selection using trait-climate and plasticity-climate regressions. Three major findings emerged: 1) There was significant genetic variation in traits expressed in each of the common gardens and in the phenotypic plasticity of traits across gardens. 2) Based on QST-FST comparisons, there was evidence of selection in all traits measured; however, this result varied from no effect in one garden to highly significant in another, indicating that detection of past selection is environmentally dependent. We also found strong evidence of divergent selection on plasticity across environments for two traits. 3) Traits and/or their plasticity were often correlated with population source climate (R<sup>2</sup> up to 0.77 and 0.66, respectively). This suggests that steep climate gradients across the Southwest have played a major role in shaping the evolution of divergent phenotypic responses in populations and genotypes now experiencing climate change.

**Title :** Evidence of climate-driven selection on both tree traits and trait plasticity across the climatic range of a riparian foundation species

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Selection on quantitative traits by divergent climatic conditions can lead to substantial trait variation across a species range. In the context of rapidly changing environments, however, it is equally important to understand selection on trait plasticity. To evaluate the role of selection in driving divergences in traits and their associated plasticity within a widespread species, we compared molecular and quantitative trait variation in *Populus fremontii* (Fremont cottonwood) populations throughout Arizona. Using SNP data and genotypes from 16 populations reciprocally planted in three common gardens, we first performed  $Q_{ST}$ - $F_{ST}$  analyses to detect selection on traits and trait plasticity. We then explored the mechanistic basis of selection using trait-climate and plasticity-climate regressions. Three major findings emerged: 1) There was significant genetic variation in traits expressed in each of the common gardens and in the phenotypic plasticity of traits across gardens. 2) Based on  $Q_{ST}$ - $F_{ST}$  comparisons, there was evidence of selection in all traits measured; however, this result varied from no effect in one garden to highly significant in another, indicating that detection of past selection is environmentally dependent. We also found strong evidence of divergent selection on plasticity across environments for two traits. 3) Traits and/or their plasticity were often correlated with population source climate ( $R^2$  up to 0.77 and 0.66, respectively). This suggests that steep climate gradients across the Southwest have played a major role in shaping the evolution of divergent phenotypic responses in populations and genotypes now experiencing climate change.

## Introduction:

Understanding the processes shaping phenotypic diversification in nature is a central objective of ecology and evolutionary biology (Schluter 2000; Bolnick *et al.* 2011). Trait variation within widespread species can be extensive due to historic demographic processes and spatially and temporally heterogeneous landscapes exerting different selection pressures across a species' range (Whitlock 2008). Over time, subpopulations can become genetically and phenotypically differentiated due to neutral processes, such as drift, gene flow, and mutation, as well as the adaptive process of natural selection (Wright 1931; Spitze 1993; Holsinger & Weir 2009; Leinonen *et al.* 2013). Natural selection acts on both phenotypes and phenotypic plasticity, defined as the range of phenotypes a single genotype can express as a function of environmental change (Nicotra *et al.* 2010). The strength and direction of selection may vary, creating a mosaic of trait means and differences in trait plasticity across species' distributions (Chevin & Lande 2011). Adaptive evolution of individual traits and associated plasticity can therefore differentially affect a population's persistence on the landscape under a changing climate, as both alter the range of phenotypes a population can express (Kelly 2019).

Phenotypic divergence is particularly evident in long-lived forest trees, which often show strong genetic differences and local adaptation among populations with ecological and evolutionary consequences for associated species and communities (Savolainen *et al.* 2007; Leimu *et al.* 2008; Hereford 2009; Whitham *et al.* 2020). One common hypothesis for the origins of phenotypic variation in trees is local adaptation in response to climate. For example, studies on *Populus* have evidence of adaptive differences among populations in growth, phenology, and physiological traits (Frewen *et al.* 2000; Fischer *et al.* 2017; Blasini *et al.* 2020), and the evolution of regionally adapted ecotypes (Grady *et al.* 2011; Evans *et al.* 2014; McKown *et al.* 2014; Ikeda *et al.* 2017; Cooper *et al.* 2019; Bothwell *et al.* 2021). To definitively show that phenotypic variation among populations is due to divergent selection by their home climate, we need approaches that integrate molecular and phenotypic assessments of replicated genotypes across multiple common garden environments.

In addition to understanding the role of natural selection in shaping trait differences, there has been an increasing interest in understanding if and how selection acts on phenotypic plasticity itself (Josephs 2018; Arnold *et al.* 2019). Phenotypic plasticity is expected to evolve proportionally to the variability and predictability of the environment, with higher plasticity correlated with more predictable and more heterogeneous environments (Lande 2009; Lind *et al.* 2011). For example, De Kort *et al.* (2020) found plastic responses to drought in woodland strawberry were higher in topographically variable sites, while Leung *et al.* (2020) experimentally determined that plasticity evolved to a lower degree in populations of a microalga experiencing less predictable salinity conditions after 500 generations. The evolution of decreased plasticity in homogeneous environments could occur when there is a net cost to maintaining plasticity (DeWitt *et al.* 1998). Plasticity is also thought to increase in populations adapted to more benign climates relative to

harsh ones because the fitness cost of maladaptive plasticity producing phenotype-environment mismatches will be greater when resource availability is limited (Albert & Simms 2002). This has been demonstrated in studies where lower elevation plants produced stronger plastic responses to drought compared to plants from harsher, high-elevation sites (Gugger *et al.* 2015; Akman *et al.* 2021). Higher plasticity under milder (yet variable) conditions may occur under the normal range of background environmental fluctuations, however when extreme events occur outside of this range, theory predicts rapid evolution of plasticity (Lande 2009). Evolution of increased plasticity after extreme environmental shifts can allow mean phenotypes to approach new optima by accelerating phenotypic adaptation, and may enhance population persistence (Lande 2009; Chevin & Lande 2010). However, this depends on the shape of the reaction norm and the genetic variance and covariances available for selection to act upon after the extreme event (Chevin & Hoffman 2017). Finally, although plasticity is commonly studied on traits in isolation, species often respond to changes in environmental with phenotypic plasticity in multiple traits, termed multivariate plasticity (Schlichting 1989; Nielsen & Papaž 2022). Plasticity in one trait can therefore alter plasticity in another trait, changing the optimal multivariate plastic response and fitness outcome to the new environment (Nielsen & Papaž 2022). Correlations among trait plasticities may constrain the evolution of plasticity, resulting in discrete phenotypic strategies or solutions (Schlichting 1989). Together, these processes could combine to generate a heterogeneous landscape, where selection gradients can produce marked differences in plasticity along environmental clines.

A common test for whether natural selection is the mechanism responsible for generating phenotypic divergence among populations is to compare  $Q_{ST}$ , the variation in quantitative traits, to  $F_{ST}$ , the variation in neutral genes (Wright 1951; Lande 1992; Spitze 1993).  $Q_{ST}$  is the quantitative genetic analog to  $F_{ST}$  and measures the proportion of additive genetic variance in a trait attributed to among-population differences. If  $Q_{ST} > F_{ST}$ , there is evidence that directional selection is responsible for population-level divergence with respect to a trait of interest. If  $Q_{ST} \approx F_{ST}$ , the null model that population differences are due to genetic drift alone cannot be rejected. Finally, if  $Q_{ST} < F_{ST}$ , this suggests uniform or stabilizing selection acting to constrain among-population divergence (Spitze 1993). Selection is expected to be uniform when populations share the same phenotypic optimum and divergent when phenotypic optima vary, such as across heterogeneous environments (Le Corre & Kremer 2012). The surge in both experimental and theoretical  $Q_{ST}$ - $F_{ST}$  studies has revealed a major role of natural selection shaping intraspecific variation in quantitative traits (McKay & Latta 2002; Leinonen *et al.* 2008; Leinonen *et al.* 2013), with approximately 70% of all studies showing  $Q_{ST} > F_{ST}$  (Leinonen *et al.* 2008).

$Q_{ST}$ - $F_{ST}$  comparisons can also be used to test for selection on phenotypic plasticity (Josephs 2018). Lind *et al.* (2011) used  $Q_{ST}$ - $F_{ST}$  to test for selection on plasticity in development time among island populations of the common frog, *Rana temporaria*, which vary in pool drying regimes. DeKort *et al.* (2016) performed a modified Bayesian  $Q_{ST}$ - $F_{ST}$  analysis (Ovaskainen *et al.* 2011) to show selection on phenological plasticity in *Alnus glutinosa* across a latitudinal gradient. Alternatively, selection on plasticity can be assessed by regressing a genotype's plasticity against overall fitness or a fitness proxy (Pigliucci & Schlichting 1996; Arnold *et al.* 2019). In Fremont cottonwood, for example, higher plasticity in bud flush is associated with higher survival when populations experience warmer temperatures (Cooper *et al.* 2019). Either approach can complement the use of environment-trait regressions (Whitlock 2008) to test whether trait divergence among populations is systematically related to climatic gradients as selection pressures.

The role of selection by past climatic in shaping intraspecific variation in foundation species is especially important to quantify in the American Southwest, where the effects of climate change are pronounced (Garfin *et al.* 2013; Williams *et al.* 2020). The loss of foundation species, defined as species that create locally stable conditions, can have dramatic effects on fundamental ecosystem processes like energy fluxes and biodiversity (Ellison *et al.* 2005). The Southwest is described as one of the most "climate-challenged" regions of North America, with warming temperatures and increasing drought events already contributing to massive forest mortality events (Breshears *et al.* 2005). Fremont cottonwood is especially sensitive to drought and high temperature, particularly in combination, as evidenced by stand-level mortality at the Bill Williams National Wildlife Refuge on the lower Colorado River (Fig. 1). Recent studies by Hultine *et al.* (2020a) and Blasini *et*

*al.* (2020) suggest that these trees are at the edge of their thermal tolerance where water is essential for evaporative cooling. This mortality is associated with the megadrought that Williams *et al.* (2020) identify as being the second worst drought in the past 1200 years in the American Southwest. Thus, current climatic gradients will be exacerbated by ongoing climate change, leading to new selection pressures on functional traits that may be locally adapted to a narrower range of environmental conditions.

In this study, we use trait data from three experimental common gardens to quantify divergence ( $Q_{ST}$ ) in both genotype means within environments and genotype plasticities across environments for five traits of *Populus fremontii*. We then compare these  $Q_{ST}$  values to neutral genetic divergence ( $F_{ST}$ ). Common gardens are necessary to ensure that among-population variance components reflect genetic differences and are not inflated by environmental effects (Leinonen *et al.* 2013). Reciprocal common gardens can reveal traits that vary across environmental gradients as a result of phenotypic plasticity (Kawecki & Ebert 2004; Franks *et al.* 2014). Plastic responses to environmental stress or release from stress may mask or amplify genetically determined trait differences that have emerged as a result of divergent selection (Oke *et al.* 2015). It is therefore important to assess phenotypes in multiple growing conditions to see how the environment can modify the degree to which we can detect evidence of selection. Our use of multiple common gardens adds to the  $Q_{ST}$  literature by examining how the detection of trait differences depends on environmental conditions (Akman *et al.* 2021) and by allowing for  $Q_{ST}$ - $F_{ST}$  tests on trait plasticity across gardens.

Both the collection and garden locations span an elevation gradient of almost 2000 m, consistent with the species' range and including a difference of 12°C mean annual temperature and > 500 mm in mean annual precipitation across source locations and ~350 mm across gardens. The benefit of these experimental gardens is enhanced by the development of genomic data based on the identification of > 9000 single nucleotide polymorphisms (SNPs) for all genotypes planted. SNPs are an ideal type of marker for  $Q_{ST}$ - $F_{ST}$  analyses because their mutation rates and the effects of drift are considered to be more similar to loci that control quantitative traits compared to other molecular markers, such as hypervariable microsatellites (Edelaar & Bjorklund 2011). Thus, the only difference between quantitative trait loci driving  $Q_{ST}$  and the loci used in  $F_{ST}$  estimates should be that only the latter conform to neutral molecular evolution (Leinonen *et al.* 2013).

In order to address whether climate-driven natural selection drives trait and trait plasticity divergences across the range of Fremont cottonwood, we evaluated three hypotheses: 1) Genetic variation in tree traits will be evident among populations and genotypes in each of the three common gardens, although the magnitude of the genetic effects may vary across environments and among traits. Likewise, populations will differ in the magnitude of plasticity of these traits measured across the garden environments 2)  $Q_{ST}$  values will be significantly higher than the neutral expectation of  $F_{ST}$ , suggesting divergent selection has outweighed drift in shaping divergence in trait means and plasticity among populations. 3) Mean population phenotypes will show strong relationships with their climate of origin, as is expected when climate is a primary selective force. Similarly, trait plasticity will also be correlated with population source climate. Such plasticity-climate relationships should emerge when population origins differ not only in mean climate conditions but also in climatic variability across seasons and years, as is the case in the Southwest.

## Materials and Methods:

### Collection sites and common gardens

To establish the common gardens, 16 populations of *Populus fremontii* were collected throughout Arizona, encompassing the environmental variation experienced by the Sonoran Desert ecotype, as well as three populations located on the Mogollon Rim within the Colorado Plateau region of northern Arizona (Fig. 2). These populations group genetically with and have been alternatively identified as the Mogollon Rim (Blasini *et al.* 2020) or Utah High Plateau ecotype (Ikeda *et al.* 2017; Supplemental Table 1). This sampling design does not include the third described ecotype of the Central California Valley (Ikeda *et al.* 2017). Cuttings were taken from individual tree genotypes located over 20 m away from each other to ensure independent genotype sampling. Clonal replicates from 12 trees per population were planted in the summer and fall of 2014 in each of the three common garden sites after rooting in the greenhouse for approximately four months.

The three replicated experimental common gardens span broad elevation and climatic gradients, resulting in extreme climatic transfers for some populations. The northernmost garden represents the cold edge of the species' climatic range. It is located adjacent to Canyonlands National Park, Utah and is maintained by The Nature Conservancy's Dugout Ranch. The middle Arizona garden is located adjacent to the Agua Fria River in Agua Fria National Monument and is maintained by the Arizona Game and Fish Department. The southernmost garden is in Yuma, Arizona near Mittry Lake, and is maintained by the Bureau of Land Management. These gardens span over a 1500 m elevation difference, a 12°C mean annual temperature range (10.7°C in Yuma, 17.2°C in Agua Fria, and 22.8°C in Canyonlands), and a precipitation difference of ~350mm (Supplemental Table 1). Each common garden was planted with 4,096 trees. These trees were arranged into four replicated blocks to account for within-garden environmental variance, with each block made up of 16 randomized population-level plots. Each population plot had 64 trees, made up of three to six replicates of the 12 genotypes collected for that population. Plots were arranged in a randomized 8 x 8 grid, with trees spaced 1.85m in each cardinal direction. The garden was designed using population plots instead of fully randomized by genotype to assess population-level effects on dependent community members such as arthropods and mycorrhizae, as well as ecosystem-level traits like carbon flux.

In order to examine the relationship between climate and traits, we downloaded 30-year normals (1961-1990 means) for 21 abiotic climate variables for each of the 16 provenance sites and the three common gardens using the program ClimateWNA (Wang *et al.* 2012). Because variation in both temperature and precipitation in the Southwest are very strongly correlated with elevation, these current climate variables are excellent proxies for the climates that trees have experienced during their local evolutionary histories ( $r > 0.985$  for correlations between current MAT and MAP (WorldClim 2, Fick & Hijmans 2017) and those variables estimated from 6,000 or 22,000 years ago (WorldClim 1.4, Hijmans *et al.* 2005). To create a multivariate climatic index representing the environmental variation found throughout the 16 provenances, the ClimateWNA variables plus elevation, latitude, and longitude, were combined in a principal component analysis (PCA) using *labdsv* (Roberts 2007) and *vegan* (Oksanen *et al.* 2016) packages in the R statistical language (R Core Team 2014).

### Trait analysis

We analyzed five traits for phenotypic differentiation: fall bud set, spring bud flush, specific leaf area (SLA), height, and trunk basal diameter. Phenology of bud set and bud flush were measured in the fall of 2015 and the spring of 2016, respectively. Bud set was recorded as the initiation of bud formation, where internode elongation had ceased and the newly emerged, bundled leaves were clustered at the same level on the stem, offset from the shoot axis (Frewen *et al.* 2000). Bud set was measured at 6-10 day intervals from September through December of 2015 on three replicates of all 12 genotypes per population in each garden. We scored trees based on the bud stage exhibited by 50% or more of the apical meristems. There was little within-plant variation in apical bud development, so we felt this was a good approximation of whole plant progression towards dormancy. Spring bud flush was recorded as the date when all leaves had flushed from the tree, specifically when a leaf had expanded from all buds on the branch. Bud flush was measured every two weeks from February through the end of April in the Yuma and Agua Fria gardens, and through the end of May in the Canyonlands garden, where colder temperatures persist later into the spring. Bud set, bud flush, and height were assessed the full first year of growth (2015-2016) for every genotype in each of the common gardens.

Specific leaf area was measured using the average of three to six fully expanded leaves that were free of or had minimal herbivore damage (if no leaves without damage could be found). To standardize for light availability, all leaves were collected from a single south-facing branch, collected approximately at breast height in May and June of 2020. Due to mortality in the gardens over the five years since planting, and the time required for sampling, SLA was only measured on 12 populations in Yuma and Agua Fria, and seven populations in Canyonlands, with 3-7 genotypes measured within each population. Although early SLA analysis from 2015 showed similar patterns, concern over residual maternal and greenhouse effects cautioned against using this first year of data. Leaves were scanned and leaf area was measured using ImageJ software (Schneider *et al.*

. 2012). After the area scans, leaves were dried using silica beads and then weighed. Lastly, we used trunk basal diameter recorded at the end of the fourth year of growth (2018), which was measured as the diameter at root collar (DRC), ~10cm from the soil, on every live tree in the gardens. Diameter at root collar was used instead of diameter at breast height because it allows us to track tree growth consistently from planting up to their current stature.

### Genetic analysis

Genomic DNA was extracted from ~0.2g silica-dried leaf tissue from all 192 genotypes using the Thermo Scientific MagJET Genomic DNA Kit (Thermo Scientific). Double-digest restriction-associated DNA (ddRAD) libraries were prepared using 2-5ng of DNA per sample in 20 $\mu$ L reactions following a modified Peterson *et al.* (2012) protocol. Briefly, restriction and ligation was carried out simultaneously in 20 $\mu$ L reactions using restriction enzymes MspI and EcoRI and universal adapter sequences for indexing PCR. Ligation products were amplified using 25 cycles of PCR. After indexing, products were checked on an agarose gel and purified. Samples were then pooled and size selected for fragments between 200 and 350bp using a Pippin Prep (Sage Science, Inc., Beverly, MA). The size-selected pool was quantified by qPCR and sequenced on an Illumina MiSeq Desktop Sequencer (Illumina, Inc. San Diego, CA) in 2x75 mode. Sequence reads were processed using a modified Stacks pipeline (Catchen *et al.* 2013; Andrews 2018). Potential chloroplast and mitochondrial sequences were filtered from the dataset by comparing them to other *Populus* sequences downloaded from GenBank. Specifically, we removed sequences that matched chloroplast sequences from *P. fremontii* and mitochondrial sequences from *P. tremula* x *P. alba*. Parameter values for clustering were based on tests following parameterization in Mastretta-Yanes *et al.* (2015). The minimum stack depth for each individual was three and the minimum number of individuals per locus cluster was three. All loci were used in the measure of  $F_{ST}$  were found to be in Hardy-Weinberg equilibrium. To calculate  $F_{ST}$  and a 95% confidence interval around  $F_{ST}$ , we bootstrapped population-level pairwise  $F_{ST}$  values 500 times using the *divPartCalc* function in the R package *diversity* (Keenan *et al.* 2013).

### Statistical Analyses

To investigate the within- and among-population and genotype variation in phenotypic traits, each garden was modeled separately using linear random effects models fit by maximum likelihood in the *lme4* package in R (R Core Team 2014; Bates *et al.* 2015). The tree traits were modeled as response variables, while population and genotype nested within population were random effects. Garden plot was included as a random variable to help account for within-garden environmental variance. Statistical significance was calculated using likelihood ratio tests for the random effects using the package *lmerTest* (Kuznetsova *et al.* 2015).

To model population and genotype variation in phenotypic plasticity, we first needed to obtain replicated estimates of plasticity for each genotype and each trait. For each genotype with at least one tree in each of all three gardens, we randomly assigned all available trees into genotype triplets, with one tree from each garden. We then calculated plasticity for each triplet as the absolute value of the maximum difference in those three trait values. This produced a number of estimates of plasticity equal to the lowest number of trees available for that genotype in any garden. We repeated this random triplet assignment 100 times to obtain a set of possible plasticity datasets. For each dataset, we estimated the variance components necessary to calculate  $Q_{ST}$  using a linear random effects model as described above, where trait plasticity was the response variable, and population and genotype nested within population were the random effects.

For each trait and trait plasticity, we compared the quantitative trait variation ( $Q_{ST}$ ) with genetic variance at neutral loci ( $F_{ST}$ ). To calculate  $Q_{ST}$  we used the following formula:

where  $\sigma^2_P$  is the additive genetic variance among populations and  $\sigma^2_G$  is the additive within-population variance (Spitze 1993; McKay & Latta 2002), i.e., the variance among genotypes within populations. Each trait or plasticity was analyzed using the models described above, and population and genotype variances were extracted to calculate  $Q_{ST}$ . Parametric bootstrap and Bayesian estimation are considered the best methods to obtain a precision estimate around  $Q_{ST}$  (O'Hara & Merilä 2005). We performed parametric bootstrapping

to obtain a 95% confidence interval for  $Q_{ST}$ , resampling the 16 populations with replacement 1000 times, and estimating  $Q_{ST}$  for each bootstrapped data set. Resampling over the highest level in a hierarchical experimental design (here the population) is considered best practice (O’Hara & Merilä 2005). Variance in  $Q_{ST}$  becomes quite large as the number of populations decreases ( $< 20$ ), especially if populations are highly differentiated (O’Hara & Merilä 2005; Goudet & Büchi 2006). Goudet & Büchi (2006) recommend sampling many populations relative to the number of families. Our design of 16 populations with 12 genotypes per population comes close to their recommended sampling design of upwards of 20 populations with 10 families (O’Hara & Merilä 2005; Goudet & Büchi 2006). In using clonally replicated genotypes, our estimate of  $\sigma^2_G$  includes both additive and non-additive genetic effects, an approach that has been shown to lower  $Q_{ST}$  estimates and is thus a conservative test of  $Q_{ST} > F_{ST}$  (Cubry *et al.* 2017). Conversely, lower  $Q_{ST}$  estimates derived from non-additive genetic effects contribute to a more liberal test of convergent selection ( $Q_{ST} < F_{ST}$ ) (Whitlock 2008; Cubry *et al.* 2017). To determine whether  $Q_{ST}$  was significantly different from  $F_{ST}$ , we compared the 95% confidence intervals for both, which provides much stronger inference than simply comparing  $Q_{ST}$  to the mean  $F_{ST}$  value (Whitlock 2008; Leinonen *et al.* 2013). Broad-sense heritability ( $H^2$ ) point estimates and confidence intervals were also calculated for each trait in each garden using the equation,  $H^2 = \sigma^2_G / (\sigma^2_G + \sigma^2_E)$ , where  $\sigma^2_E$  includes both the plot variance and the error variance. Calculations of heritability for plasticity did not include plot-level variance since plasticity was measured across gardens.

In order to test whether phenotypes showed strong climatic relationships, we regressed population trait means and trait plasticities against the first principal component (PC1) from the environmental PCA. We tested these regressions and calculated an adjusted  $R^2$  using a linear model in R (R Core Team 2014). Here, we used a single estimate of plasticity for each genotype. Using all available replicates for each genotype, we first calculated the mean trait value for each genotype in each garden, and then calculated plasticity as the maximum difference between gardens. Systematic differences among populations seen in these trait-climate correlations are another, stronger test for evidence of divergent selection acting over genetic drift (Whitlock 2008).

## Results:

### Genetic and Phenotypic Variation

Our dataset of 192 genotypes analyzed with ddRAD yielded 9195 SNP loci. The 16 Arizona populations show strong differentiation with an average pairwise  $F_{ST} = 0.175$  and 95% confidence interval of 0.144-0.205. Consistent with our first hypothesis, we found significant within and among population variation for traits at each of the three common gardens (Table 1, Fig. 3), with phenology traits exhibiting higher differentiation at the population than the genotype level in all but one case. For SLA, height, and diameter at root crown (DRC), the relative contribution of population vs. genotype varied among gardens. Traits measured in the hottest common garden (Yuma) exhibited stronger population than genotype effects in four out of the five traits; for SLA, the proportion of variance explained by genotype was higher than the proportion explained by population, although it was very close (27% vs. 24%, respectively; Table 1). This garden thus produced higher values of  $Q_{ST}$  (the proportion of the genetic variance that is found among rather than within populations, see next section).

Phenotypic plasticity across the three gardens showed significant population differences in all traits except SLA (Table 1, Fig. 3). Here, the lower sample size of seven populations with no populations from the hottest locations may have contributed to this non-significant effect. Population explained more variance in plasticity in the growth traits compared to the phenology traits (Table 1).

### $Q_{ST} - F_{ST}$ : Comparison of quantitative trait differentiation to neutral genetic expectation

We found evidence of selection ( $Q_{ST} [?] F_{ST}$ ) driving phenotype differences in over half of the traits measured across the three gardens (Table 2, Fig. 4). Overall, the mean  $Q_{ST}$  value across all traits and all gardens (0.42) was above the  $F_{ST}$  confidence interval (0.144-0.205), consistent with directional selection shaping trait differences and increasing local adaptation among these populations. Bud flush traits exhibited the highest levels of population differentiation. For example,  $Q_{ST}$  for bud flush in the hot and mid gardens was

0.87 and 0.86, respectively, while  $Q_{ST}$  values for bud set were more moderate (0.26-0.54). The confidence intervals for  $Q_{ST}$  crossed those of  $F_{ST}$  in two phenology measurements (bud set in Agua Fria and bud flush in Canyonlands), suggesting no difference from the neutral expectation of genetic drift of these traits in these environments. Divergent selection on specific leaf area was apparent in the mid ( $Q_{ST} = 0.54$ ) and cold gardens (0.79), but not detectable in the hot garden of Yuma (0.31). Tree growth traits showed relatively lower  $Q_{ST}$  values compared to leaf traits across gardens. Tree height showed significant divergent selection when measured at the hottest common garden in Yuma, Arizona ( $Q_{ST} = 0.44$ ), but was not statistically different from  $F_{ST}$  in the two cooler gardens of Agua Fria and Canyonlands (0.14 and 0.26, respectively). Contrary to our hypothesis, we found evidence of stabilizing selection for basal trunk diameter in the coldest garden, where the  $Q_{ST}$  value (0.01) fell below the  $F_{ST}$  confidence interval. However, this result should be interpreted with caution since dominance reduces estimates of  $Q_{ST}$  and is therefore a poor indicator of stabilizing selection (Cubry *et al.* 2017). This trait was indistinguishable from the neutral expectation of  $F_{ST}$  in the warm and mid gardens ( $Q_{ST} = 0.50$  and 0.03, respectively).

Mean  $Q_{ST}$  values for all trait plasticities except DRC were above the  $F_{ST}$  confidence interval (Table 2), suggesting overall divergent selection acting on plasticity. We found the strongest evidence for divergent selection on plasticity for bud flush (mean  $Q_{ST} = 0.84$ ) and height (mean  $Q_{ST} = 0.66$ ) where the  $Q_{ST}$  95% confidence interval distribution never crossed the  $F_{ST}$  confidence interval (Fig. 5). In the analyses of bud set (mean  $Q_{ST} = 0.44$ ) and SLA (mean  $Q_{ST} = 0.69$ ), the lower  $Q_{ST}$  confidence interval distribution overlapped with  $F_{ST}$ , indicating that for some of the 100 possible plasticity datasets, trait plasticity differences among populations were not distinguishable from the neutral expectation of drift. Specifically, 67% of the permutations fell within the  $F_{ST}$  interval for bud set, while only 6% overlapped for SLA. Similar to DRC, the  $Q_{ST}$  values for DRC plasticity were much lower than the other plasticities (mean  $Q_{ST} = 0.07$ ), however the upper confidence interval did overlap with  $F_{ST}$  76% of the time (Fig. 5). These three cases of plasticity  $Q_{ST}$  confidence interval distributions overlapping with  $F_{ST}$  represent a weaker detection of selection. However, these results do provide partial evidence for SLA, where 94/100 permutations were non-overlapping.

### Climate as an agent of selection

The strength of the correlations between traits and provenance climate varied across gardens (Table 3, Fig. 3), supporting our third hypothesis of strong associations between phenotype and climate for some traits in some environments. A single axis (PC1) explained 95.8% of the variation in provenance climate and was influenced primarily by temperature and growing season-related climate variables (Supplemental Table 2). Populations sourced from areas with higher temperatures, lower precipitation, lower elevation, and longer growing seasons had higher PC1 scores. Bud set exhibited the strongest relationship with provenance climate across the gardens ( $R^2 = 0.67$  to 0.77), while bud flush showed significant correlations in the two warmer gardens ( $R^2 = 0.49$  in Yuma and 0.66 in Agua Fria), but not in the cold garden ( $R^2 = -0.01$ ) (Table 3, Fig. 3a). In Canyonlands, population variation was constrained as all trees flushed at approximately the same time, late in the spring. Specific leaf area did not show significant trait-climate correlations in any garden, although we see overall SLA values increasing from the hot to the cold garden site (Fig. 3a).

Tree growth traits were more likely to show garden-dependent relationships between population origin and performance (Table 3, Fig. 3b). Tree height showed no climate relationships when planted in the hottest garden, however the correlation become stronger in the mid to cold gardens. When planted at the coldest garden, trees sourced from colder, wetter climates, including the three populations from the Colorado Plateau, were taller than populations from hotter, drier environments. Similarly, the DRC relationship in the hottest garden showed trees sourced from warm, hot environments had larger trunk diameters compared to trees from colder climates (Fig. 3b). Together, tree height and basal trunk diameter act as indicators that overall tree performance is consistent with local adaptation, with hot, southern populations growing larger in the hottest Arizona garden, and northern, cold adapted populations growing larger in the coldest Utah garden.

Phenotypic plasticity was significantly correlated with population source climate for all traits except SLA (Table 3, Fig. 3). Populations sourced from hot, dry climates exhibited increased plasticity in leaf-level phenology traits relative to the colder populations, as previously reported in Cooper *et al.* (2019). Tree-

level growth traits showed the opposite pattern of increased plasticity in those populations sourced from the colder, high elevation environments. SLA showed a similar trend to phenology, with warmer populations exhibiting higher plasticity compared to populations sourced from cooler climates, but was not significant. Again, this may be due in part to the lower sample size of seven populations that did not include populations from the hottest, driest sites (Fig. 3a).

## Discussion:

We found evidence of selection acting on both traits and trait plasticity across our three common gardens. In addition, regressions between traits and provenance climate indicate that much of the selection detected with  $Q_{ST}$ - $F_{ST}$  analysis is driven by climatic clines to which these populations are locally adapted. The result of mostly high  $Q_{ST}$  values for traits is consistent with a majority of studies finding  $Q_{ST}$  is generally larger than  $F_{ST}$  for ecological traits (McKay & Latta 2002; Savoleinen *et al.* 2007; Leinonen *et al.* 2013). However, our result of divergent selection acting on trait plasticity is quite striking in light of the relatively few examples of selection on plasticity documented in the literature (Arnold *et al.* 2019). Combining  $Q_{ST}$ - $F_{ST}$  analysis on trait plasticity with plasticity-climate regressions can help uncover the evolutionary forces shaping plasticity differences across environmental gradients (Whitlock 2008; Josephs 2018; Kelly 2019). Below, we discuss the evidence for climate-driven adaptive divergence in traits and trait plasticity, local adaptation to climate, and the potential consequences of both under current climate change.

### Divergent selection shapes population trait and plasticity differences

We interpret cases when both the  $Q_{ST}$ - $F_{ST}$  analysis showed large divergences from neutral expectation and phenotype-climate correlations were significant as strong evidence for climate-driven selection. Cases with only one of these tests showing population differences provide partial evidence for climate-driven selection (Table 4). For instance, there were four cases showing  $Q_{ST} > F_{ST}$  but non-significant trait-climate correlations. These inconsistencies between the two tests could be due to divergent selection that is not related to the climatic gradients we tested. There were also four cases showing  $Q_{ST} \approx F_{ST}$  and significant trait-climate correlations. In these cases,  $Q_{ST}$ -confidence intervals overlapping with  $F_{ST}$  could be due to the bootstrap sampling of genotypes with less genetic variation compared to the full sampling design, thus lowering the  $Q_{ST}$  estimate. There were also more significant plasticity-climate correlations (4 out of 5) than significant  $Q_{ST}$ - $F_{ST}$  differences for plasticity (2 out of 5). Finally, there was a range of results across the three gardens within the  $Q_{ST}$ - $F_{ST}$  analysis itself. Together, these tests provide a continuum of support for selection on traits and trait plasticity, and highlight which traits may be under the strongest selection and potentially the most important to investigate under climate change.

We found the largest  $Q_{ST}$  values for spring bud flush, consistent with other studies showing high phenological divergence across latitudinal clines (Hurme 1999; Howe *et al.* 2003; Hallet *et al.* 2007; Evans *et al.* 2016). Spring bud flush is highly differentiated among *P. fremontii* populations, with a difference of up to eight weeks observed in flush timing (Grady *et al.* 2015; Cooper *et al.* 2019; Blasini *et al.* 2020). We also found large population differences in fall bud set timing of ~2-5 weeks across the common garden gradient, reflected in moderate  $Q_{ST}$  values in two out of the three gardens. The strong population differences in phenology found here agree with Fischer *et al.* (2017), who showed leaf phenology accounted for >80% of the variation in tree and forest productivity among Fremont cottonwood genotypes. We found larger population differences in bud flush compared to bud set. This result is intriguing given that spring bud flush is primarily governed by temperature, while fall bud set is mostly cued by precise day length periods (Thomashow 2001; Howe *et al.* 2003). While day length is driven by latitude and is constant from year to year, temperature can vary. The fixed environmental cue of day length should allow populations to become highly locally adapted and differentiated in bud set compared to a variable environmental cue such as temperature. However, the strength of photoperiod-driven selection on bud set may be relaxed in all but the highest elevation populations, where the trade-off for longer growing seasons is selected for in areas that very rarely or never experience killing frosts (Howe *et al.* 2003). Both phenology traits also showed strong relationships with provenance climate across the gardens, except for bud flush in the coldest garden, where low temperatures prevented an earlier flush in the southern, warm-adapted populations (Fig. 3a).

Our detection of selection was dependent, in part, on the environmental conditions of each garden. Bud flush, bud set, and SLA all exhibited divergent selection ( $Q_{ST} > F_{ST}$ ) in two out of three gardens (Fig. 4). Tree growth traits exhibited even larger variation in  $Q_{ST}$  among gardens. For example, we observed high population differentiation in height expressed in the hottest garden ( $Q_{ST} = 0.44$ ). When populations were planted in the moderate and cool gardens, these population differences diminished, but became more strongly predictable from home climate (Fig 3).  $Q_{ST}$  estimates for trunk diameter also decreased with decreasing garden temperature. This variability in  $Q_{ST}$  across gardens suggests that phenotypes shaped by selection pressures across a species' range can be expressed differently in different growing environments, with some environments enhancing and others dampening population phenotypic differences (Oke *et al.* 2015; Akman *et al.* 2021). Particularly for growth traits, this may represent an interaction between the selection pressures that have shaped existing variation across the species range and novel selection pressures imposed in a common garden experiment or under future climate change.

The larger population-level trait differences exhibited in the hottest common garden for most traits (except SLA) could be driven by the maladaptation of the cold-adapted, northern populations to the extreme thermal conditions experienced in this hot garden. This climate transfer from northern to southern Arizona represents an extreme warming treatment, a scenario that may be imposed on populations under severe heat waves with climate change (Cook *et al.* 2015). Similarly, Evans *et al.* (2016) found that the relationship between  $Q_{ST}$  and  $F_{ST}$  changed through time, with tree height displaying high population differentiation ( $Q_{ST} > F_{ST}$ ) under the growing conditions in one year but not the next. Long-term common garden experiments can demonstrate how population differences are expressed both across different environments and through time. Given the intensification of extreme events and climate variability going forward (Jentsch *et al.* 2007; Ganguly *et al.* 2009; Garfin *et al.* 2013; Williams *et al.* 2020), these types of field trials should be expanded to evaluate the correspondence between the degree of existing climate adaptation and the potential for future climate survival, either through phenotypic plasticity, selection on remaining genetic variation, or a combination of the two (Nicotra *et al.* 2010; Josephs 2018).

Our  $Q_{ST}$ - $F_{ST}$  comparison revealed support for divergent selection acting on phenotypic plasticity in bud flush and tree height, and showed partial evidence for selection on plasticity in the other three traits (Table 2; Fig. 5). This is in contrast to previous studies that found no evidence of selection on trait plasticity using  $Q_{ST}$ - $F_{ST}$  type comparisons (Lind *et al.* 2011, De Kort *et al.* 2016), and low overall support for selection on plastic responses to temperature (Arnold *et al.* 2019). Our results suggest that for some traits, differences in plasticity among populations across a wide environmental gradient are larger than expected from neutral genetics, where some populations show minimal plasticity and others exhibit high plasticity. Conversely we found some evidence for stabilizing selection in DRC plasticity, indicating that the difference in the magnitude of plasticity for this trait across our populations was smaller than expected by  $F_{ST}$ , however it was not below the  $F_{ST}$ -confidence interval in all 100 plasticity permutations. The mosaic of natural selection acting on trait plasticity across our populations shows how plasticity itself can evolve in response to different climates.

The mosaic of natural selection acting on trait plasticity across our populations shows how plasticity itself can evolve in response to different climates. We found significant plasticity-climate relationships in phenology and growth traits, where the sign of the correlation switched between these two types of traits (Fig. 3). Specifically, we found trees sourced from colder environments were significantly more plastic in height and DRC compared to the warm provenance populations, but were not as plastic with regard to their bud set and bud flush (Fig. 3). This is an example of a multivariate plasticity response, where plasticity in one trait may be affecting the plasticity in another trait (Nielsen & Papaj 2022). The higher plasticity in phenology traits measured in populations from hotter provenances is counterintuitive because colder source populations experience much more predictable fall freezing events and higher yearly temperature variation (see TD in Supplemental Table 1), and theory predicts plasticity will increase under predictably variable environments (Chevin & Lande 2010). However, our climate transfer of southern Arizona populations to the northernmost cold garden represents an extreme climate event (over 15°C colder in the coldest month for the populations from the hottest source locations, Supplemental Table 1) far outside of some populations'

normal temperature range, which can result in large, maladaptive plastic responses (Chevin & Hoffman 2017). The higher phenological plasticity seen in hot-adapted populations did not translate into increased growth or growth plasticity, likely due to maladaptive phenological plasticity that pushed these trees outside of the appropriate growing season window (Cooper *et al.* 2019). The high bud set plasticity of warm-adapted populations meant that these trees did not set bud until late in the growing season, when freezing temperatures damaged non-dormant tissues. The subsequent frost damage translated to lower growth compared to cold-adapted trees that set buds earlier in the season and avoided frost damage. The increased height of the cold adapted populations in the coldest garden relative to the warm populations produced the significant differences in height plasticity. Therefore, our result of higher height plasticity in populations sourced from cold locations can be partially explained by the warm populations’ maladaptive plasticity in phenology.

In comparing our results to previous findings of no divergent selection on plasticity in other systems, it is important to consider both climate means and variances. In this system, higher growth plasticity observed in populations sourced from colder, high elevation locations could also be due to adaptation to increased climate variability, compared to the central and southern Arizona populations. Specifically, the temperature difference between the mean warmest month and the mean coldest month was the largest for the three populations collected on the Colorado Plateau compared to the rest of the populations below the Mogollon Rim of the Plateau (see TD in Supplemental Table 1). This follows the theory that higher levels of plasticity should occur in more variable environments (Lande 2009).

Finally, our estimates of heritability in both traits and trait plasticity also indicate that these components of the phenotype can evolve in response to selection, at least under some environmental conditions. Broad-sense heritability values for the five traits were moderate, with a mean value across all gardens of 0.21 (Table 2). Our phenology heritability measures ( $H^2 = 0.04-0.48$  for bud flush and  $H^2 = 0.19-0.30$  for bud set) were lower than previously found in some *Populus* studies ( $H^2 = 0.94$  for bud flush and  $H^2 = 0.91$  for bud set in *P. trichocarpa x deltooides*, Frewen *et al.* 2000). Heritability values for growth traits ( $H^2 = 0.11-0.27$  for height and 0.08-0.21 for DRC) and SLA ( $H^2 = 0.10-0.35$ ) were fairly consistent with other reported *Populus* estimates ( $H^2 = 0.03-0.42$  for height and  $H^2 = 0.09-0.25$  for diameter at breast height in *P. tremuloides*, Ding *et al.* 2020;  $H^2$  [?] 0.2-0.6 for SLA in *P. nigra*, Guet *et al.* 2015). The range of heritability estimates for the same trait across the three gardens highlights the environment-dependent nature of heritability. This is especially apparent in our bud flush results, where we found the lowest value in the cold garden ( $H^2 = 0.04$ ) and the highest value in the warmest garden ( $H^2 = 0.48$ ). There was also no trend toward higher or lower heritability estimates in a particular common garden. These results suggest that in some environments evolutionary potential is limited but can increase as environmental conditions and associated selection pressures change. Furthermore, these heritability increases are not necessarily associated with a specific direction of change (*i.e.*, increasing or decreasing temperature). Broad-sense heritability for the five trait plasticities ranged from 0.09-0.18, a similar result to bud burst plasticity found in another riparian deciduous tree, black alder ( $H^2 = 0-0.129$ , De Kort *et al.* 2016). Our results of genetic variation in trait plasticity combined with the evidence for selection based on  $Q_{ST}-F_{ST}$  analysis and non-zero heritability estimates show selection on the heritable components of phenotypic plasticity may lead to evolving plasticity across the landscape among these Arizona populations of Fremont cottonwood.

### Local adaptation to climate

Whereas leaf phenology and morphology traits (bud flush, bud set, and SLA) had the highest degree of differentiation in our study, likely due to climate-related divergent selection, tree growth traits (height and trunk basal diameter) were less differentiated. This result suggests that the neutral processes of gene flow and drift may override weak selection on growth traits (McKay & Latta 2002), or that growth is strongly constrained by the relative success dictated by the rest of a plant’s phenotype (Saint-Laurent *et al.* 2007; Leimonen *et al.* 2013). In this case, the latter explanation is very likely. Although phenology and growth traits showed significant regressions with climate of origin (Fig. 3), only the growth traits reversed the sign of that relationship across gardens. Specifically, phenology trends were mostly constant across gardens, with warmer source populations setting bud later and flushing earlier regardless of growing environment

(except for bud flush in Canyonlands). However, height and trunk diameter declined as transfer distance increased (in terms of both hotter and colder climates) for populations relative to their home sites. This indicates local adaptation, where the highest productivity is observed in populations whose source climate best matches that of the garden climate. In the hottest garden (Yuma), there was a positive relationship between trunk diameter and warmer provenance climates. In the coldest garden (Canyonlands), the reverse was true, where trees from cooler provenances grew significantly taller than those from the warmer sites (Fig. 3b). Whether this higher performance of local populations is enabled by their leaf phenology and morphology traits measured here vs. additional plant functional traits is an important area for further study.

### *Management implications*

When local adaptation and phenotypic differentiation in forest trees are closely tied to variation in climate, populations may become increasingly maladapted as climate change continues (Shaw & Etterson 2012; Franks *et al.* 2014; Aitken & Bemmels 2015). However, the magnitude of climate change combined with the degree of genetic variability, heritability, and phenotypic plasticity of traits will all interact to determine the extent of adaptation or maladaptation. Maladaptation due to climate change is expected to be greatest in populations from the warmest extent of their range, while populations at the cold edge may benefit from slightly warmer temperatures (Aitken & Bemmels 2015). This expectation corresponds with the maladaptive phenological plasticity we have observed in southern populations vs. adaptive phenological plasticity in northern populations (Cooper *et al.* 2019). However, nuanced changes in temperature and precipitation patterns will produce novel genotype-climate associations, creating more complex climate responses compared to the poleward range shifts and vulnerable trailing edges traditionally associated with warming (Gougherty *et al.* 2021). Although our study does not encompass the full genetic and geographic range of Fremont cottonwood, our results of declining performance as climate transfer distance increases suggests that this species will likely experience maladaptation as local conditions become more arid, especially for southern populations that are close to their thermal tolerance (Ault *et al.* 2014; see Fig. 1). Because these trees are important foundation species of riparian systems, selecting genotypes with sufficient performance under warming conditions is essential for the persistence of associated communities and ecosystems.

### Conclusions

By combining  $Q_{ST}$ - $F_{ST}$  analyses and climate-trait regressions, we show that both quantitative traits and associated plasticity are targets of climate-driven selection, where population may exhibit significant differentiation in both, one, or neither type of analysis (Table 4). This spectrum can help determine which traits are likely under the strongest climate-drive selection. Specifically, phenology as well as bud flush plasticity and height plasticity show the strongest responses to selection (Table 4). These traits and plasticities could be most impacted by further climate change, as they show strong signals of climate-driven selection in the past. However, both the detection of past selection and the differentiation in current performance differed strongly across our experimental common gardens. Strategies for management of widespread species like Fremont cottonwood would benefit from considering the climatic selection pressures of source locations to anticipate their future performance, as well as the population-specific potential for adaptive trait plasticity under changing environmental conditions.

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**Data Accessibility:**

The trait values from each garden and the SNP dataset used to produce  $F_{ST}$  estimates, along with the detailed RADseq protocol used in this manuscript, will be uploaded to Dryad upon manuscript acceptance.

**Author Contributions:**

HF Cooper collected trait data, performed genetic and statistical analyses, and wrote much of the manuscript. RJ Best contributed to the development of the  $Q_{ST}$  plasticity calculation and helped with the theoretical development of the paper and writing. She, JPM Corbin, and IJ Garthwaite also assisted with some field collections and sample processing. LV Andrews helped with RADseq processing. GJ Allan, CA Gehring, KR Hultine, KR Grady, and TG Whitham, designed the common garden experiment. All authors provided critical edits to the manuscript and figures.

**Tables and Figures:**

**Table 1:** Model variance and p-values for each trait measured in each garden, showing the population, genotype, and plot-level effects on trait variation. In addition, model variance and p-values for each trait’s plasticity across the three gardens, showing the population effect, are shown.

Trait	Garden	Variable	Proportion variance explained	P-value
Bud set	Yuma	Population	30.19	<0.001
		Genotype	13.12	<0.001
		Plot	8.43	<0.001
	Agua Fria	Population	17.70	<0.001
		Genotype	24.97	<0.001
		Plot	7.82	<0.001
	Canyonlands	Population	29.53	<0.001

Trait	Garden	Variable	Proportion variance explained	P-value	
Bud flush	Garden	Genotype	16.57	<0.001	
		Plot	2.23	0.003	
		Plasticity	Population	30.00	<0.001
		Yuma	Population	86.59	<0.001
		Genotype	6.42	<0.001	
		Plot	0	1	
	Agua Fria	Population	78.08	<0.001	
		Genotype	6.59	<0.001	
		Plot	1.54	<0.001	
		Canyonlands	Population	2.97	0.16
			Genotype	3.56	0.08
			Plot	4.07	0.02
SLA	Plasticity	Population	23.25	<0.001	
		Yuma	Population	24.28	0.002
		Genotype	26.81	<0.001	
	Agua Fria	Plot	10.91	<0.001	
		Population	19.31	0.02	
		Genotype	8.3	<0.001	
		Plot	27.63	<0.001	
		Canyonlands	Population	61.21	<0.001
			Genotype	8.16	<0.001
	Plot		7.03	<0.001	
	Height	Plasticity	Population	6.72	0.2
			Yuma	Population	14.85
Genotype			9.40	<0.001	
Agua Fria		Plot	7.57	<0.001	
		Population	3.73	0.5	
		Genotype	11.55	<0.001	
		Plot	20.26	<0.001	
		Canyonlands	Population	16.39	0.004
			Genotype	22.81	<0.001
Plot			8.57	<0.001	
DRC		Plasticity	Population	45.51	<0.001
			Yuma	Population	12.13
	Genotype		6.13	<0.001	
	Agua Fria	Plot	12.92	<0.001	
		Population	0.52	0.9	
		Genotype	7.90	<0.001	
		Plot	23.77	<0.001	
		Canyonlands	Population	0.71	0.7
			Genotype	24.66	<0.001
	Plot		4.03	<0.001	
	Plasticity	Population	36.93	<0.001	

**Table 2:**  $Q_{ST}$  and broad-sense heritability,  $H^2$  (+ 95% confidence intervals), for each trait in each garden, and the mean  $Q_{ST}$  and  $H^2$ (+ mean 95% confidence intervals) for the 100 plasticity permutations for each trait across gardens.

Trait	Garden	Q <sub>ST</sub>	H <sup>2</sup>
Bud set	Yuma	0.54 (0.40-0.86)	0.19 (0.05-0.26)
	Agua Fria	0.26 (0.15-0.42)	0.30 (0.24-0.34)
	Canyonlands	0.47 (0.46-0.57)	0.28 (0.18-0.24)
	Plasticity	0.44 (0.18-0.82)	0.12 (0.03-0.21)
Bud flush	Yuma	0.87 (0.69-0.93)	0.48 (0.36-0.60)
	Agua Fria	0.86 (0.67-0.93)	0.30 (0.16-0.43)
	Canyonlands	0.29 (0.00-1.00)	0.04 (0.00-0.07)
	Plasticity	0.84 (0.64-0.95)	0.16 (0.04-0.27)
SLA	Yuma	0.31 (0.01-0.62)	0.35 (0.18-0.46)
	Agua Fria	0.54 (0.23-0.79)	0.10 (0.04-0.14)
	Canyonlands	0.79 (0.70-0.93)	0.21 (0.09-0.23)
	Plasticity	0.69 (0.37-0.98)	0.14 (0.01-0.26)
Height	Yuma	0.44 (0.23-0.69)	0.11 (0.04-0.17)
	Agua Fria	0.14 (0.00-0.50)	0.12 (0.05-0.19)
	Canyonlands	0.26 (0.03-0.43)	0.27 (0.18-0.31)
	Plasticity	0.66 (0.42-0.94)	0.09 (0.01-0.17)
DRC	Yuma	0.50 (0.09-0.73)	0.07 (0.04-0.10)
	Agua Fria	0.03 (0.00-0.26)	0.08 (0.05-0.10)
	Canyonlands	0.01 (0.00-0.07)	0.21 (0.19-0.30)
	Plasticity	0.07 (0.00-0.16)	0.18 (0.13-0.23)

**Table 3:** The adjusted R<sup>2</sup> and p-value output from the linear regression models of provenance climate (PC1 score) and population trait means at each common garden, as well as between PC1 and the population-level trait plasticity.

Trait	Garden	Adjusted R <sup>2</sup>	P-value
Bud set	Yuma	0.67	< 0.001
	Agua Fria	0.76	< 0.001
	Canyonlands	0.77	< 0.001
	Plasticity	0.65	< 0.001
Bud flush	Yuma	0.49	0.002
	Agua Fria	0.66	< 0.001
	Canyonlands	-0.01	0.36
	Plasticity	0.62	< 0.001
SLA	Yuma	-0.08	0.69
	Agua Fria	0.02	0.28
	Canyonlands	0.20	0.17
	Plasticity	0.33	0.1
Height	Yuma	-0.02	0.42
	Agua Fria	0.32	0.013
	Canyonlands	0.12	< 0.001
	Plasticity	0.32	0.017
DRC	Yuma	0.62	< 0.001
	Agua Fria	-0.01	0.37
	Canyonlands	0.05	0.21
	Plasticity	0.28	0.02

**Table 4:** Summary of the two tests for selection. Light blue indicates a significant Q<sub>ST</sub>-F<sub>ST</sub> test and dashed

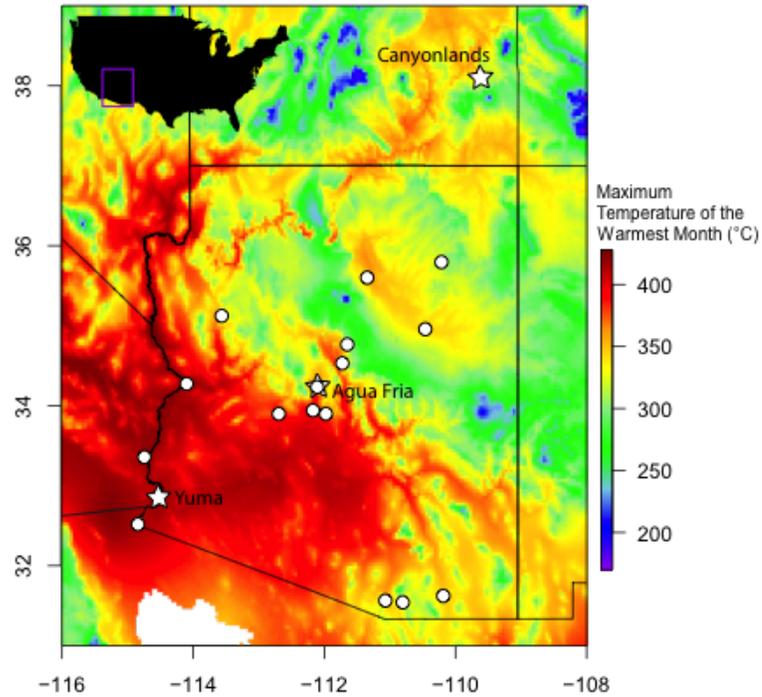
lines indicate significant climate regressions for that trait or plasticity. The Garden abbreviations are Y= Yuma, A = Agua Fria, C = Canyonlands, and P = Plasticity across gardens.

Trait	Bud set	Bud set	Bud set	Bud set	Bud flush	Bud flush	Bud flush	Bud flush	SLA	SLA
Garden	Y	A	C	P	Y	A	C	P	Y	A
Selection Tests										

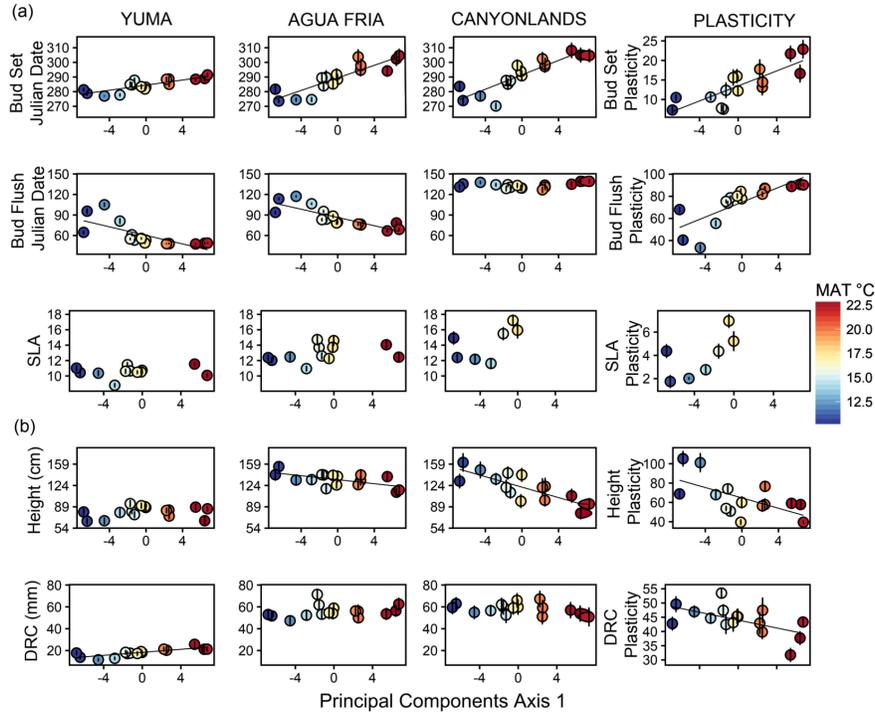
**Figure 1:** Stand-level mortality event of Fremont cottonwoods along the Bill Williams National Wildlife Refuge on the lower Colorado River. Photo taken by HF Cooper in March 2017.



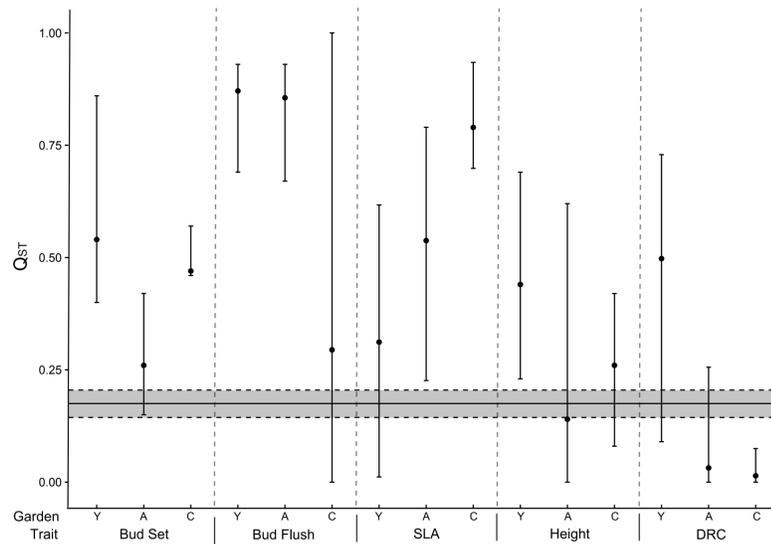
**Figure 2:** Map of the 16 collection locations (white circles) and three common gardens (white stars). The middle common garden of Agua Fria is also a collection site. Color represents the maximum temperature of the warmest month (°C). Axes are labeled with degrees latitude and longitude. Inset is the map of the United States with a purple rectangle outlining the latitude and longitude presented in the full map.



**Figure 3:** Population means ( $\pm$  1SE) for (a) leaf traits and (b) growth traits measured in each garden as well as the population mean trait plasticity values, regressed onto their home climate (indicated by principal component axis 1 values). Populations are colored by mean annual temperature (MAT °C). Regression lines are present when there is a significant relationship ( $p < 0.05$ ) between the PC1 axis and the trait. SLA is in unit of  $\text{mm}^2/\text{mg}$ . Note the scale on the y-axis for the plasticity regressions are different than the trait regressions.



**Figure 4:**  $Q_{ST}$  means and 95% confidence intervals (point and vertical lines, respectively) for the phenotypic traits measured at each of the three gardens. The average pairwise  $F_{ST}$  value (0.175) + 95% confidence interval (0.144 - 0.205) is shown as the grey band. Common gardens are abbreviated as Y = Yuma, A = Agua Fria, and C = Canyonlands.



**Figure 5:** Trait plasticity  $Q_{ST}$ - $F_{ST}$  distributions for each trait measured across the three common gardens. The filled shape is the mean  $Q_{ST}$  distribution and the open shapes are the 95% confidence intervals for the 100 plasticity datasets. Like Fig. 4, the horizontal lines represent the average population pairwise  $F_{ST}$  and

its 95% confidence interval. Any  $Q_{ST}$  distribution that crosses the  $F_{ST}$  confidence interval is interpreted as no different from neutral expectation and not under selection.

