

Climate-driven divergent selection in a foundation tree species: QST-FST evidence from multiple common gardens

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Abstract

Widespread tree species span large climatic gradients that often lead to high levels of local adaptation and phenotypic divergence across their range. To evaluate the relative roles of selection and drift in driving divergence in phenotypic traits, we compared molecular and quantitative genetic variation in *Populus fremontii* (Fremont cottonwood), using data from > 9000 SNPs and genotypes from 16 populations reciprocally planted in three common gardens that span the species' climatic range. We present three major findings: 1) There is significant within- and among-population variation in functional traits expressed in each of the common gardens. 2) There is evidence from all three gardens that population divergence in leaf phenology and specific leaf area has been driven by divergent selection ($Q_{ST} > F_{ST}$). In contrast, Q_{ST} - F_{ST} comparisons for performance traits like height and basal diameter were highly dependent on growing environment, indicating divergent, stabilizing, or no selection across the three gardens. We show this is likely due to local adaptation of source populations to contrasting growing environments. 3) Climate is a primary selective force driving trait divergence, where the traits showing the strongest correlations with a genotype's provenance climate also had the highest Q_{ST} values. We conclude that climatic gradients have contributed to significant phenotypic differences and local adaptation in Fremont cottonwood. These results are important because as climate is changing much more rapidly, traits such as phenology that are finely tuned to local conditions may now be subject to intense selection or quickly become maladaptive.

Title: Climate-driven divergent selection in a foundation tree species: Q_{ST} - F_{ST} evidence from multiple common gardens

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Abstract:

Widespread tree species span large climatic gradients that often lead to high levels of local adaptation and phenotypic divergence across their range. To evaluate the relative roles of selection and drift in driving divergence in phenotypic traits, we compared molecular and quantitative genetic variation in *Populus fremontii* (Fremont cottonwood), using data from > 9000 SNPs and genotypes from 16 populations reciprocally planted in three common gardens that span the species' climatic range. We present three major findings: 1) There is significant within- and among-population variation in functional traits expressed in each of the common gardens. 2) There is evidence from all three gardens that population divergence in leaf phenology and specific leaf area has been driven by divergent selection ($Q_{ST} > F_{ST}$). In contrast, Q_{ST} - F_{ST} comparisons for performance traits like height and basal diameter were highly dependent on growing environment, indicating divergent, stabilizing, or no selection across the three gardens. We show this is likely due to local adaptation of source populations to contrasting growing environments. 3) Climate is a primary selective force driving trait divergence, where the traits showing the strongest correlations with a genotype's provenance climate also had the highest Q_{ST} values. We conclude that climatic gradients have contributed to significant phenotypic differences and local adaptation in Fremont cottonwood. These results are important because as climate is changing much more rapidly, traits such as phenology that are finely tuned to local conditions may now be subject to intense selection or quickly become maladaptive.

Introduction:

Understanding the processes shaping phenotypic diversification in nature is a central objective of ecology and evolutionary biology (Schluter 2000, Bolnick *et al.* 2011). The effects of phenotypic variation in foundation species can be far-reaching, influencing everything from species interactions to the evolution of complex communities (Whitham *et al.* 2020). Trait variation within widespread species can be extensive due to historic demographic processes and spatially and temporally heterogeneous landscapes exerting different selection pressures across their 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). The relative importance of these stochastic versus selective forces is still debated but is crucial for understanding the probability and rate of phenotypic divergence in the past and future (O'Hara 2005; Hangartner *et al.* 2012; Leinonen *et al.* 2013). Forest ecosystems provide evidence of significant genetic differences, a high degree of local adaptation, and ecological consequences for associated species and communities (Savolainen *et al.* 2007; O'Neill *et al.* 2008; Leimu *et al.* 2008; Hereford 2009), including species of *Populus* (Whitham *et al.* 2006; Grady *et al.* 2011; Grady *et al.* 2013; Evans *et al.* 2016; Fischer *et al.* 2017; Cooper *et al.* 2019). Understanding the processes underlying genetic and phenotypic divergence in these species, especially in relation to past and future adaptation to climatic variation, is essential both for selecting current stock for restoration and forecasting the potential for further adaptation in response to climate change (Grady *et al.* 2015; Evans *et al.* 2016).

One way to test whether natural selection is the mechanism responsible for generating phenotypic differences 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} exceeds the neutral expectation of F_{ST} , there is evidence that directional selection is responsible for population-level phenotypic differentiation. 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} is lower than F_{ST} , this suggests uniform or stabilizing selection acting to constrain among-population divergence (Spitze 1993). Q_{ST} - F_{ST} comparisons have been primarily used to detect selection and evaluate the degree of local adaptation among populations, but have increasingly been used as a management and conservation tool (Leinonen *et al.* 2013). For example, Q_{ST} has been used to designate populations as separate conservation units (Leinonen *et al.* 2008), to assess the adaptive potential of invasive species, measure the rates of evolution in different environments, and

look at the constraints on adaptation due to increased habitat fragmentation (Leinonen *et al.* 2013). The surge in both experimental and theoretical studies comparing molecular and quantitative genetic variation has revealed a major role of natural selection in 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} studies are often used as an exploratory analysis to first see the selective patterns across a suite of traits, and then target those traits with the highest levels of differentiation to examine their genetics and responses to selection more closely (Leinonen *et al.* 2008; Whitlock 2008).

The pattern of phenotypic variation in tree species along climate gradients often appears consistent with local adaptation in response to selection by climatic conditions. For example, phenological traits are closely linked to temperature and photoperiod, and show strong latitudinal clines in multiple tree species (Howe *et al.* 2003; Savoleinen *et al.* 2007; Evans *et al.* 2016; Cooper *et al.* 2019). Within *Populus*, growth and phenology traits differ among genotypes (Frewen *et al.* 2000; Howe *et al.* 2000; Fischer *et al.* 2017; Davis *et al.* 2020), with evidence of variation and adaptive differences among populations (Grady *et al.* 2011; Evans *et al.* 2014; McKown *et al.* 2014; Cooper *et al.* 2019). In *Populus fremontii* specifically, there are large population differences in phenology expressed in common garden experiments at both the cold and hot edge of the species' tolerance (Cooper *et al.* 2019), as well as clear correspondence between a population's source climate and its mortality and productivity in cold vs. hot conditions (Grady *et al.* 2011, 2013, 2015). Population structure in *P. fremontii* has also been attributed to differences in spring and winter precipitation, which can affect flowering phenology, and therefore gene flow, across its range (Cushman *et al.* 2014; Ikeda *et al.* 2017). However, 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 in common garden environments.

The role of selection by past climatic conditions 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). Fremont cottonwood is especially sensitive to drought and high temperature, as is evidenced by stand-level mortality at the Bill Williams National Wildlife Refuge on the lower Colorado River (Fig. 1). Mortality in these trees 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. 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. 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 spanning the climatic range of *P. fremontii* to quantify phenotypic divergence (Q_{ST}) and compare it 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 experimental gardens can indicate whether populations are locally adapted to their current environments, reveal traits that vary across environmental gradients as a result of phenotypic plasticity (Kawecki & Ebert 2004; Franks *et al.* 2014), and quantify the intensity of selection across space (Whitlock 2008). Our use of multiple common gardens adds to the Q_{ST} literature by examining how population-level trait differentiation is expressed across environmental gradients. 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). Therefore, it is important to assess phenotypes in multiple growing conditions in order to demonstrate how the environment can modify the degree to which we can detect evidence of selection.

The three gardens used in this study contain cloned cuttings from 16 populations of *P. fremontii* collected throughout Arizona. Both the collection and garden sites span an elevational 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. The benefit of these experimental gardens is enhanced by the development of genomic data based on the identification of 1000s of single nucleotide polymorphisms (SNPs) in the Fremont

cottonwood genome. These data can provide improved estimates of F_{ST} , owing to their greater coverage of the genome and potentially lower mutation rate than microsatellites, which have been routinely used to estimate F_{ST} . SNPs are an ideal type of marker for quantifying molecular divergence because mutation rates and the effects of drift on SNP variation are considered to be more similar to loci that control quantitative traits (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 natural selection by climatic conditions is an agent of phenotypic diversification across the range of Fremont cottonwood, we evaluated three hypotheses: 1) Genetic variation in multiple tree traits (phenology, specific leaf area, height, and trunk diameter) will be evident among populations and genotypes in each of the three common gardens, although the magnitude of the genetic component may vary across environments and among traits. 2) Q_{ST} values will be significantly higher than the neutral expectation of F_{ST} , suggesting divergent selection has outweighed drift in shaping trait differences. Again, this drift-selection balance may vary among traits, and our ability to detect selection on these traits may vary across common gardens. 3) Mean population phenotypes will show strong associations with their climate of origin, especially for the most differentiated traits (those with high Q_{ST}). This pattern is expected when phenotypic differentiation is strongly shaped by selection due to climate.

Materials and Methods:

Collection sites and common gardens

Sixteen populations of *Populus fremontii* were collected throughout Arizona, encompassing the environmental variation experienced by the Sonoran Desert ecotype (Ikeda *et al.* 2017), as well as three populations located on the Colorado Plateau, which fall into a recently described Mogollon Rim ecotype (Blasini *et al.* 2020; Supplemental Table 1). Cuttings were from individuals collected over 20 m distance from each other to insure independent genotype sampling. Clonal replicates of 12 genotypes per population were planted in the summer and fall of 2014 in three common garden sites after rooting in the greenhouse for ~4 months. Using this design, within and among population variation for phenotypic traits could be assessed.

The three experimental common gardens span wide environmental gradients (Fig. 2, Supplemental Table 1), resulting in extreme climatic transfers for some populations. The northernmost garden 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 located in Yuma, Arizona near Mittry Lake, and is maintained by the Bureau of Land Management. Each common garden is composed of four blocks, with each block made up of 16 randomized population plots. Each population plot has 64 trees composed of replicates of 12 genotypes, arranged in a randomized 8 x 8 grid, with trees spaced 1.85m in each cardinal direction. Collection and garden locations are presented in Fig. 2, and additional garden information can be found in Cooper *et al.* (2019) and Hultine *et al.* (2020b).

In order to examine the relationship between climate and traits, we downloaded 21 abiotic climate variables (1961-1990 means) for the 16 provenance sites and the three common gardens using the program ClimateWNA (Wang *et al.* 2012). To create a multivariate climatic trait representing the environmental variation found throughout the 16 provenances, the ClimateWNA variables plus elevation, latitude, and longitude, were analyzed 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. Height and SLA were assessed the first year of growth in the summer of 2015 for every genotype in the common gardens. Specific leaf area was measured using the average of three fully expanded leaves that were free or nearly free of herbivore damage, collected from the southern

aspect of each tree in June and July. Leaves were scanned and leaf area was measured using ImageJ software (Schneider *et al.* 2012). After the area scans, leaves were dried in an oven at 70°C for at least 72 hours, following protocol recommendations in Perez-Harguindegay *et al.*'s (2013) updated trait handbook. Trunk basal diameter was measured at the end of the second year of growth (mid-January in the Yuma and Agua Fria gardens and in early March in Canyonlands) as the diameter at root collar, ~10cm from the soil, on every tree in the gardens. Diameter at root collar was used instead of diameter at breast height due to the short stature of the stems.

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. This measurement is a good approximation of whole plant progression towards dormancy as there was little within-plant variation in apical bud development. Spring bud flush was recorded as the first sign of full leaf emergence on the tree. Bud flush was measured biweekly 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.

Genetic analysis

Genomic DNA was extracted from ~0.2g silica-dried leaf tissue from all 192 genotypes (12 genotypes per population, 16 populations) 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 20µL reactions following a modified Peterson *et al.* (2012) protocol. Briefly, restriction and ligation was carried out simultaneously in 20µL 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.

To ensure we measured F_{ST} for putatively neutral loci, all SNP loci included in the measure of F_{ST} were tested and found to be in Hardy-Weinberg equilibrium. Values of Q_{ST} were compared to the distribution range of F_{ST} estimates and not the mean (Whitlock 2008; Leinonen *et al.* 2013). In order to obtain a 95% confidence interval around F_{ST} , population-level pairwise F_{ST} values were bootstrapped 500 times using the *divPartCalc* function in the R package *diversity* (Keenan *et al.* 2013).

Statistical Analyses

To investigate the within and among population variation in phenotypic traits, each garden was modeled separately using linear mixed 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 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 *packagemerTest* (Kuznetsova *et al.* 2015).

We compared the quantitative trait variation (Q_{ST}) with genetic variance at neutral loci (F_{ST}) in each garden. For quantitative traits, the ratio of variances can be described as

where σ_P is the additive genetic variance among populations and σ_G is the additive within-population variance (Spitze 1993; McKay & Latta 2002). Each trait was analyzed using the model 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). We directly compared the confidence intervals for F_{ST} and Q_{ST} to ascertain significance. In using clonally replicated genotypes, our estimate of σ_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 $Q_{ST} > F_{ST}$ (Goudet & Büchi 2006). Broad-sense heritability (H^2) was also calculated for each trait in each garden using the equation, $H^2 = \sigma_G / (\sigma_G + \sigma_W)$, where σ_W includes both within-population genotypic variance and the error variance.

In order to test whether traits showed strong climatic relationships, we calculated the Pearson’s product moment correlation coefficient in R (R Core Team 2014) between population trait means and the first principal component (PC1) from the environmental PCA. Systematic differences among populations seen in these trait-climate correlations are another test to rule out genetic drift (Whitlock 2008). To then test whether those traits showing strong climatic patterns also showed evidence of stronger selection, we fit a linear model of Q_{ST} to the absolute value of the trait-climate correlation coefficient ($|r|$) with garden and trait modeled as covariates, using the *lmer* package (Bates *et al.* 2015), following Evans *et al.* (2016). Each garden was also modeled separately to calculate garden-specific regression correlation coefficients.

Results:

Neutral Genetic 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. This result is consistent with the population structure ($F_{ST} = 0.221$) found across populations collected throughout the entire range of *P. fremontii* obtained with microsatellite loci (Cushman *et al.* 2014). It is within the range of other *Populus* species, from another strongly differentiated species, *P. angustifolia* with a $F_{ST} = 0.21$ (Evans *et al.* 2014), to very low genetic structure ($F_{ST} = 0.01$) found in *P. tremula* (Hall *et al.* 2007).

Phenotypic Variation

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 example, most of the variation in bud flush in Yuma and Agua Fria was explained by population. For the morphological and growth traits, the relative contribution of population vs. genotype varied among gardens. Traits measured in the hottest common garden exhibited stronger population than genotype effects in four out of the five traits (SLA had a higher genotype variance). 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).

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

In support of our second hypothesis, we found evidence of divergent selection ($Q_{ST} > F_{ST}$) driving phenotype differences in most traits (Table 2, Fig. 4). Overall, the average Q_{ST} value across all traits and all gardens (0.48) was above the F_{ST} confidence interval (0.144-0.205), consistent with directional selection shaping trait

differences and local adaptation across these populations. Phenology traits exhibited some of the highest levels of population differentiation, with mean Q_{ST} values for bud flush between 0.53-0.90 and bud set between 0.42-0.79. In all but one case (bud set in Agua Fria), the confidence intervals for these Q_{ST} values lie above the confidence interval for F_{ST} . Divergent selection on specific leaf area was also apparent in the mid and cold gardens of Agua Fria and Canyonlands, but not detectable in the hot Yuma garden. In contrast to this relative consistency in results for leaf traits, traits related to tree performance showed more variation in $Q_{ST} - F_{ST}$ comparisons across gardens. Tree height showed significant divergent selection ($Q_{ST} > F_{ST}$) when measured at the hottest common garden in Yuma, Arizona, but was not statistically different from F_{ST} in the two cooler gardens of Agua Fria and Canyonlands. We found evidence of stabilizing selection for basal trunk diameter in the cold Canyonlands garden, where the Q_{ST} value (0.03) fell below the F_{ST} confidence interval. However, this trait was indistinguishable from the neutral expectation of F_{ST} in the warm and mid gardens.

Climate analysis

In each garden, most traits were significantly correlated with population provenance climate (Table 3), supporting our third hypothesis of strong associations between phenotype and climate. A single axis (PC1) explained 95.8% of the variation in provenance climate and was influenced primarily by four temperature-related climate variables (degree-days above 5 °C, degree-days below 18 °C, degree-days above 18 °C, and summer heat-to-moisture index (Wang *et al.* 2012)), plus elevation. Populations sourced from areas with higher temperatures, lower precipitation, lower elevation, and longer growing seasons had higher PC1 scores. Positive correlations with PC1 indicate that trait values are higher in those hotter provenance populations, while negative correlations with PC1 mean that trait values are higher in the colder provenance populations (Fig. 3). Phenology traits showed the strongest correlations with provenance climate ($r = -0.75$ to -0.77 for bud flush in Yuma and Agua Fria, and $r = 0.40$ to 0.47 for bud set in all gardens, Table 3). Here, the negative correlation for bud flush indicates that the populations from hotter source climates (with higher PC1 scores) had earlier spring flush dates, at least when growing in the two hotter gardens. The positive correlation for bud set in all gardens indicates that populations sourced from colder areas (lower PC1 scores) had later bud flush dates in the spring. Although lower in magnitude, the correlation between climate and SLA was also consistent in direction. In both the hot and the cold garden, SLA was higher (meaning leaves were thinner) in populations from hot climate origins.

In contrast with the consistent direction of correlations between home climate and trait value for phenology and SLA, growth traits were more likely to show garden-dependent relationships between population origin and population performance (Table 3, Fig. 3). In general, tree height and basal diameter acted 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 populations growing larger in the coldest Utah garden.

We also found that traits more strongly correlated with climate had stronger evidence for selection driving their divergence. The full model including the three gardens and all traits showed a significant relationship between the strength of the trait-climate correlation and Q_{ST} (Fig. 5, $P = 0.0012, R^2 = 0.57, F_{(1,13)} = 16.88$). Neither garden nor trait identity had significant effects on the mean Q_{ST} value. When gardens were modeled separately, the two warmer gardens showed significant relationships between trait-climate correlations and Q_{ST} (Yuma: $P = 0.059, R^2_{adj} = 0.66$; Agua Fria: $P = 0.028, R^2_{adj} = 0.79$), while the coldest garden in Canyonlands did not ($P = 0.571, R^2_{adj} = 0.18$). This result shows that traits with the highest differentiation among populations are those where that trait variation is most strongly correlated with the climate, indicating a strong role for selection by climate in driving differentiation. Interestingly, this relationship was masked in the cold garden, where southern populations did not flush out their leaves early in the spring as they do in their home climate, thereby reducing the amount of population differentiation in that trait compared to what we could detect in the warmer gardens (Figs 3-5).

Discussion:

We found evidence for divergent selection ($Q_{ST} > F_{ST}$) acting on leaf phenology and morphology traits measured in three common gardens for 16 different Arizona populations. This result is consistent with a majority of studies finding Q_{ST} values are generally larger than F_{ST} for ecological traits (McKay & Latta 2002; Savoleinen *et al.* 2007; Leinonen *et al.* 2013). In addition, we found no or contrasting evidence for selection on performance traits, which also showed a reversal in the relative growth rates of populations grown in hot vs. cold common environments. Below, we discuss the evidence for climate-driven adaptive divergence in leaf traits, local adaptation to climate that this trait divergence may enable, and the potential consequences of both under current climate change.

Divergent selection has shaped population differences

We found the largest Q_{ST} values for spring bud flush, followed by fall bud set, consistent with other studies showing high phenological divergence across latitudinal clines (Hurme 1999; Howe *et al.* 2003; Hall *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 in three separate common garden studies (Grady *et al.* 2015; Cooper *et al.* 2019; Davis *et al.* 2020). We also found large population differences in fall bud set timing of ~2-5 weeks across the common gardens.

Bud flush and bud set also showed evidence of divergent selection ($Q_{ST} > F_{ST}$) in the congeneric species, *P. angustifolia* (Evans *et al.* 2016), as well as in *P. tremula* (Hall *et al.* 2007). In contrast to these studies, however, we found bud flush had a higher degree of population differentiation compared to bud set (Fig. 4). This result is intriguing, since spring bud flush is primarily governed by the accumulation of degree-days above a specific temperature, while fall bud set is often linked to precise day length periods (Howe *et al.* 2003). While day length is driven by latitude and is constant from year to year, temperature can vary each year. Our result of less differentiated bud set timing compared to other *Populus* examples could be because our study did not encompass as large a latitudinal gradient. Evans *et al.* (2016) used three gardens spanning over 10 degrees of latitude and Hall *et al.* (2007) employed two gardens approximately seven degrees apart, while this study used gardens just over five degrees of latitude in separation (Supplemental Table 1). This reduced latitudinal gradient means smaller day length differences experienced across the gardens, perhaps resulting in reduced differences in bud set expression.

Alternatively, our results of higher bud flush versus bud set differentiation could be because our source populations span the winter frost line. The southern populations in our study do not experience predictable freezing winters, which could mean that there is more room for temperature-related differentiation in bud flush earlier in a risk-free spring, and/or less selection for differentiation in bud set among populations south of the frost line late in the fall. Overall, these two phenology traits showed both the highest degree of population differentiation of all our traits and the strongest correlations with provenance climate (highest Q_{ST} values; Fig. 5). This result is suggestive of climate as a strong driver of phenotypic differences among these Arizona populations.

Effects of growing conditions on the evidence for selection

Whereas evidence for divergent selection in bud flush was consistent across all gardens, both bud set and SLA showed evidence of divergent selection in two out of three gardens (although for bud set the third garden was very close). Variability in this conclusion was driven by variation in the Q_{ST} value of the same trait across the three gardens (Fig. 4). This finding suggests that phenotypes shaped by selection pressures across a species' range can be expressed differently in different growing environments. This variation among gardens led to even larger contrasts in the evidence for selection in the performance traits. For example, we observed high population differentiation in height expressed in the hottest garden ($Q_{ST} = 0.45$), but these differences diminished when populations were planted in the moderate and cool gardens. For basal diameter, Q_{ST} also decreased with decreasing garden temperature, approaching evidence for divergent selection in the hot garden to showing evidence for stabilizing selection in the cold garden. Thus, our detection of selection is dependent on the common garden environment, with some environments enhancing and others dampening population phenotypic differences. This may represent an interaction between the selection pressures shaping natural

variation across the species range and novel selection pressures imposed in a common garden experiment or under future climate change. The large population-level trait differences exhibited in the hottest common garden for all traits except SLA is likely driven by the maladaptation of the high elevation populations to the extreme thermal conditions experienced in the hot, Yuma 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 (Nicoltra *et al.* 2010; Josephs 2018).

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; Leinonen *et al.* 2013). In this case, the latter is very likely. Although phenology and growth traits showed significant correlations with climate of origin (Fig. 3), only the growth traits reversed the sign of that correlation across gardens. Specifically, phenology trends were 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 were positive trends between growth traits 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. Whether this higher performance of local populations is enabled by their particular values of the leaf phenology and morphology traits measured here vs. additional plant functional traits is an important area for further study.

Management implications

Given that local adaptation and phenotypic differentiation in forest trees (Savoleinen *et al.* 2007) has been closely tied to variation in climate (Alberto *et al.* 2013), populations may become increasingly maladapted as climate change continues (Shaw & Etterson 2012; Franks *et al.* 2014, Aitken & Bemmels 2015). 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). Provenance trials have shown this response experimentally with tree productivity declining as the climate distance transferred between home site and garden site increases (O'Neill *et al.* 2008; Evans *et al.* 2016; Grady *et al.* 2015). Transfer functions can help determine how far a population can be moved before growth declines below a specified level; this tool, combined with climate change forecasts, is one of the best ways to implement assisted migration in order to manage for future forest health and productivity. This method has been used to recommend seed transfer zones and distances for economically important conifer species in British Columbia (O'Neill *et al.* 2008, 2017), and as a caution to move trees at a reasonable, step-wise pace to track climate change (Grady *et al.* 2015). Based on our results of declining tree performance as climate transfer distance increases, we may expect decreases in tree productivity and increasing maladaptation as local conditions become increasingly arid, especially for populations in southern Arizona that are close to the thermal and low elevation edge of their distribution (Ault *et al.* 2014; see Fig. 1).

In addition to tracking declines in growth metrics with climate transfer distance, it is important to consider how shifts in phenology in forest trees will affect dependent communities (Whitham *et al.* 2020). Numerous studies have shown trophic-level asynchronies across different ecosystems due to mismatches in phenological changes (Thackeray *et al.* 2010; Renner & Zohner, 2018). For example, warmer, earlier springs can facilitate earlier phenology of many tree species, which may or may not be synchronized with the emergence and reproductive cycles of important community members (Visser *et al.* 2006; Kudo & Ida 2013; van Asch *et al.* 2013). This can cause a phenological mismatch between plants and the species that rely on them for food or habitat (Renner & Zohner 2018), disrupting species interactions and trophic cascades (Bailey *et al.* 2006; Smith *et al.* 2011). Since cottonwood trees provide food and habitat for thousands of dependent species (Whitham *et al.* 2006; Lamit *et al.* 2015), changes in growth, morphology, and phenology with altered climate will likely affect species interactions, community composition, and functionality (Whitham *et al.* 2020).

Conclusions

Our results show that populations are significantly differentiated with respect to growth, morphology, and phenology traits, supporting a multi-trait hypothesis of divergent selection ($Q_{ST} > F_{ST}$). Phenology traits were the most differentiated among populations with the largest Q_{ST} values, while SLA and height supported the divergent selection hypothesis in some gardens, but not others. In contrast, basal trunk diameter was the only trait to show evidence of stabilizing selection and only in the coldest common garden (Fig. 4). In addition, we found source climate is significantly correlated with trait differences across the gardens, suggesting the large climatic gradient experienced by these Arizona populations is an agent of selection. Interestingly, the magnitude of trait variation detected among populations depended, in part, on their growing environment. We found most traits had the greatest population differences with highest Q_{ST} values in the warmest garden and declined as the trees were planted in cooler environments. Specific leaf area was the only trait measured with the opposite response of higher population differentiation in the cold garden (Fig. 4). Populations exhibited local adaptation in growth and phenology traits, with many populations growing largest in the gardens that most closely matched their home climates. This study demonstrates that experimental common gardens simulating climate change, across even a portion of a species range, can have a substantial impact on how important functional traits are differentially expressed among populations. The gradient of climate-driven selection may lead to the identification of a geographic mosaic of local adaptation that may also cascade to affect associated species and communities (e.g., Thompson *et al.* 2005; Smith *et al.* 2011; Wooley *et al.* 2020). Importantly, we found that the detection of past selection on population-level trait differences, as measured by Q_{ST} - F_{ST} analysis, is modified by growing environment. This finding suggests past climate can interact with the current and future climates to affect population responses. Strategies for management of widespread species like Fremont cottonwood would benefit from considering the climatic selection pressures of source locations to anticipate their performance under changing environmental conditions. **Acknowledgements**

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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. LV Andrews helped with RADseq processing. RJ Best contributed essential analysis of our results and helped write the manuscript. KR Grady assisted with field collections, and along with GJ Allan, CA Gehring, KR Hultine, 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.

Trait	Garden	Variable	Variance	p-value
Bud Set	Yuma	Population	14.781	<0.001
		Genotype	6.421	<0.001
		Plot	4.127	<0.001
	Agua Fria	Population	54.1	<0.001
		Genotype	76.3	<0.001
		Plot	23.88	<0.001
	Canyonlands	Population	104.737	<0.001
		Genotype	58.762	<0.001
		Plot	7.913	0.003
Bud Flush	Yuma	Population	77.16	<0.001
		Genotype	0	1
		Plot	0	1
	Agua Fria	Population	48.33	<0.001
		Genotype	0	1
		Plot	0	1
	Canyonlands	Population	1.60E-15	1
		Genotype	0	1
		Plot	9.64E-14	1
SLA	Yuma	Population	0.3071	<0.001
		Genotype	0.386	<0.001
		Plot	0.0799	0.001

Trait	Garden	Variable	Variance	p-value	
Height	Agua Fria	Population	0.3231	0.011	
		Genotype	0.2083	0.001	
		Plot	0.2363	<0.001	
	Canyonlands	Population	1.339	<0.001	
		Genotype	0.2071	<0.001	
		Plot	0.3263	<0.001	
	Yuma	Population	64.99	<0.001	
		Genotype	41.12	<0.001	
		Plot	33.13	<0.001	
	DRC	Agua Fria	Population	33.82	0.5
			Genotype	104.67	<0.001
			Plot	183.56	<0.001
Canyonlands		Population	407.5	0.004	
		Genotype	567.3	<0.001	
		Plot	213.1	<0.001	
Yuma		Population	5.194	<0.001	
		Genotype	1.331	<0.001	
		Plot	4.093	<0.001	
Agua Fria		Population	Population	3.051	0.6
			Genotype	8.871	<0.001
			Plot	44.059	<0.001
	Canyonlands	Population	2.24E-12	1	
		Genotype	36.83	<0.001	
		Plot	38.35	<0.001	

Table 2: Q_{ST} and broad-sense heritability, H^2 (+ 95% Confidence Interval).

Trait	Garden	H^2	Q_{ST}
Bud Set	Yuma	0.07 (0.05-0.26)	0.79 (0.40-0.86)
	Agua Fria	0.23 (0.24-0.35)	0.42 (0.19-0.46)
	Canyonlands	0.24 (0.20-0.25)	0.47 (0.46-0.57)
Bud Flush	Yuma	0.83 (0.58-0.99)	0.88 (0.80-0.97)
	Agua Fria	0.52 (0.24-0.89)	0.90 (0.67-0.97)
	Canyonlands	0.17 (0.04-0.33)	0.53 (0.22-0.87)
SLA	Yuma	0.21 (0.17-0.36)	0.32 (0.10-0.47)
	Agua Fria	0.13 (0.07-0.18)	0.32 (0.26-0.61)
	Canyonlands	0.15 (0.05-0.24)	0.76 (0.43-0.92)
Height	Yuma	0.12 (0.04-0.19)	0.45 (0.26-0.70)
	Agua Fria	0.15 (0.05-0.21)	0.14 (0.00-0.62)
	Canyonlands	0.31 (0.21-0.35)	0.21 (0.08-0.42)
DRC	Yuma	0.06 (0.06-0.11)	0.66 (0.14-0.74)
	Agua Fria	0.04 (0.03-0.06)	0.16 (0.00-0.53)
	Canyonlands	0.22 (0.13-0.73)	0.03 (0.00-0.09)

Table 3: Pearson’s product moment correlation coefficient (r) and p-value between provenance climate (PC1 score) and traits at each common garden.

Trait	Garden	Correlation coefficient	P-value
Bud Set	Yuma	0.82	< 0.001
	Agua Fria	0.89	< 0.001
	Canyonlands	0.91	< 0.001
Bud Flush	Yuma	- 0.77	< 0.001
	Agua Fria	- 0.83	< 0.001
	Canyonlands	- 0.06	0.83
SLA	Yuma	0.43	0.12
	Agua Fria	- 0.13	0.64
	Canyonlands	0.52	0.07
Height	Yuma	0.23	0.43
	Agua Fria	- 0.55	0.03
	Canyonlands	- 0.80	< 0.001
DRC	Yuma	0.78	< 0.001
	Agua Fria	0.05	0.85
	Canyonlands	- 0.45	0.09

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.

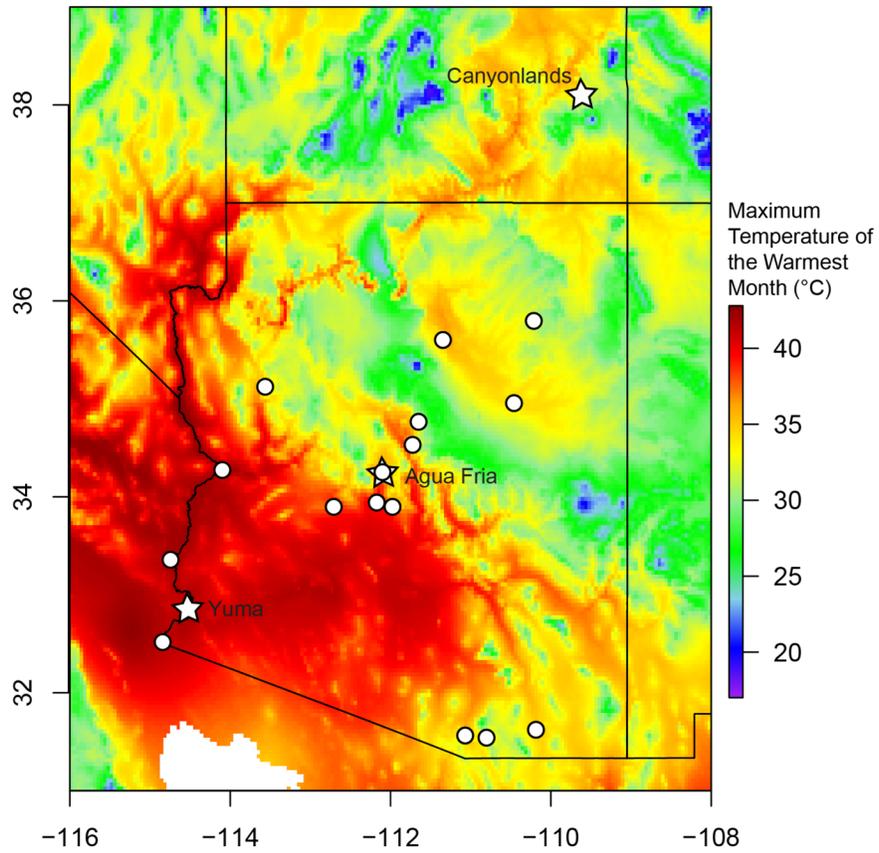


Figure 3: Population means (\pm 1SE) for each functional trait measured in each garden, in relation to their home climate (indicated by principal component axis 1 values). Populations are colored by mean annual temperature (MAT °C). Regression lines are present when there is a significant relationship ($p < 0.05$) between the PC1 axis and the trait. Dashed lines indicate marginal significance ($0.05 < p < 0.1$). Note the y-axis varies in scale among the three gardens.

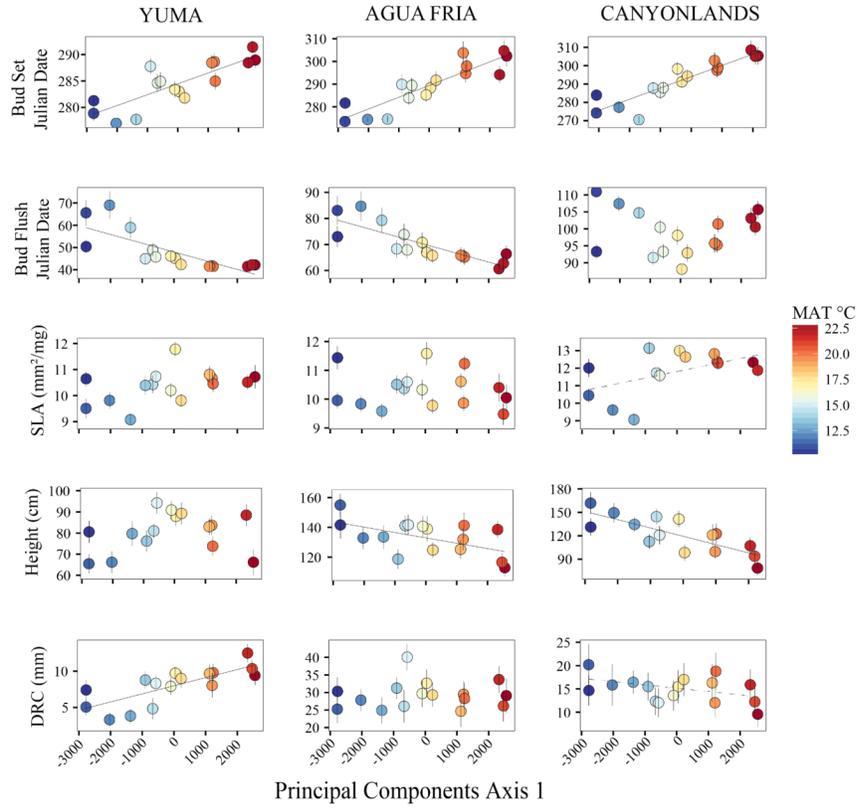


Figure 4: Q_{ST} means and 95% confidence intervals (point and vertical lines, respectively) for the phenotypic traits measured at each of the three gardens. The average pairwise F_{ST} value (0.175) + 95% confidence interval (0.144 - 0.205) is shown as the grey band. Common gardens are abbreviated as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

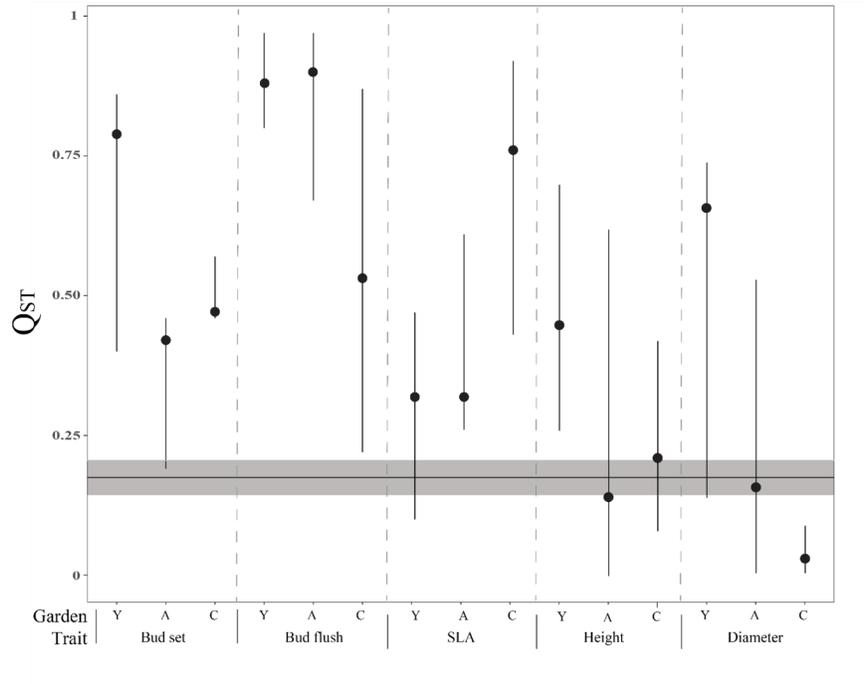


Figure 5: Positive relationships between the level of population trait differentiation (Q_{ST}) and the strength of correlation of each trait with its provenance climate. Each point represents one trait at one garden. Traits with the strongest correlations to provenance climate (r) are also the most differentiated (largest Q_{ST}). Gardens are coded by color and traits by symbol.

