ENVIRONMENT SPECIFIC ALLELES RELATED TO ADAPTIVE SEASONAL TRAITS AND THE FITNESS CONSEQUENCES OF PHENOLOGY FOR *PINUS STROBIFORMIS*

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ABSTRACT

ENVIRONMENT SPECIFIC ALLELES RELATED TO ADAPTIVE SEASONAL TRAITS AND THE FITNESS CONSEQUENCES OF PHENOLOGY FOR *PINUS STROBIFORMIS*

JARED KARL SWENSON

Rapid shifts in temperature and precipitation related to anthropogenic climate change threaten to decouple evolved responses to environmental stimuli resulting in maladaptive phenology. Asynchronous phenology could result in increased mortality in forest trees and trigger a cascade of impacts on large scale ecosystem processes. Understanding phenological responses to novel conditions and identifying the genetic architecture of phenological traits and survival can aid forest managers in identifying both vulnerable populations and populations with substantial genetic variation. *Pinus strobiformis* is a high elevation conifer whose disjunct and isolated distribution across the American southwest makes it particularly vulnerable to climate change. *P. strobiformis* contributes to biodiversity in mixed conifer forests, but species distribution models predict that suitable habitat for *P. strobiformis* will decrease across their current range and shift to higher latitudes and altitudes. Recent genetic analysis described the extent of a hybrid zone in New Mexico and Arizona between *P. strobiformis* and the more northerly distributed *Pinus flexilis.* These hybrid populations may possess unique allele combinations necessary for survival under combined water limitations and freezing conditions.

This thesis builds upon a rapidly growing body of knowledge through an international collaborative effort across multiple universities and government agencies to understand the physiology, ecology, genetics, and resistance to invasive pathogens. In the first chapter we

utilized three common gardens at *Pinus strobiformis* climate margins to detect genetic markers related to phenology, survival, and bud damage through both single and multi-trait genome wide association analyses. Multi-trait groupings included 1) the same trait measured at multiple time points, or longitudinal measures, of the same trait across multiple time periods, 2) different components of spring phenology bud development, and 3) traits that had significant associations with maternal seed source climate. We detected 103 SNPs related to survival, phenology, and bud damage over the span of two years. Most minor, or less common, alleles detected for traits had a negative effect on survival and variable effects on spring phenology. Using multi-trait groupings improved our ability to detect loci and identify shared genetic influences among multiple traits. Our results suggest that phenology, survival, and bud damage in *Pinus strobiformis* are regulated by complex genetic and environment interactions, environment specific allele sensitivities, and indirect effects of loci on multiple phenotypes.

The second chapter extends the utility of the genome-wide association analyses by investigating phenological relationships with survival and the distribution of minor alleles along climate gradients. We investigated variation across years and growing conditions in phenological traits and their relationship with survival. The relationships among phenological traits and survival were used to group alleles by their garden specific effects. Alleles that increased survival under particular growing conditions at a specific time were considered favorable, whereas alleles that decreased survival were considered unfavorable. These allele distinctions are garden specific and likely do not represent alleles that are unfavorable nor favorable for *Pinus strobiformis* in their home range. An additional group of alleles was created for alleles that were related to spring phenological development. These groups were used to investigate the relationships among allele counts for individual maternal trees and maternal seed source climate

variables and garden specific survival. Spring phenology and bud damage varied across the two study years and across gardens. Fall phenology demonstrated little variation between the high and low elevation gardens. Maternal trees with greater bud damage had lower survival at all gardens, whereas spring bud burst phenology had opposing relationships with survival at the two extreme gardens. Our results demonstrate that early growth in *P. strobiformis* responds to climatic shifts in context dependent ways. Maternal *P. strobiformis* trees with more unfavorable minor alleles and more phenology related minor alleles had lower survival. *P. strobiformis* families with more favorable, unfavorable or phenology related minor alleles were from seed source sites with less winter precipitation and more climate moisture deficit. This pattern suggests that *Pinus strobiformis* may have retained low frequency, rare alleles, through a combination of selective pressure on early season growth, stressful conditions, and seasonal moisture. Additionally, local adaptation within large highly variable maternal populations; and unequal patterns of gene flow may have contributed to the retention of these alleles. Our study contributes to the developing body of work that highlights the importance of seasonal precipitation and moisture deficit for *P. strobiformis* local adaptation and evolutionary history.

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DEDICATION

To all those who have encouraged me to continue chasing curiosity, and to

Dr. Rosana Cappellato—thank you.

"The more one thinks, the more one feels the hopeless immensity of man's ignorance."

−Charles Darwin

PREFACE

This thesis is presented as two independent unsubmitted manuscripts. There is overlapping information between the two chapters because they rely on the same samples, experimental gardens, and genetic material. Chapter one covers a single and multi-trait genome-wide association study on phenological traits and survival. Chapter two relies on the results from the genome-wide association analyses from chapter one. Chapter two uses the association study findings to investigate relationships among phenological and survival measurements and determine the climatic distribution of the minor alleles detected in the association study. Citations, tables, and figures are included at the end of each chapter.

CHAPTER ONE

Detection of environment specific alleles for phenology and survival at the edge of a conifer's climate niche

INTRODUCTION

An organism's capacity to survive seasonal changes in abiotic conditions is determined by locally adapted phenotypic responses (Savolainen et al. 2007). Within and across years tree phenology, or response to seasonal changes, is regulated by locally adapted genetic variation (Alberto et al. 2013) and environmental factors including changes in day length, temperature, water availability, lag effects from conditions during the previous growing season (Kramer et al. 2000, Kozlowski et al. 1991), accumulated chilling hours (Haase 2016) and heat sum hours (Rossi et al. 2008 and Swidrak et al. 2011). Climate change will test evolved relationships by altering the synchrony between phenological development and environmental cues. Asynchrony could result in increased mortality in forest trees (Poulos 2014, Yakovlev et al. 2006) and trigger a cascade of impacts on large scale ecosystem processes (Anderegg et al. 2013). Phenological asynchrony can have effects on local biodiversity and trophic level function. For example, in the southwestern United States during years of variable and heavy snowfall in montane communities, conifer and deciduous reproductive phenology is delayed, reducing avian migration stop over due to a decrease in herbivorous insect abundance (Kellerman and Riper 2015).

Phenological events such as spring bud burst and fall bud set are associated with across season changes to cold temperature acclimation or cold hardiness in conifers and can be involved in setting limits to the conditions under which they can survive (Aitken and Hannerz 2001). Phenological and cold hardiness development are species and population dependent and may fall along clines, or gradients, related to seed source elevation, latitude, temperature, or precipitation (Rehfeldt 1988, Sunblad and Andersson 1995, Alberto et al. 2013). Phenology also determines species ecological distribution (Chuine 2010) because selection for locally adapted phenotypic

responses confer a fitness advantage under particular conditions (Savolainen et al. 2007). Phenological plasticity enables individuals to adjust to interannual abiotic conditions. Extreme environmental conditions at a species margins may challenge individuals adaptive responsiveness and result in a mismatch in phenological responses. This asynchrony combined with the low levels of genetic variability for selection to act on typically found at a species leading or trailing edge may limit range expansion (St. Clair and Howe 2007, Chuine and Beaubien 2001). Cold damage to buds, inadequate cold hardiness, and short growing seasons can limit upward latitudinal or elevational expansion while insufficient chilling hours and delayed or absent bud burst may limit downward expansion (Clark et al. 2014). However, process-based species distribution models based on phenological clines related to temperature predict that interannual plastic variation in *Pinus sylvestris* phenology should negatively affect fitness near the species climate margins but increase fitness beyond the species margins (Duputié et al. 2015).

Standing genetic variation may influence fitness across environmental gradients (Fournier-Level et al. 2011). Deleterious alleles at a species margins can be maintained at low levels or increase to fixation and decrease fitness (Excoffier and Ray 2008) further limiting range expansion. However, phenotypic plasticity may enable a short term response of species to rapid changes in climate conditions (Franks et al. 2013). Historical cycles of range expansion and contraction related to glaciation have resulted in complex evolutionary trajectories in conifers. Periodic gene flow, involving periods of introgression or hybridization, may have distributed new variation and potentially beneficial alleles to currently isolated and disjunct populations (Dannemann et al. 2016, Menon et al. 2018). These alleles may persist at low frequencies and increase fitness under changing or novel conditions (Frank et al. 2017). Thus, long lived tree

species spanning highly variable climates may possess the genetic diversity to survive beyond their current distribution (Rosenblad et al. 2019, Frank et al. 2017). *Pinus contorta,* for example, can survive across a broad range of climate conditions and transplanted populations from other areas often outperform native populations (Rehfeldt et al. 1999). Discovering the genetic variants related to phenology and direct measures of fitness (e.g., survival) at a species margins can aid in identifying potential genetic reservoirs that may be critical for climate mitigation (Aitken and Bemmels 2016).

Phenotypic expression is mediated by the interaction between environmental variation and genetic variation. Phenotypes may vary due to local adaptation to evolved abiotic environments and due to maternal effects related to environmental conditions during seed development (Skrøppa and Johnsen 2000). Specific environmental conditions can activate alternative genetic developmental or physiological pathways through allelic sensitivity or environment dependent regulatory switches (Pigliucci 2001, Des Marais et al. 2013) altering the genetic variation acting on phenotypes. By planting individuals from across a species range in common environments researchers can better identify the genetic signals that affect phenotypic variation in particular contexts (Lind et al. 2018, Fournier-Level et al. 2011). Using multiple common gardens established at the edge of a species geographic or climate distribution can provide further insight into the fitness consequences of phenotypic expression (Duputié et al. 2015).

Genome-wide association studies (GWAS) are a quantitative genetic tool often paired with common gardens to identify genetic markers, or single-nucleotide polymorphisms (SNPs), and their effect size on traits (Ingvarsson and Street 2011). GWAS associate phenotypic data with genetic variants from across the genome of an individual to identify candidate loci related to

trait variation and understand evolutionary processes influencing traits of economic and conservation utility (Lind et al. 2018). Growing trees under a common environment enables researchers to reduce phenotypic plasticity to detect genetic variation contributing to phenotypic expression (Jankowski et al. 2019). Association studies in trees have detected loci contributing to adaptive traits such as growth, phenology, cold hardiness, water use efficiency, leaf morphology, and more (Hall et al. 2016). In outcrossing species, like most conifers, typically a large number of SNPs of small effect are discovered (Josephs et al. 2017). Phenology and cold hardiness traits often have moderate to high heritability estimates which may increase the likelihood of detecting significant loci. SNPs associated with cold hardiness traits also cumulatively tend to explain a large amount of phenotypic variation (Hall et al. 2016). However, single locus GWAS may have limited capacity to influence gene-targeted conservation in trees due to complications from linkage between the detected and causal loci, difficulties isolating the influence of genetic drift and selection, high false positive rates, small effect sizes, and unclear biological function of detected alleles (Kardos and Shafer 2018, Ingvarsson and Street 2011). Additionally, association studies in conifers are exacerbated by limited genomic coverage of large genome sizes that are replete with non-coding transposable elements (De La Torre et al. 2014). Nevertheless, loci contributing to adaptive traits in wild tree species have still been identified (Hall et al. 2016). For conifers specifically, uniform genomic sampling efforts may reduce bias in sequencing and polymorphism identification (Peterson et al. 2012, Parchman et al. 2018) and new statistical methodology may increase the power of GWAS analyses (Zhou and Stephens 2012).

Multi-trait approaches to GWAS in trees offer a promising avenue to improve the detection of meaningful loci for traits of economic and conservation importance (Lind et al. 2018, Josephs et al. 2017). Several multivariate approaches exist including combining traits

along principal component axes into a single value (Carlson et al. 2019), machine learning techniques like Random Forest (Breiman et al. 2001) and combining functionally or statistically correlated traits as multi-trait response variables in programs like GEMMA (Zhou and Stephens 2012). Combining functionally related, traits measured across multiple time points (longitudinal measures), or highly correlated traits into multivariate approaches may better represent variability in phenotypic expression and help detect global or stable loci that influence traits across time and space (Chen et al. 2021, Baison et al. 2019, Sillanpää et al. 2012). Additionally, research has demonstrated that multi-trait approaches add statistical power, increase detection of pleiotropic loci, increase effect sizes, and capture some of the missing heritability common in association studies (Tan 2018, Songsomboon et al. 2018 and Chhetri et al. 2019, Chhetri et al. 2020, Evans et al. 2014, Porter and O'Reilly 2017). Comparing the results from univariate and multivariate GWAS can ultimately improve our understanding of trait and species specific genetic architectures (De La Torre et al. 2021).

Southwestern white pine (*Pinus strobiformis*) is a five-needle white pine of conservation concern that supports biodiversity in isolated mixed conifer forests in the southwestern United States and Mexico (Looney and Waring 2013) (Figure 1). *P. strobiformis'* populations are distributed across highly variable wind-swept slopes and canyons. Their evolution under stochastic environments involving large shifts in temperature and precipitation and history of hybridization them an ideal candidate to study the genetic architecture of phenological traits, misaligned cues, and survival. Their current distribution and climate niche space is best characterized by a few climate variables including, annual freezing degree days, winter precipitation and winter temperature (Shirk et al. 2018). Recent analyses detailed the extent of a hybrid zone located primarily in New Mexico and Arizona, between *P. strobiformis* and the

more northerly distributed *Pinus flexilis* (Menon et al. 2018). Allele variants, or SNPs, identified in *P. strobiformis* genomes that had *P. flexilis* ancestry were associated with cold temperatures while SNPs associated with drought or water-availability were primarily of *P. strobiformis* ancestry. Given the climate factors determining *P. strobiformis* distribution, these hybrid populations may possess unique allele combinations necessary for survival under combined water limitation and freezing conditions (Menon et al. 2021).

Across the *P. strobiformis* US range climate change is predicted to increase temperatures and decrease summer monsoon precipitation by 11-45% by the end of the century (Garfin et al. 2010). These shifts are anticipated to increase evaporative demands in the winter (Seager et al. 2007) and advance snow melt in high elevation forests (Williams et al. 2020). Additionally, interannual variation in temperature and precipitation are predicted to alter the frequency of cold snaps and winter precipitation and increase the number of frost-free days (Cayan et al. 2013). Interannual variation in precipitation and large fluctuations in temperature may strain phenological timing and increase tissue damage and mortality. Physiologically dry winters with warm mid-day temperatures may impose moisture demands on conifers that are unable to extract water from frozen soil (Kozlowski et al. 1991) and cold snaps may cause rapid damage to trees with insufficient cold hardiness (Poulos 2014).

We conducted a GWAS on phenological traits, bud damage, and survival in three common gardens situated along an elevational and climate gradient for 202 families of *P. strobiformis* from across the species US range. We focused on easily distinguishable phenological stages during the development of apical or lateral buds in the spring, bud burst, and their changes prior to dormancy in the fall, bud set. While the three gardens span the average seasonal and annual climatic conditions experienced in *P. strobiformis'* maternal sites, they also

impose extreme fluctuations in temperature and evaporative demands that occur towards the margins of *P. strobiformis* US climate niche (Figure 2, Table S2, Table S3). We used this experimental garden design to answer several questions: Q1) What is the genetic architecture (number of loci, effect sizes, allele frequencies, and genes) of survival, phenology, and bud damage in *P. strobiformis*? Q2) Do multi-trait genome-wide association analyses increase understanding of the genetic architecture of these traits?

To answer Q1 we conducted separate genome-wide association analyses on traits at each garden across two years. The highly heterogenous environments *P. strobiformis* inhabit may have induced selective pressure on allele variants conferring plastic phenotypic expression, generating detectable and locally adapted genetic differences. However, they may also possess variants with negative consequences under novel environments. Therefore, we anticipate (H1a) that we will detect different loci for traits at each garden and more loci at the most extreme and novel gardens (high and low elevation). Additionally, we hypothesize (H1b) that we will detect many lower frequency loci of small effect, as observed in other GWAS for forest trees, however, the direction of the effects will vary by environment. These separate environments should enable us to detect environment specific genetic contributors to phenotypic expression and survival. Specifically, we expect the traits with significant associations, the directions of the effects, and heritability estimates to differ across gardens.

To answer Q2 we created multi-trait groupings that either combined longitudinal measures of the same trait or traits with demonstrated clinal relationships to maternal site climate variables. Longitudinal measures were those measured for the same trait across several time points. We hypothesize (H2a) that by grouping longitudinal traits we will detect stable loci affecting traits across multiple seasons and additional unique loci not detected in the univariate

approach. Grouping longitudinal measurements may better capture the full phenotypic expression of each trait. Additionally, (H2b) grouping traits by their relationships to maternal climate variables will reveal shared genetic markers among traits and identify loci putatively responsible for responding to abiotic cues. Overall, grouping longitudinal traits and traits with observed climate relationships may detect genetic loci that are directly linked to survival and phenological expression at the species climate margins and provide insight into evolutionary processes affecting genetic divergence in *P. strobiformis*.

METHODS

Common Garden Establishment and Phenotypic Data Collection

Open-pollinated *Pinus strobiformis* seeds were collected from 202 individual trees across 50 populations in natural stands spanning the US range of *P. strobiformis* in Colorado, Arizona, New Mexico, and Texas (Table S1).We will refer to the 202 individuals we collected seeds from as "maternal trees", and their offspring as members of a mixed-sibling "family" (Menon 2020, Bucholz et al. 2020). The seedlings are considered mixed-siblings because they contain the same maternal genetic contribution (half-siblings) but are not full-siblings because they have varying degrees of paternal contribution due to open-pollination. Up to 25 seedlings per maternal tree were planted from 2015-2017 at three Southwest Experimental Garden Array (SEGA) sites in the Kaibab National Forest, Arizona, USA. The three common gardens are distributed along an elevational and climatic gradient spanning the climate conditions experienced at maternal site locations but also imposing climate extremes of the US *P. strobiformis* range for several climate variables (Figure 1, Figure 2, Table S2, S3, S4). Based on 30-year averages (1981-2010) for maternal sites extracted from climateWNA (Haman et al. 2013), the three common gardens had more variable temperatures (continentality or TD) and a greater climate moisture deficit (CMD)

in 2018 and 2019 than the 30-year averages for the majority of maternal sites (Figure 2). In 2018 and 2019 the mean warmest month temperatures were higher at the lower two gardens than the 30-year averages for the majority of maternal sites, and winter precipitation was highly variable across years (Figure 2). Each garden contained raised-bed boxes with 100 individual seedlings planted in 10x10 rows. A random planting design was used to eliminate the need to account for block effects during analysis. Trees received irrigation during each growing season from April-October. Additional experimental design details and specific environmental differences among gardens can be found in Bucholz et al. (2020).

Trait measurements were collected during each growing season from 2018-2020 (Table 1). Spring phenology, or bud burst, was collected twice each year at each garden during the 2018 and 2019 growing season. Bud stage was scored from 0-4 representing bud development from dormant (0) through bud elongation (2), needle emergence (3), and needle elongation (4). This scoring system is modified from Goodrich and colleagues (2016) with personal contributions from Bucholz, Moler, and Whipple. For each individual tree, a bud burst slope (budslp) and intercept (budint) were calculated from the relationship between bud score and the Julian measurement day. The bud burst slope and intercept represent the rate of bud development during each growing season. A larger slope represents more rapid bud development. A lower intercept corresponds to rapid bud development as well, however, the intercept measurement captures the variation in the first bud measurement of the season relative to its rate of growth throughout the season; providing additional phenological insight. A third spring phenological measurement, bud advancement (budadv), is the change in bud score between the two measurement points. Using all metrics may increase biological interpretability of spring phenology. Fall bud stage phenology was recorded in the fall of 2018 and 2019 on two scales,

bud set(set) and fall bud variation (budv), to capture variation in dormancy initiation. Fall bud variation on a 0-3 scale, encapsulates the nuance and variation in bud formation, color, and needle length at the end of the growing season. Scores of 0 and 1 were considered active by presence of a green bud whereas scores of 2 or 3 were considered dormant. Further distinctions within active (0,1) and dormant buds (2, 3) were based on variation in needle length relative to full length needles. Higher scores represented new full-length needles whereas lower scores had underdeveloped or short needles. The second metric, binary bud set is the condensed version of fall bud variation that focuses exclusively on whether the bud is active (0) or dormant (1). Together these measurements capture bud specific changes and variation in needle length during dormancy initiation.

Other bud and shoot growth characteristics were recorded during spring and fall phenology measurement collection times including bud damage and lammas growth. Lammas growth is a secondary flush in one growing season (Kaya et al. 1994) that may provide individuals, especially seedlings, with a competitive advantage (Howe et al. 2003) by elongating the growing season (Goto et al. 2017). Bud damage (bdmg) was determined when a tree had obviously produced a bud, the shoot began to elongate, but the bud/shoot failed to fully develop. Damaged buds were crispy, brown, and brittle. Bud damage represents a phenological mismatch with growing environment conditions and possible damage in response to rapid drops in temperature. Assigning a direct mechanism for damage to apical buds or other tissue can be challenging. However, we suggest damage is likely due to cold temperatures given the nature of characterized damage to apical buds in the field and personal observations of trees with damaged apical buds quickly developing lateral shoots in the summer (personal observation and communication with Whipple, Bucholz, and Moler). A score of 0 indicates a damaged bud and a

score of 1 indicates a healthy bud. Due to logistical constraints our collection times were too coarse to detect immediate responses of buds following cold snaps. Time lags between cold snaps and physiological and morphological expression of damage may also contribute to uncertainty in the causal mechanism for bud damage. Presence of lammas growth (lms19) at the low elevation garden was recorded as a 1 in the fall of 2019 and absence was recorded as a 0. Lammas was not included from the intermediate and high elevation gardens because lammas occurred only 38 times across both sites.

Survival was recorded from the fall of 2017 to the fall of 2020 and broken up into several binary categories where 0 represents a dead tree and 1 a living tree. Survival over two winters (wint17-18 and wint18-19), survival over two growing seasons (grow18 and grow19), and overall survival (surv19) were included. An experimentally induced complete water restriction was imposed on a subset of trees during the 2020 growing season to reveal differences in drought responsiveness. Survival at the end of that growing season was called lethal drought survival (drt20).

Statistical Methods for Phenotypes

To prepare phenotype data for the GWAS, trait measurements for individual mixed-sibling seedlings at each garden were converted to maternal values. This process aims to remove microsite influences on the trait value and extract the additive genetic maternal contribution to each trait. The procedure for calculating maternal values is modified from Menon (2020) to address observed variation in growing conditions at the common gardens. We created separate maternal values for each trait at each garden and measurement time using a generalized linear mixed effects model in package *glmmTMB* in program R (Brooks et al. 2017). All combinations of microsite effects were included as predictor variables to determine the combination of

microsite effects that influenced phenotypic expression at each garden. A top model for each trait at each garden was determined using Akaike Information Criterion (AIC) model selection (Bozdogan 1987). The full set of predictors considered are included in the following equation, but the final combination of predictors are garden and trait specific:

$$y_{ijkml} = \mu + SY_j + WT_k + DistancefromCenter_l + BoxSide_m + (1|Family_i) + \epsilon$$

y represents the trait values for each individual tree at a particular garden. Binary traits (Table 1) were fit using a binomial model. SY is the year in which an individual was planted from 2015-2017. This was included to control for planting year effects on trait values. WT is the water treatment used on particular boxes. Adding WT as a fixed effect in this model effectively removes the influence of different water treatments on trait values as it was not central to this investigation. For more information on the water treatment and its impact on physiology see Bucholz et al. (2020). Distance from Center is a categorical variable included to control for edge effects and differences in microclimate in each raised box. Box Side is a binary variable that represents whether a tree was positioned on the north or south side of each box. Box Side was included to account for potential unequal cooling or heating of soil in raised boxes. The maternal family was included as a random effect. These microsite variables were selected based on biological inference and applied to other studies using the same common garden experiment (e.g., Bucholz 2020, Moler 2020). The top model for each trait at each garden was used to calculate the final maternal value using the following equation:

$$MaternalValue_i = \mu + Family_i$$

The final maternal value for each trait at each garden is the addition of the global intercept (μ) and the random effect coefficients for each maternal family i from each top model (Menon 2020).

Genotypic data

Total genomic DNA was extracted from the 202 maternal trees and library preparation, sequencing, and SNP calling was performed following Menon et al. (2018) at Virginia Commonwealth University in the lab of Andrew J. Eckert, Ph.D. ddRADseq libraries were prepared and all libraries were digested using two restriction enzymes *EcoR1 and Mse1* to reduce genomic complexity (Peterson et al. 2012). Single end sequencing was conducted with Illumina HiSeq 4000 and SNPs were called using dDocent bioinformatics pipeline assuming a de novo assembly by Menon following Menon et al. (2018) and custom Eckert Lab protocols (source code and protocols available at github.com/EckertLab/protocols). SNPs were filtered on Northern Arizona University's high performance computing cluster (found at https://in.nau.edu/hpc) by Swenson and Menon using vcfTools (Danecek et al. 2011) to remove indels, SNPs with 50% missing data, monomorphic SNPs, and SNPs with a PHRED score below 20 with depth above the 75th percentile. This retained 46,889 of the original 49,859 SNPs. A minor allele frequency cut off of 0.05 was used to retain at least 10 minor alleles at each locus across all maternal trees; reducing the final number of SNPs to 13,255. The allele frequencies reported in the results are from the output from each association analyses conducted in GEMMA (Zhou and Stephens 2012). Each GWAS included a different number of maternal trees due to uneven mortality and missing phenotypes so some of the allele frequencies reported are below the 0.05 MAF threshold (Table 2 & 3). Due to high rates of error in next generation sequencing methods (Han et al. 2015), low coverage, and resulting ascertainment bias (Namroud et al. 2008)

we acknowledge that the SNP set included in this analysis lack rare alleles that may explain a substantial portion of phenotypic variation (Fournier et al. 2019). However, uniform genomic sampling as used in our protocol, using two restriction enzymes to cut evenly throughout the genome provide a good representation of the genome and may reduce ascertainment bias and false positives (Parchman et al. 2018). Additionally, RADseq approaches have successfully identified genetic contributors to trait variation in other conifer studies (Parchman et al. 2018). Imputation was performed in LinkImpute (Money et al. 2015) because the association software GEMMA does not accept missing genotypes (Zhou and Stephens 2012). Efforts to impute using BEAGLE (Browning et al. 2018) or filter allele frequencies after imputation resulted in unacceptable QQplots. The resulting SNP dataset was converted to a PLINK binary PED file format.

Population Structure

To assess the degree of population structure across the 202 *P. strobiformis* maternal families a principal components analysis (PCA) was conducted in the R package *adegenet* (Jombart 2011). A second approach to infer population structure was also implemented in fastSTRUCTURE (Raj et al. 2014). To determine the optimal number of populations (K) ten separate runs were conducted for values of K from one to ten. All runs for the optimal K were combined and then visualized using R package *pophelper* (Francis 2017).

Association Test

Univariate and multivariate genome-wide association analyses were conducted using GEMMA (Zhou and Stephens 2012; Zhou and Stephens, 2014). A binary PED file was used as the genotype input for all analysis across gardens. The maternal values per garden were input as independent columns in a ".fam" file, compatible with the PED format (Zhou and Stephens 2012

supplemental). For each garden, a genetic relatedness matrix was constructed in GEMMA using the standardized genotypes method because it is better suited for use with lower minor allele frequencies. Maternal values, the relatedness matrix from GEMMA and the 13,255 SNPs were used in all univariate and multivariate association tests. Population structure using principal component axes was not included as a covariate because GWAS analyses with only the relatedness matrix produced more consistent qqplots than GWAS analyses that included both the relatedness matrix and the significant PC axes.

Univariate Association Test

Separate univariate (single trait) tests for each phenotype measured at each garden and time point were conducted. The univariate model in GEMMA used the following equation:

$$y = \alpha + x\beta + \mu + \epsilon$$

Here, $\mathcal{Y}$ is the maternal value for a particular trait, α is a c-vector of coefficients including the intercept, x is an n-vector of marker genotypes, μ is an n-vector of random effects including the relatedness matrix, β is the effect size of each marker, and ϵ is the error. The multivariate model in GEMMA follows a similar equation except $\mathcal{Y}$ would represent a matrix of maternal values for each phenotype. Within GEMMA the likelihood ratio test was used to calculate p-values and all other settings were left at default.

Multivariate Association Trait Grouping

Multivariate (multi-trait) association tests were performed on groups of phenotypes from each garden, or planting environment. Multivariate groupings included: longitudinal measures of the same trait across multiple time periods (Table 2), different components of the same phenomenon (i.e., spring phenology slope, intercept, and bud stage advancement), and traits that had significant associations with maternal climate (Table 3). The last group was generated

because phenotypes in conifers often follow clinal patterns that could represent local adaptation to maternal climates (Aitken and Hannerz 2001); phenological traits and survival are connected to cold hardiness (Sutinen et al. 2001) and their expression may result from shared loci. We initially selected 10 climate variables related to temperature or water availability gradients including: frost free period (FFP), continentality (TD), climate moisture deficit (CMD), winter precipitation (PPT_wt), mean warmest month temperature (MWMT), degree days below zero (DD_0), precipitation as snow in winter (PAS_wt), summer heat moisture index (SHM), annual heat moisture index (AHM), and summer solar radiation (RAD_sm). Evidence in previous studies on *P. strobiformis* demonstrated that some of these 10 climate variables, or correlated variables, are related to physiology and morphology (Bucholz 2020) at the same common gardens. Additionally, some of these climate variables also influence seed development and germination (Moler 2020), describe *P. strobiformis* ecological distribution (Shirk et al. 2018), and define climate niche differences with closely related *P. flexilis* (Menon et al. 2018, Moreno-Letelier et al. 2009). Divergence along temperature and precipitation gradients were also observed in a genotype-environment association analysis using a different set of genetic markers (Menon 2020).

After removing highly correlated climate variables, we included only three maternal climate variables; frost-free period (FFP), continentality (TD), and climate moisture deficit (CMD), because they represented temperature and moisture variables that were directly related to seasonal growth (FFP) and extreme conditions present at the gardens (TD, CMD) (Figure 2). To determine trait groupings generalized linear mixed effects models in R package *glmmTMB* (Brooks et al. 2017) were used to establish relationships between maternal values (response variable) and maternal climate variables FFP, TD, and CMD (predictor variable). If a significant

relationship was present between a maternal value and FFP, CMD, or TD then the trait was grouped with other traits that had the same relationship. The relationships between maternal values and CMD, FFP, or TD were garden specific. Most traits at the high elevation garden were associated with frost free period, and only four were associated with continentality (Table S5). However, at the lower two gardens almost an equal number of traits were associated with each climate variable. Due to lack of variation in the maternal values and subsequent convergence issues some traits were not grouped with climate variables for the GWAS analyses.

Analyses of Association Results

To remove spurious associations and reduce false positives we used a Benjamini and Hochberg (1995) false discovery rate (FDR) p-value correction in R using the *p.adjust* function in the base *stats* package. The FDR correction uses sample size to adjust the p-value and a custom function was used to modify the sample size of SNPs for each analysis. Therefore, the final p-value cut-off varied between analyses because the number of SNPs and maternal trees (e.g., Table 2 and Table 3) included in each association analysis differed. QQplots were generated for each association test and visually assessed. The proportion of variance explained by genotyped SNPs "chip heritability" (h^2_chip) estimated in GEMMA is reported for univariate models. Chip heritability is calculated using the genetic and environmental variance components from restricted maximum likelihood method (REML) within GEMMA (Zhou and Stephens 2012, Elhezzani et al. 2018). Chip heritability enables researchers to estimate heritability for unrelated individuals using genotyped SNPs (Speed et al. 2012). SNPs that were only detected in one environment or trait (unique) and SNPs that were detected across multiple gardens (overlapping) from each association test were identified. A BLASTn search was conducted to detect functional annotations and coding regions for significant SNPs in the reference genomes

and other genetic sequences for *Pinus lambertiana* (Stevens et al. 2016), *Pinus taeda* (Neale et al. 2014), and *Pseudotsuga menziesii* (Howe et al. 2013). While the genomes may lack complete functional annotations, we limited the scope of this analysis to North American conifers. The 80 base pair nucleotide sequences for each region or contig were used to search all gene space in the genomes. The percent identity (%ID), expectation value (*e-value*), and alignment length region (ALR) are reported in the supplementary material for the gene coding detections (Table S6).

RESULTS

Single and multi-trait GWAS of phenological traits, bud damage and survival detected 103 unique significant SNPs. The number of detected loci ranged from 8 at the high elevation garden to 31 and 65 at the intermediate and low elevation gardens, respectively. One locus (167770:33) was detected across multiple association tests. It was detected in a single (bdmgS19) and multi-trait analysis (FFP_MT, including surv19, set18, wint18-19, bdmgS19) at the intermediate garden and for spring phenology (budadv19) at the high elevation garden. Four 80bp contig regions housed eight significant SNPs, two each, that were detected for different combinations of traits including lammas, bud damage, and spring phenology (budslp and budint) (Table S7). Overall, SNP detections were garden, trait, and analyses specific, with two loci detected over multiple time points for longitudinal traits.

Population Structure

The optimal number of population clusters (K) was 2 across all 10 runs. The results from fastSTRUCTURE show that the genetic material from the second cluster is spread across *P. strobiformis* range at low proportions of the total genomic content (Figure 1). Only two PC axes were significant and accounted for a small amount of the variation (Figure 1). PC1 accounted for 26.87% of the variation and PC2 accounted for 6.55%.

Univariate Association Tests

Of the 59 SNPs unique to single trait analyses 34 were associated with lammas growth at the low elevation site, seven for bud damage at different time points at the intermediate site, and three for bud advancement at the high elevation site (Table 4). The minor allele frequency was moderate for all SNPs detected in the single-trait analyses and only 12 SNPs had an allele frequency above 0.1 (Figure 3). Four were for lammas growth, one bdmgS18 at the intermediate site, two for wint17-18 at the low site, and the remaining were for phenology at the low (SpringPhen18_MT) and intermediate sites (SpringPhen19_MT) (Figure 3).

Growing conditions had a large impact on bud-related traits and SNP detections in the univariate associations. All minor alleles associated with lammas growth increased the likelihood of this trait (average β=0.253) (Figure 4) and had moderate minor allele frequencies (MAF $\bar{x}$ =0.065) (Figure 3). All seven minor alleles associated with bud damage were at the intermediate garden and increased the likelihood of bud damage ($\beta\ \bar{x} = -0.128$) and had a moderate minor allele frequency (MAF $\bar{x}$ =0.056) (Table S8). The three minor alleles associated with spring bud advancement (budadv19) at the high elevation garden increased the pace of bud development ($\beta\ \bar{x}$ = 0.205) (Figure 4) but were rare (MAF $\bar{x}$ = 0.033) (Figure 3).

A total of 28 SNPs were associated with survival across all gardens in the univariate analyses. The majority of survival minor alleles were for overwinter survival and had negative effects (Table 4, Table S8); specifically, 16 at the low elevation site for wint17-18 survival and six for wint18-19 at the intermediate. SNPs associated with at least one measurement of survival were detected at each garden, but not a single SNP overlapped between univariate analyses within nor across gardens. Only two of the 28 minor alleles detected in the univariate analyses had a positive effect on survival. This included SNPs detected for survival during a lethal

drought, drt20, (β = 0.137 ± 0.027) and for overall survival, surv19, (β = 0.165 ± 0.034) at the high elevation garden (Figure 5).

Chip Heritability

Chip heritability (h^2_chip) uses genomic markers to estimate heritability (Sun et al. 2014). Chip heritability was only estimated in the univariate analyses and was affected greatly by measurement time and growing environment (Table S8, Figure S1). On average, chip heritabilities were highest at the intermediate garden (Figure S1). Across all three gardens h^2_chip did not directly correspond to detection of significant SNPs. For two traits, bdmgS18 at the intermediate site and budadv19 at the high elevation site, h^2_chip was less than 0.001, but several SNPs were associated with each trait (Table 4). The remaining traits with at least one SNP detection had moderate to high chip heritabilities ranging from 0.237 for surv19 to 0.999 for lms19. Other traits, such as budv18 and set18 had some of the highest chip heritabilities, 0.826 and 0.754 respectively, but no SNPs were detected (Table S8). Surprisingly, some of the overall highest and lowest chip heritability estimates were for the same traits measured at a different time point or garden. For example, while h^2_chip for survival over the 2019 growing season (grow19) at the intermediate site and under lethal drought (drt20) at the high elevation site were high, 0.654 and 0.736 respectively, they were both 1.78e-6 at the low elevation site (Table S8).

Multi-trait Results

Multi-trait GWAS analyses detected an additional 21 unique SNPs (Table 5). At the low elevation garden five unique loci and 27 total SNPs were detected from multi-trait analyses. At the intermediate elevation garden 15 unique and 17 overall SNPs were detected, and one at the high elevation garden. The greatest number of SNPs detected for multi-trait groupings were for

traits associated with seed source CMD at the low garden (CMD_Lo_MT) and spring phenology 2019 (SpringPhen19_MT) at the intermediate garden with 14 and 15 associated SNPs respectively (Table 5). An additional 10 SNPs were detected at the low elevation garden for spring phenology 2018 measurements (SpringPhen18_MT).

Multi-trait groupings of longitudinal measures for fall bud variation, bud set, and bud damage, did not detect any significant SNPs. Multi-trait groupings of spring phenology slope and intercept within one growing season (2018 or 2019; BudDev18_MT or BudDev19_MT) and across years (2018 and 2019; BudDevAll_MT) revealed three unique SNPs at the low elevation garden. One of these SNPs represents a stable minor allele that impacted phenology across two years and two minor alleles that only affected phenology in one year. The multi-trait grouping for spring phenology across both 2018 and 2019 (BudDevAll_MT) detected a stable minor allele (72802:41) that delayed bud burst in both years (2018 $\beta = 3.6\text{x}10^{-4} \pm 8.6\text{x}10^{-5}$, 2019 $\beta = 3.59\text{x}10^{-4} \pm 1.83\text{x}10^{-4}$). The minor allele associated with BudDev18_MT (45856:75) delayed bud burst in 2018 ($\beta = -2.22\text{x}10^{-4} \pm 7.1\text{x}10^{-5}$). The minor allele detected in BudDev19_MT (257691:39) had the opposite effect in 2019, marginally advancing bud burst ($\beta = 5.6\text{x}10^{-4} \pm 1.6\text{x}10^{-4}$).

While fewer SNPs were detected for trait groupings based on FFP, TD, and CMD maternal climate variables across gardens, 18 total SNPs were detected. 15 of these SNPs were associated with CMD at the low elevation garden. These SNPs were associated with spring phenology and over winter survival from 2017-2018, demonstrating SNPs that directly connect spring phenology and survival. An additional three SNPs were associated with FFP (FFP_Int_MT) and TD (TD_Hi_MT) trait groupings (Table S9). Both groups included survival, bud set, and bud damage measurements. The minor allele associated with each group negatively

affected survival and bud damage but the effect on bud set differed. At the high elevation garden (TD_Hi_MT) the minor allele advanced bud set in 2018 and 2019 (set18, β= 0.102 ± 0.047; set19, β = 0. 007 ± 0.017) whereas at the intermediate garden (FFP_Hi_MT) the two minor alleles delayed bud set in 2018 (167770:33, β= -0.016 ± 0.016; 52217:22, β = -0. 021 ± 0.014) (Table S9).

Multi-trait analyses increased the detection of SNPs and RAD-contigs shared between garden environments and traits. SNP 167770:33 was detected in both univariate and multi-trait analyses for different traits at the high and intermediate gardens (Figure 6). At the high elevation garden this minor allele had a positive effect on spring phenology bud advancement (budadv19) (β =0.207±0.041) increasing the rate of development. At the intermediate garden, this minor allele increased bud damage in spring 2019 (bdmgS19) in the univariate association and was also associated with traits in the FFP multi-trait group (FFP_Int_MT) discussed previously (Table 2, Figure 6).

Four RAD-contigs housed two significant SNP associations each, revealing shared genetic markers among traits. Three contigs contained SNPs at the low elevation garden detected in univariate and multi-variate association tests. One of these three housed a SNP detected in the 2018 spring phenology multi-trait grouping (BudDev18_MT) and lammas growth (lms19). The minor allele associated with BudDev18_MT (45856:75) delayed bud burst (budslp18; $\beta = -2.2\text{x}10^{-4} \pm 7.10\text{x}10^{-5}$). The minor allele associated with lammas growth increased the likelihood of lammas growth (β = 0.267 ± 0.062). Two contigs (5309 and 49636) contained SNPs that were associated with lammas growth (Table S7). Interestingly, the pair of minor alleles associated with lammas on each of the two contigs had the same positive effect, (5309: β = 0.234 ± 0.059; 49636: β = 0.206 ± 0.052) and were found at the same allele frequency (5309: MAF= 0.042;

49636: MAF= 0.076). One contig held two of the five total SNPs associated with spring 2018 bud damage (bdmgS18) at the intermediate garden.

Reference Genome Hits

A BLASTn search using 99 RAD-contigs regions with at least one associated SNP to detect matches in all gene space and functional annotations in *Pinus lambertiana*, *Pinus taeda*, and *Pseudotsuga menziesii genomes* resulted in the detection of six gene coding regions and 212 hits (Table S6). Matches to *Pseudotsuga menziesii* were most telling with 17 hits including three gene coding regions on four contigs. One gene was for a heat shock protein (CN640419.1) (%ID = 90.9, ALR= 22, *e-value*= 0.017) on contig 21871. The SNP found on contig 21871 was associated with SpringPhen19_MT at the intermediate site. This minor allele had a negative effect on bud burst for heterozygous individuals, but homozygous individuals had nearly equivalent bud burst development. The second and third gene were found on contigs associated with lammas growth. One gene (Pm_CL1692Contig1) (%ID=100, ALR=18, *e-value*=0.005) is a zinc-finger containing protein gene on contig 69504, and the other is for an anaphase promoting complex/cyclosome protein (ES420771.1)(%ID = 85.7 , ALR=28, *e-value*= 0.005) on contig 235570. From *Pinus lambertiana*, two hypothetical protein sequences were detected among 16 hits. Five of these hits were found on Contig 93308 (%ID= 83.3, ALR=30, *e-value*= 0.016) that housed a SNP associated with lammas growth. One of the hypothetical protein sequences was (CL1029Contig1_01) (%ID= 88, ALR=25, *e-value*= 0.016) with no known function found on contig 72802 which was detected for spring phenology across 2018 and 2019 (BudDevAll_MT) at the low elevation garden and delayed bud development. Only one maternal tree was homozygous for the minor allele, so the detection of this gene coding region could be a statistical artifact. The other hypothetical protein (2-3756-03) (%ID=95.24, ALR= 21, *e-value* =0.009) on

contig 52217 contained a SNP detected for traits in the multi-trait frost-free period grouping. The blast search on *Pinus taeda* elicited 179 hits and one hypothetical protein (0_1078_01) (%ID = 91.6, ALR= 24, *e-value* = 0.042) on contig 112465 where a SNP for spring phenology 2019 at the intermediate garden is housed (Table S6).

DISCUSSION

Advancements in GWAS aim to improve our understanding of the genetic contributors to complex phenotypes by increasing the detection of SNPs that account for greater proportions of observed phenotypic variation. In this study we utilized three common gardens that imposed some novel climate extremes on *P. strobiformis* and detected 103 SNPs related to survival, phenology, and bud damage over the span of two years. The number of SNPs detected for trait measurements and the direction of their effects was garden specific. Using multi-trait groupings of longitudinal traits and traits with clinal relationships to maternal climate improved our ability to detect loci and identify shared genetic influences on multiple traits. Since environmental conditions greatly influence trait expression (Stinchcombe and Hoekstra 2008) the use of multiple gardens may have also increased our capacity to detect genetic variation that would be missed using a single environment (Lind et al. 2018). Our results suggest that phenology, survival, and bud damage in *P. strobiformis* are regulated by complex relationships between standing genetic variation, indirect effects of loci on multiple phenotypes, and sensitivity to growing conditions.

PATTERNS OF LOCI DETECTION ACROSS VARIABLE ENVIRONMENTS

In agreement with H1a, we detected mostly unique loci for each trait across measurement times and garden environments. Only one out of 103 total SNPs overlapped gardens (167770:33); even though seeds from the same maternal trees were grown in all three gardens

and we attempted to capture temporal variation in phenotypes by running GWAS on longitudinal traits. In support of our hypothesis (H1a) we detected the highest number of unique SNPs, 65, at the low elevation garden which is the warm and dry extreme of the three gardens. The low elevation garden also imposes the most variable temperatures (Figure 2, Table S2-4). Only eight SNPs were detected at the high elevation garden where *P. strobiformis* would most likely occur in natural stands and represents the cold and wet climate extremes of the three gardens.

In our study the lack of overlapping SNPs across gardens is likely driven by altered genotype-phenotype relationships in response to garden specific climates (Josephs et al. 2019). The gardens in 2018 and 2019 had highly variable temperatures and precipitation regimes that were at or beyond the US populations 30-year climate averages (Figure 2, Table S2-S4). Providing irrigation during the growing season likely offset some of the moisture deficit (Bucholz et al. 2020), but the dramatic differences in winter precipitation likely influenced early season growth and resulting phenotypic expression. Additionally, detecting the same genetic variants across analyses is rare in GWAS because studies often incorporate different populations, growing conditions, allele frequency thresholds, or significance cut-offs (e.g., Chhetri et al. 2020, Tan 2018, Carlson et al. 2019). Small sample sizes, coarse phenotypes, and the inability to recreate precise environmental conditions can also lead to non-overlapping detections (Ingvarsson and Street 2011). For example, when the same populations of *Populus trichocarpa* were grown in two separate environments, including one at the southern terminus of the species range, GWAS analyses on traits measured at different time points failed to identify any overlapping SNPs (Chhetri et al. 2020). Additionally, in a *Eucalyptus* study, using the same traits and populations but a different GWAS tool affected the detection of overlapping SNPs (Tan 2018). Our study design with the same populations distributed across three environments, trait

measurements collected at multiple overlapping time points, and comparisons between univariate and multi-trait approaches enable us to determine that differences in SNP detections were likely the result of growing environment and genotype-environment interactions.

GENETIC ARCHITECTURE OF CLIMATE-RELATED TRAITS

While many GWAS analyses aim to identify the genetic structure of ecologically relevant traits related to fitness, few directly address fitness in the form of survival or the link between survival and phenological timing. In our study we detected 103 loci for seasonal and overall survival, bud phenology, and bud damage in *P. strobiformis* across univariate and multivariate association analyses. Like previous GWAS on adaptive traits in forest trees we conclude that phenological traits and survival in *P. strobiformis* are regulated by complex gene and environment interactions mediated by many rare variants (Fournier et al. 2019) with small effects. Our results also confirm our hypothesis (H1b) that the detectable genetic architecture of traits differs by growing environment and measurement time providing additional ecological and evolutionary insight. At the low elevation garden, the majority of SNPs were detected for traits measured in 2018 (Table 4, 5), while at the intermediate and high elevation gardens the majority of SNPs were detected for traits measured in 2019. Finally, seven of the eight unique SNPs detected at the high elevation garden were for 2019 growing season survival, overall survival, and spring phenology measurements, and 2020 lethal drought survival (Table 4, 5).

Survival

Across six different measurements of seasonal and overall survival a total of 36 SNPs were detected, of which 21 were unique to only survival metrics, and the remaining 15 were detected in analyses with another trait. The majority of the effects of minor alleles were negative (Figure 3), however, ten minor alleles were beneficial to survival in particular conditions. The

two minor alleles with the largest positive effects (β=0.137± 0.027 and β=0.165 ± 0.034) (Figure 5) were both detected at the high elevation garden for overall survival (surv19) and lethal drought survival (drt20) respectively. The detection of these positive effect minor alleles at the coldest of the three gardens and under extreme water limitation may suggest that genetic variation in *P. strobiformis* may confer an advantage for cold and/or dry conditions (Figure 5). Additionally, the greatest number of negative effect minor alleles detected at the two lower, and drier, gardens were for winter survival. Considering seedlings were not irrigated during the winter, and the 2018 winter was particularly dry (Figure 2) the detection of these negative effect minor alleles may promote the argument that genetic variation related to cold temperatures and water availability have fitness consequences. Overall, the opposing relationships among minor alleles and survival in growing environments across a temperature and precipitation gradient demonstrate that winter climates likely affect the maintenance of rare alleles in *P. strobiformis* populations. In other conifers, including four co-occurring species in Europe, numerous loci were associated with winter precipitation and seasonal minimum temperatures (Mosca et al. 2012). SNPs associated with water use efficiency and cold hardiness traits explain substantial variation in association studies compared to other traits (Hall et al. 2016). Additionally, a forest drought-stress index using tree-ring data over 1,000 years revealed that drought stress is equally influenced by winter precipitation and warm-season climate moisture deficit in the southwestern United States (Williams et al. 2013). This further promotes that seasonal temperatures and precipitation regimes impose selective pressures on *P. strobiformis* seedlings and may be responsible for the detection of alleles in seasonal and garden specific patterns observed in our study. Our results are also supported by previous studies on *P. strobiformis* geographic distribution (Shirk et al. 2018), hybridization history (Menon et al. 2018), and a gene-

environment association study (Menon et al. 2021) that highlight the role of moisture deficit and cold temperatures in shaping *P. strobiformis* evolutionary history.

Spring Phenology

A total of 37 SNPs were detected for spring phenology in *P. strobiformis.* The minor alleles detected for spring phenology at the two lower gardens were associated with bud development (Figure 4). The majority of minor alleles detected had small effects, but three minor alleles detected at the high elevation garden had the largest effects, advancing bud burst (Figure 4). Additionally, SNPs associated with spring phenology had some of the highest allele frequencies of any trait (Figure 3), with five of the 12 SNPs with a minor allele frequency above 0.1 detected for spring phenological traits. The opposing effects on spring phenology by minor alleles at one site highlight the genetic complexity regulating the trade-off between growth and surviving diverse abiotic conditions. These trade-offs drive the high levels of plasticity observed in spring phenology for tree species (Hall et al. 2007) and SNPs associated with spring phenology have evolved under positive selection for *Populus trichocarpa* (Apuli et al. 2020). SNPs associated with bud burst phenology in *Populus trichocarpa* were attributed to selection for geographic differences in winter chilling and heat sum accumulation (McKown et al. 2018). The SNPs detected for *P. strobiformis* across the three gardens may represent minor alleles that are sensitive to specific environmental conditions where one allele alters trait expression in one condition but not in another (Broman and Sen 2009). The maintenance of rare and more common alleles with opposing effects may be beneficial for *P. strobiformis* to deal with interannual variation in temperature and precipitation. For example, in another study on northern Arizona populations of *P. flexilis* populations (Adams and Kolb 2004), that could be *P. strobiformis* hybrids, grew faster in wet years and more slowly in dry years compared to *Pinus ponderosa*

(Adams and Kolb 2004), demonstrating physiological and morphological compromises in response to abiotic conditions. Selective pressures on populations that inhabit climatically variable environments, like *P. strobiformis,* may maintain alleles at lower frequencies because they are occasionally favorable in particular years (Wittmann et al. 2017). *Pinus contorta* has high levels of within population variation for adaptive traits that is important for surviving the harsh conditions they inhabit and result in strong selective pressures on seedlings (Rehfeldt 1999, Aitken and Hannerz 2001). *Pseudotsuga menziesii* also inhabit a large range of climate conditions and rare alleles at low frequencies have been implicated in cold hardiness and phenology trait expression (De La Torre et al. 2021).

Two SNPs detected for spring phenology were on contigs that matched known protein sequences including a heat shock protein in *P. menziesii* and a hypothetical protein in *P. lambertiana* and *P. taeda*. These discoveries increase the biological implications for our association analyses. The heat shock protein (CN640419.1) is a candidate gene used in a *Pseudotsuga menziesii* cold hardiness study (Eckert et al. 2009a). While this particular gene was not under positive nor selective pressure in the Eckert (et al. 2009a) study, heat shock proteins have important biological influences related to stress, cold acclimation, and drought physiology. Specifically, heat shock proteins were upregulated in *Arabidopsis* in relation to cold signaling (Lee et al. 2005), are implicated in response to multiple stressors, and are considered proactive proteins for drought response in four conifers (Moran et al. 2017). The action of heat shock proteins in these aforementioned studies highlight heat shock proteins role in facilitating trade-offs between growth and surviving diverse abiotic pressures.

The SNP detected on the contig matching the heat shock protein, in our study was detected for spring phenology in 2019 at the intermediate garden. Heterozygous individuals for

the minor allele delayed bud burst compared to the homozygous individuals, suggesting balancing selection or overdominance at this locus (González-Martínez et al. 2006). This pattern has been observed in other genomic studies in Norway spruce wood growth (Baison et al. 2019), phenology in *Eucalyptus* (Tan and Ingvarsson 2018), and flowering time in *Arabidopsis* (Seymour et al. 2016). In the *Pinus lambertiana* genome a hypothetical protein sequence (CL1029Contig1_01) of no known action matched the sequence that contained a minor allele at the low elevation site that delayed bud development across multiple years (BudDevAll_MT). Further investigation of this protein may reveal reliable or stable genetic drivers of phenology.

Lammas Growth

The greatest number of SNPs, 34, for any single analyses were detected for lammas growth, and all of the minor alleles had a positive effect, increasing the likelihood of lammas growth (Figure 4). The large number of SNPs detected for lammas growth at only one garden in *P. strobiformis* highlight that loci responsible for lammas growth may respond to warmer temperatures and longer growing seasons that individuals may not experience in their native range. This further promotes the conclusion that the impacts of standing genetic variation on phenotypes are heavily influenced by environmental conditions. Secondary flushing may provide a competitive advantage to *P. strobiformis* individuals that can elongate their shoots without occurring injury, which may not occur in the current US range, but may be advantageous under climate change. Secondary flushing in *Abies sachalinensis* is also regulated by several loci and exhibits an altitudinal cline (Goto et al. 2017).

The two protein sequences that matched contigs containing SNPs for lammas growth reveal connections between lammas growth and cold hardiness. The two protein sequences were a zinc-finger containing protein (Pm_CL1692Contig1) and an anaphase promoting

complex/cyclosome protein (ES420771.1) that were candidate genes in an association study on cold hardiness in *Pseudotsuga menziesii* (Eckert et al. 2009b). In that study the anaphase promoting complex protein was significantly associated with seed weight, which was higher on the western side of a dividing mountain range (the Cascades) and lower on the eastern. The geographic divide across the Cascade Mountain range is known to impose selective pressure on individuals and benefit populations that can take advantage of favorable conditions (Eckert et al. 2009b). Additionally, the anaphase promoting complex protein may be responsible for rapid growth due to its known influence on cell cycle regulation and shoot branching (Eloy et al. 2015).

Bud Damage

In our study only eight total minor alleles were detected in relation to bud damage, and all increased the likelihood of bud damage at the high and intermediate gardens (Figure 4). The SNPs detected for bud damage had low to moderate allele frequencies ($\bar{x}$ = 0.043), except for one with a minor allele frequency of 0.124, one of the highest observed in our study (Figure 3). The low number of SNP detections was surprising given the climate extremes imposed across gardens, the moderate chip heritability for bud damage at all sites (Figure S1), and the robust evidence for genetic connections to cold damage in other conifers. Namely, in *Picea sitchensis* SNPs associated with cold hardiness explained 28% of the variation in the observed variation (Holliday et al. 2010), in *Pseudotsuga menziesii* candidate genes putatively responsible for cold damage have been identified (Eckert et al. 2009b, Vangestel et al. 2018), and six SNPs on those candidate genes explained 17% of variation in cold damage (Eckert et al. 2009b). The lack of signals for bud damage in this study may relate to the timing of our measurements because trees may have died following damage before we were able to account for it. Therefore, it is likely that

the large number of SNPs detected for survival may be partially attributable to bud damage. Availability of a reference genome or larger contig lengths may help address if SNPs related to bud damage and survival are physically linked.

MULTI-TRAIT CONTRIBUTIONS TO GENETIC ARCHITECTURE

Longitudinal Traits

In partial support of our hypothesis (H2a) grouping longitudinal traits in multi-trait association analyses in GEMMA increased the number of detected SNPs associated with spring phenology compared to the univariate approach, but not for bud damage, survival, or bud set. In the univariate approach only three SNPs were detected for spring phenology overall, whereas grouping spring phenology within and across seasons resulted in 28 additional SNPs (Table 5). 27 of these were unique to either 2018 or 2019 and only one SNP was associated with spring phenological measurements across both years. Annual variation in SNP detections for phenology has been documented in *Abies sachalinensis* where SNPs detected for spring phenology in one year explained 11.5% of phenotypic variation but failed to detect any SNPs the following year (Goto et al. 2017). In *Eucalyptus* multi-trait approaches also enhanced the detection of SNPs for growth and spring phenology compared to univariate approaches (Tan 2018), suggesting that spring phenology and other seasonal growth metrics benefit from multivariate GWAS approaches.

The spring phenological measurements in our study (bud slope, bud int, and bud advancement) represented different components of bud development but were still highly correlated (Figure S2). The high correlation between these variables and the resulting high number of SNPs supports previous research that suggests combining highly correlated traits in GWAS may increase power (Zhou and Stephens 2014, Chhetri et al. 2019). For example,

grouping highly correlated metabolic traits in oats (*Avena sativa* L.) resulted in the detection of new and previously discovered loci (Carlson et al. 2019). Future genetic association studies would benefit from using multi-trait techniques to better capture spring phenological advancement over longer periods of time. Additionally, incorporating more unified assessments of phenology to pinpoint bud responses to particular climate cues (Chuine 2000) may help unravel the temporal effects on spring phenology.

Considering many genome wide association studies for fall dormancy have detected numerous loci explaining large amounts of phenotypic variation (e.g., Goto et al. 2017, Apuli et al. 2020, Holliday et al. 2010) it was surprising that longitudinal trait groupings of fall bud set did not result in loci detections. The lack of detections for bud set across years could result from conifer genome structure and interannual variation in trait expression may complicate the ability to detect variants affecting traits across time. Since genetic variation for bud set is low and conifer genomes are so large it is likely that our genomic sampling effort and SNP set did not include loci related to bud set. A study on *Populus trichocarpa* tried to detect SNPs associated with bud set across years, but only six of a total 34 SNPs were detected for bud set in consecutive years (Apuli et al. 2020). Additionally, while correlations across measurement times for bud set traits (budset, budv) in our study were low (Table S2), that alone may not fully explain our lack of detections. For example, inconsistent correlations across measurement times did not hinder a longitudinal study of height in *Populus* spp. that detected 41 SNPs (Chen et al. 2021).

Trait Groupings by Maternal Climate Relationships

Grouping traits based on relationships with maternal site climate variables enabled us to detect loci that were shared among survival, spring and fall phenology, and bud damage. Our

results also highlight environment specific allelic action because at each garden only one grouping resulted in the detection of significant SNPs; CMD at the low elevation garden, FFP at the intermediate garden, and TD at the high elevation garden. Notably, all groups in this category with significant SNP detections included a survival metric. To our knowledge this was the first attempt at grouping traits in this manner. These findings directly link genetic markers responsible for survival, seasonal phenology, and bud damage.

The multi-trait grouping for CMD at the low elevation garden (CMD_Lo_MT) revealed 14 SNPs associated with overwinter survival (wint17-18) and subsequent spring 2018 phenology. Six of those 14 SNPs improved overwinter survival from 2017–2018 and delayed 2018 spring phenology. SNPs detected for traits in the TD and FFP groupings revealed genetic relationships among survival, bud set and bud damage. Minor alleles detected in the FFP grouping delayed bud set, increased bud damage and increased mortality over the 2018-2019 winter (Table S9). However, the two minor alleles detected for the TD grouping had the opposite effect on bud set. These minor alleles (167770:33 and 52217:22) (Table S9) promoted earlier bud set in two consecutive years, but still increased bud damage and mortality. Detecting alleles with opposing relationships between survival and bud set at the high and intermediate elevation garden may result from different environmental signals and trade-offs in extending or shortening the growing season to minimize damage related to cold temperatures. Also, novel environments, such as those at our gardens, may alter the genetic correlation between life history traits and potentially constrain adaptive responses to stressors (MacTavish and Anderson 2020).

Conclusions

In this study we detected 103 SNPs related to survival, bud damage, lammas growth, and spring and fall phenology. These SNPs had low to moderate minor allele frequencies and SNPs

had site and trait specific effects. Our experimental design, exposing trees to the conditions at the clinal maxima of temperature and moisture deficit for *P. strobiformis*, may have increased our capacity to detect loci with no phenotypic effect in commonly encountered conditions (González-Martínez et al. 2006, Pyhäjärvi et al. 2020). The positive effect minor alleles detected for survival at the high elevation garden, and moderately high heritabilities (h^2_chip) for survival and bud damage across gardens could suggest that *P. strobiformis* has substantial genetic variation that may be expressed or experience selection under extreme climates beyond its current distribution (Rosenblad et al. 2019). Novel environments imposed by climate change may alter the genetic relationships with phenological traits and survival in *P. strobiformis* and affect adaptive responsiveness to stressors. Novel environments imposed by climate change may alter the expression of phenological traits and survival in *P. strobiformis*, despite the fact that loci with phenotypic effects in novel conditions may be un-expressed or neutral in their native range (Fournier-Level et al. 2011). Seedling and adult trees may respond to environmental cues differently (Pardos et al. 2014) and seedling survival, while crucial, may not always result in reproductive output for adult trees under stressful conditions.

Our use of multi-trait GWAS allowed us to detect stable loci influencing traits across multiple time points and unique loci with diverse effects on spring phenology. These results demonstrate that using multi-trait GWAS on spring phenology measurements may help capture some of the genetic contribution to phenological variation that is missed in univariate analyses. We determined that phenological variation across growing conditions has a quantifiable genetic basis and observed differences between gardens are not due to phenotypic plasticity alone (Hancock et al. 2011). Grouping phenotypes based on maternal site climate variables revealed shared genetic relationships among survival, phenology, and bud damage. Overall, our research

indicates that loci associated with survival and phenological traits in *Pinus strobiformis* coordinate complex responses to environmental stimuli.

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LITERATURE CITED

Adams HD, Kolb TE (2004) Drought responses of conifers in ecotone forests of northern Arizona: tree ring growth and leaf δ13C. Oecologia 140:217–225. https://doi.org/10.1007/s00442-004-1585-4

Aitken, SN, Hannerz M (2001) Genecology and Gene Resource Management Strategies for Conifer Cold Hardiness. *In* F.J. Bigras and S.J. Colombo (eds). Conifer cold hardiness. Kluwer Academic Publ., Dordecht. The Netherlands. 23–53. https://doi.org/10.1007/978-94-015-9650-3_2

Alberto FJ, Aitken SN, Alía R, et al.(2013) Potential for evolutionary responses to climate change - evidence from tree populations. Glob Chang Biol 19:1645–1661. https://doi.org/10.1111/gcb.12181

Apuli R-P, Richards T, Rendon M, et al.(2020) The genetic basis of adaptation in phenology in an introduced population of Black Cottonwood (*Populus trichocarpa,* Torr & Gray). bioRxiv. https://doi.org/10.1101/2020.06.17.156281

Baison J, Vidalis A, Zhou L, et al.(2019) Genome-wide association study identified novel candidate loci affecting wood formation in Norway spruce. Plant J 100:83–100. https://doi.org/10.1111/tpj.14429

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B, 57: 289-300. http://www.jstor.org/stable/2346101.

Bozdogan H (1987). Model selection and Akaike's Information Criterion (AIC): The general theory and its analytical extensions. Psychometrika, 52(3): 345–370. https://doi.org/10.1007/BF02294361

Breiman L (2001) Random Forests. Machine Learning. 45*:* 5–32 https://doi.org/10.1023/A:1010933404324

Brodersen CR, Germino MJ, Johnson DM, et al.(2019) Seedling Survival at Timberline Is Critical to Conifer Mountain Forest Elevation and Extent. Front For Glob Chang 2: 1-12 https://doi.org/10.3389/ffgc.2019.00009

Broman, Sen (2010) A Guide to QTL Mapping with R / qtl. J Stat Softw 32. https://doi.org/10.18637/jss.v032.b05

Brooks ME, Kristensen K, van Benthem KJ, et al.(2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R Journal. 9:378–400. https://doi.org/10.32614/rj-2017-066

Browning BL, Zhou Y, Browning SR (2018) A one-penny imputed genome from next generation reference panels. Am J Hum Genet. 103(3):338-348. doi:10.1016/j.ajhg.2018.07.015

Bucholz E (2020) Early growth, water relations and survival: common garden studies of *Pinus strobiformis* under climate change. Doctoral dissertation, Northern Arizona University.

Bucholz, BR, Waring, KM, Kolb, TE Swenson, JK, and Whipple, AV (2020) Water relations and drought response of *Pinus strobiformis*. Canadian Journal of Forest Research. 50(9): 905-916. https://doi.org/10.1139/cjfr-2019-0423

Carlson MO, Montilla-Bascon G, Hoekenga OA, et al.(2019) Multivariate genome-wide association analyses reveal the genetic basis of seed fatty acid composition in oat (*Avena sativa L.*). G3 Genes, Genomes, Genet. 9: 2963–2975. https://doi.org/10.1534/g3.119.400228

Cayan, D, M. Tyree, KE, Kunkel, et al.(2013). Future Climate: Projected Average. *In* Assessment of Climate Change in the Southwest United States: A Report Prepared for the National Climate Assessment, G. Garfin, A. Jardine, R. Merideth, M. Black, and S. LeRoy(eds). 101–125. A report by the Southwest Climate Alliance. Washington, DC: Island Press.

Chen Y, Wu H, Yang W, et al.(2021) Multivariate linear mixed model enhanced the power of identifying genome-wide association to poplar tree heights in a randomized complete block design. G3 Genes, Genomes, Genetics. https://doi.org/10.1093/g3journal/jkaa053

Chhetri HB, Macaya-sanz D, Kainer D, et al.(2019) Multitrait genome-wide association analysis of *Populus trichocarpa* identifies key polymorphisms controlling morphological and physiological traits. New Phytologist. 293–309. https://doi.org/10.1111/nph.15777

Chhetri, HB, Furches, A, Macaya-Sanz, D, Walker, et al.(2020). Genome-Wide Association Study of Wood Anatomical and Morphological Traits in *Populus trichocarpa*. Frontiers in Plant Science, 11. https://doi.org/10.3389/fpls.2020.545748

Chuine, I (2000). A Unified Model for Budburst of Trees. Journal of Theoretical Biology, 207(3): 337–347. https://doi.org/10.1006/JTBI.2000.2178

Chuine I (2010) Why does phenology drive species distribution? Philos Trans R Soc B Biol Sci. 365:3149–3160. https://doi.org/10.1098/rstb.2010.0142

Chuine I, Beaubien EG (2001) Phenology is a major determinant of tree species range. Ecol Lett 4:500–510. https://doi.org/10.1046/j.1461-0248.2001.00261.x

Clark JS, Salk C, Melillo J, Mohan J (2014) Tree phenology responses to winter chilling, spring warming, at north and south range limits. Functional Ecology. 28: 1344-1355. https://doi.org/10.1111/1365-2435.12309

Danecek, P, Auton, A, Abecasis, et al.(2011). The variant call format and VCFtools. Bioinformatics (Oxford, England), 27(15): 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

Dannemann M, Andrés AM, Kelso J (2016) Introgression of Neandertal- and Denisovan-like Haplotypes Contributes to Adaptive Variation in Human Toll-like Receptors. Am J Hum Genet 98:22–33. https://doi.org/10.1016/j.ajhg.2015.11.015

De La Torre AR, Birol I, Bousquet J, et al.(2014) Insights into conifer giga-genomes. Plant Physiol 166:1724–32. https://doi.org/10.1104/pp.114.248708

De La Torre AR, Wilhite B, Puiu D, et al.(2021) Dissecting the polygenic basis of cold adaptation using genome-wide association of traits and environmental data in Douglas-fir. Genes (Basel) 12:1–28. https://doi.org/10.3390/genes12010110

Des Marais DL, Hernandez KM, Juenger TE (2013) Genotype-by-Environment Interaction and Plasticity: Exploring Genomic Responses of Plants to the Abiotic Environment. Annu Rev Ecol Evol Syst 44:5–29. https://doi.org/10.1146/annurev-ecolsys-110512-135806

Duputié A, Rutschmann A, Ronce O, Chuine I (2015) Phenological plasticity will not help all species adapt to climate change. Glob Chang Biol 21:3062–3073. https://doi.org/10.1111/gcb.12914

Eckert, AJ, Wegrzyn, JL, Pande, B, et al.(2009a). Multilocus patterns of nucleotide diversity and divergence reveal positive selection at candidate genes related to cold hardiness in coastal Douglas fir (*Pseudotsuga menziesii* var. *menziesii*). Genetics, 183(1): 289–298. https://doi.org/10.1534/genetics.109.103895

Eckert AJ, Bower AD, Wegrzyn JL, et al.(2009b) Association genetics of coastal Douglas fir (*Pseudotsuga menziesii* var. *menziesii*, Pinaceae). I. Cold-hardiness related traits. Genetics 182:1289–302. https://doi.org/10.1534/genetics.109.102350

Elhezzani, NS (2018) Improved estimation of SNP heritability using Bayesian multiple-phenotype models. European Journal of Human Genetics 26(5): 723–734. https://doi.org/10.1038/s41431-018-0100-z

Eloy NB, de Freitas Lima M, Ferreira PCG, Inzé D (2015) The Role of the Anaphase-Promoting Complex/Cyclosome in Plant Growth. CRC Crit Rev Plant Sci 34:487–505. https://doi.org/10.1080/07352689.2015.1078613

Excoffier L, Ray N. (2008) Surfing during population expansions promotes genetic revolutions and structuration. Trends Ecol Evol. 23(7):347-51. doi: 10.1016/j.tree.2008.04.004. Epub 2008 May 24. PMID: 18502536.

Eu-Ahsunthornwattana J, Miller EN, Fakiola M;, Jeronimo SMB (2014) Comparison of Methods to Account for Relatedness in Genome-Wide Association Studies with Family-Based Data. PLoS Genet 10:1004445. https://doi.org/10.1371/journal.pgen.1004445

Evans LM, Slavov GT, Rodgers-Melnick E, et al.(2014) Population genomics of Populus trichocarpa identifies signatures of selection and adaptive trait associations. Nat Genet 46:1089–1096. https://doi.org/10.1038/ng.3075

Fournier T, Saada OA, Hou J, et al.(2019) Extensive impact of low-frequency variants on the phenotypic landscape at population-scale. Elife 8:1–18. https://doi.org/10.7554/eLife.49258

Fournier-Level A, Korte A, Cooper MD, et al.(2011) A map of local adaptation in *Arabidopsis thaliana*. Science 334:86–89. https://doi.org/10.1126/science.1209271

Francis, RM (2017) Pophelper: An R package and web app to analyse and visualize population structure. Mol. Ecol. Resour. 17: 27–32. https://doi.org/10.1111/1755-0998.12509

Frank A, Sperisen C, Howe GT, et al.(2017) Distinct genecological patterns in seedlings of Norway spruce and silver fir from a mountainous landscape. Ecology 98:211–227. https://doi.org/10.1002/ecy.1632

Garfin GM, Eischeid JK, Lenart MT, Cole KL, Ironside K., Cobb NS (2010) Downscaling climate projections in topographically diverse landscapes of the Colorado Plateau in the arid southwestern United States. The Colorado Plateau IV; Shaping Conservation through Science and Management. 22–43.

González-Martínez SC, Krutovsky KV, Neale DB (2006) Forest-tree population genomics and adaptive evolution. New Phytol 170:227–238. https://doi.org/10.1111/j.1469-8137.2006.01686.x

Goto S, Kajiya-Kanegae H, Ishizuka W, et al.(2017) Genetic mapping of local adaptation along the altitudinal gradient in *Abies sachalinensis*. Tree Genet Genomes 13(104) 1:13. https://doi.org/10.1007/s11295-017-1191-3

Haase, DL, Khadduri, N, Mason, E, Dumroese, RK. (2016) Relationships among chilling hours, photoperiod, calendar date, cold hardiness, seed source, and storage of Douglas-Fir seedlings. Tree Planter's Notes 59(1): 52-63

Hall D, Hallingbäck HR, Wu, HX (2016) Estimation of number and size of QTL effects in forest tree traits. https://doi.org/10.1007/s11295-016-1073-0

Hall D, Luquez V, Garcia VM, et al.(2007) Adaptive population differentiation in phenology across a latitudinal gradient in European aspen (*Populus tremula*, L.): A comparison of neutral markers, candidate genes and phenotypic traits. Evolution 61:2849–2860. https://doi.org/10.1111/j.1558-5646.2007.00230.x

Hamann, A, and Wang, T, Spittlehouse, DL, and Murdock, TQ (2013) A comprehensive, high-resolution database of historical and projected climate surfaces for western North America. Bulletin of the American Meteorological Society 94: 1307–1309.

Han E, Sinsheimer JS, Novembre J (2015) Fast and accurate site frequency spectrum estimation from low coverage sequence data. Bioinformatics 31:720–727. https://doi.org/10.1093/bioinformatics/btu725

Hancock AM, Witonsky DB, Alkorta-Aranburu G, et al.(2011) Adaptations to Climate-Mediated Selective Pressures in Humans. PLoS Genet 7:e1001375. https://doi.org/10.1371/journal.pgen.1001375

Holliday JA, Ritland K, Aitken SN (2010) Widespread, ecologically relevant genetic markers developed from association mapping of climate-related traits in Sitka spruce (*Picea sitchensis*). New Phytol 188:501–514. https://doi.org/10.1111/j.1469-8137.2010.03380.x

Howe GT, Aitken SN, Neale DB, et al.(2003) From genotype to phenotype: unraveling the complexities of cold adaptation in forest trees. Can J Bot 81:1247–1266. https://doi.org/10.1139/b03-141

Howe GT, Yu J, Knaus B, Cronn R, Kolpak S, Dolan P, Lorenz WW, Dean JF (2013) A SNP resource for Douglas-fir: de novo transcriptome assembly and SNP detection and validation. BMC Genomics 14: 137

Ingvarsson PK, Street NR (2011) Association genetics of complex traits in plants. New Phytol 189:909–922. https://doi.org/10.1111/j.1469-8137.2010.03593.x

Jankowski A, Wyka TP, Zytkowiak R, et al.(2019) Does climate-related in situ variability of Scots pine (*Pinus sylvestris* L.) needles have a genetic basis? Evidence from common garden experiments. Tree Physiol 39:573–589. https://doi.org/10.1093/treephys/tpy145

Jombart, T (2008) Adegenet: A R package for the multivariate analysis of genetic markers. Bioinformatics, 24: 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Josephs EB, Berg JJ, Ross-Ibarra J, Coop G (2019) Detecting adaptive differentiation in structured populations with genomic data and common gardens. Genetics 211(3): 989-1004 https://doi.org/10.1534/genetics.118.301786

Josephs EB, Stinchcombe JR, Wright SI (2017) What can genome-wide association studies tell us about the evolutionary forces maintaining genetic variation for quantitative traits? New Phytol 214:21–33. https://doi.org/10.1111/nph.14410

Kardos, M, Shafer, ABA. (2018) The peril of gene-targeted conservation. Trends in Ecology and Evolution 33(11):827-839. https://doi.org/10.1016/j.tree.2018.08.011 1

Kaya Z, Adams WT, Campbell RK (1994) Adaptive significance of intermittent shoot growth in Douglas-fir seedlings. Tree Physiol 14:1277–1289. https://doi.org/10.1093/treephys/14.11.1277

Kellermann, JL, van Riper, C. (2015) Detecting mismatches of bird migration stopover and tree phenology in response to changing climate. Oecologia 178, 1227–1238. https://doi.org/10.1007/s00442-015-3293-7

Kozlowski, TT, Kramer, PJ, and Pallardy, SG, (1991). The Physiological Ecology of Woody Plants. Academic Press, San Diego, CA.

Kramer, K, Leinonen, I, Loustau, D (2000). The importance of phenology for the evaluation of impact of climate change on growth of boreal, temperate and Mediterranean forests ecosystems: an overview. Int. J. Biometeorol. 44: 67–75. doi: 10.1007/s004840000066

Lee BH, Henderson DA, Zhu JK (2005) The Arabidopsis cold-responsive transcriptome and its regulation by ICE1. Plant Cell 17:3155–3175. https://doi.org/10.1105/tpc.105.035568

Lind BM, Menon M, Bolte CE, et al.(2018) The genomics of local adaptation in trees: are we out of the woods yet? Tree Genet Genomes 14:29. https://doi.org/10.1007/s11295-017-1224-y

Looney CE, Waring KM (2013) *Pinus strobiformis* (southwestern white pine) stand dynamics, regeneration, and disturbance ecology: A review. For Ecol Manage 287:90–102. https://doi.org/10.1016/J.FORECO.2012.09.020

MacTavish R, Anderson JT (2020) Resource availability alters fitness trade-offs: implications for evolution in stressful environments. Am J Bot 107:308–318. https://doi.org/10.1002/ajb2.1417

McKown, AD, Klápště, J, Guy, RD, El-Kassaby, YA, & Mansfield, SD (2018). Ecological genomics of variation in bud-break phenology and mechanisms of response to climate warming in *Populus trichocarpa*. New Phytologist, 220(1), 300–316. https://doi.org/10.1111/nph.15273

Menon M (2020) Interspecific gene flow potentiates adaptive evolution in a hybrid zone formed between *Pinus strobiformis* and *Pinus flexilis.* Doctoral dissertation, Virginia Commonwealth University

Menon M, Bagley JC, Friedline CJ, et al.(2018) The role of hybridization during ecological divergence of southwestern white pine (*Pinus strobiformis*) and limber pine (*P. flexilis*). Mol Ecol 27:1245–1260. https://doi.org/10.1111/mec.14505

Money, D, Gardner, K, Migicovsky, Z, Schwaninger, H, Zhong, GY, & Myles, S (2015). LinkImpute: Fast and Accurate Genotype Imputation for Nonmodel Organisms. G3 5(11): 2383–2390. https://doi.org/10.1534/g3.115.021667

Moler, ERV. (2020) Climate change studies of Southwestern White Pine: Phenotypic and life history trait responses to temperature manipulations during seed development and seedling growth. Doctoral dissertation, Northern Arizona University

Moran E, Lauder J, Musser C, et al.(2017) The genetics of drought tolerance in conifers. New Phytol 216:1034–1048. https://doi.org/10.1111/nph.14774

Moreno-Letelier A, Piñero D (2009) Phylogeographic structure of *Pinus strobiformis Engelm*. across the Chihuahuan Desert filter-barrier. J Biogeogr 36:121–131. https://doi.org/10.1111/j.1365-2699.2008.02001.x

Mosca E, Eckert AJ, Di Pierro EA, et al.(2012) The geographical and environmental determinants of genetic diversity for four alpine conifers of the European Alps. Mol Ecol 21:5530–5545. https://doi.org/10.1111/mec.12043

Namroud MC, Beaulieu J, Juge N, et al.(2008) Scanning the genome for gene single nucleotide polymorphisms involved in adaptive population differentiation in white spruce. Mol Ecol 17:3599–3613. https://doi.org/10.1111/j.1365-294X.2008.03840.x

Neale, DB, Wegrzyn, JL, Stevens, et al.(2014) Decoding the massive genome of loblolly pine using haploid DNA and novel assembly strategies. Genome Biology, 15(3), R59. https://doi.org/10.1186/gb-2014-15-3-r59

Parchman TL, Gompert Z, Mudge J, et al.(2012) Genome-wide association genetics of an adaptive trait in lodgepole pine. Mol Ecol 21:2991–3005. https://doi.org/10.1111/j.1365-294X.2012.05513.x

Parchman TL, Jahner JP, Uckele KA, et al.(2018) RADseq approaches and applications for forest tree genetics. Tree Genet Genomes 14:39. https://doi.org/10.1007/s11295-018-1251-3

Pardos M, Climent J, Almeida H, Calama R (2014) The role of developmental stage in frost tolerance of *Pinus pinea* L. seedlings and saplings. Ann For Sci 71:551–562. https://doi.org/10.1007/s13595-014-0361-9

Peterson BK, Weber JN, Kay EH, et al.(2012) Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS One 7:. https://doi.org/10.1371/journal.pone.0037135

Pigliucci M. (2001) Phenotypic Plasticity: Beyond Nature and Nurture. Baltimore, MD: The Johns Hopkins Univ. Press

Porter HF, O'Reilly PF (2017) Multivariate simulation framework reveals performance of multi-trait GWAS methods. Sci Rep 7:38837. https://doi.org/10.1038/srep38837

Poulos HM (2014) Tree mortality from a global-change type drought coupled with a short-duration freezing event in a Southwestern piñon-juniper woodland, USA. https://doi.org/10.7287/peerj.preprints.237v1

Pyhäjärvi T, Kujala ST, Savolainen O (2020) 275 years of forestry meets genomics in *Pinus sylvestris*. Evol Appl 13:11–30. https://doi.org/10.1111/eva.12809

Raj, A, Stephens, M, Pritchard, JK (2014) fastSTRUCTURE: Variational Inference of Population Structure in Large SNP Data Sets. Genetics. 197:573–589. https://doi.org/10.1534/genetics.114.164350

Rehfeldt, GE (1988) Ecological genetics of *Pinus contorta* from the Rocky Mountains (USA): a synthesis. Silvae Genet. 37*:*131-135

Rehfeldt, GE Ying, CC, Spittlehouse, DL, and Hamilton, DA Jr. (1999) Genetic responses to climate in *Pinus contorta:* niche breadth, climate change, and reforestation. Ecol Monogr. 69: 375-407

Rosenblad KC, Perret DL, Sax DF (2019) Niche syndromes reveal climate-driven extinction threat to island endemic conifers. Nat Clim Chang 9:627–631. https://doi.org/10.1038/s41558-019-0530-9

Rossi S, Deslauriers A, Griçar J, et al.(2008) Critical temperatures for xylogenesis in conifers of cold climates. Glob Ecol Biogeogr 17:696–707. https://doi.org/10.1111/j.1466-8238.2008.00417.x

Savolainen O, Pyhäjärvi T, Knürr T (2007) Gene Flow and Local Adaptation in Trees. Annu Rev Ecol Evol Syst 38:595–619. https://doi.org/10.1146/annurev.ecolsys.38.091206.095646

Seager R, Ting M, Held I, et al.(2007) Model projections of an imminent transition to a more arid climate in southwestern North America. Science 316:1181–4. https://doi.org/10.1126/science.1139601

Seymour, DK, Chae, E, Grimm, DG, Pizarro, CM, Habring-Muller, A, Vasseur, F, Rakitsch, B, Borgwardt, KM, Koenig, D and Weigel, D (2016) Genetic architecture of nonadditive inheritance in *Arabidopsis thaliana* hybrids. P Natl Acad Sci USA 113, E7317–E7326.

Shirk AJ, Cushman SA, Waring KM, et al.(2018) Southwestern white pine (*Pinus strobiformis*) species distribution models project a large range shift and contraction due to regional climatic changes. For Ecol Manage 411:176–186. https://doi.org/10.1016/j.foreco.2018.01.025

Sillanpää MJ, Pikkuhookana P, Abrahamsson S, et al.(2012) Simultaneous estimation of multiple quantitative trait loci and growth curve parameters through hierarchical Bayesian modeling. Heredity 108:134–146. https://doi.org/10.1038/hdy.2011.56

Skrøppa, T, and Johnsen, Ø (2000) Patterns of adaptive genetic variation in forest tree species: Patterns of adaptive genetic variation in forest tree species: the reproductive environment as an evolutionary force in *Picea abies*. *In* Forest genetics and sustainability. C. Mátyás (eds). Kluwer Academic Publishers, Dordecht. The Netherlands. 49-58. 10.1007/978-94-017-1576-8_6.

Songsomboon K (2018) Genome-Wide Associations with Resistance to Bipolaris Leaf Spot (*Bipolaris oryzae* (Breda de Haan) Shoemaker) in a Northern Switchgrass Population (*Panicum virgatum*) bioRxiv http://dx.doi.org/10/1101/424721

Speed, D, Hemani, G, Johnson, MR, and Balding, DJ (2012). Improved heritability estimation from genome-wide SNPs. American journal of human genetics 91(6): 1011–1021. https://doi.org/10.1016/j.ajhg.2012.10.010

Stevens KA, Wegrzyn JL, Zimin A, et al.(2016) Sequence of the Sugar Pine Megagenome. Genetics 204:1613–1626. https://doi.org/10.1534/genetics.116.193227

Sun J, Kranzler HR, Bi J (2015) Refining multivariate disease phenotypes for high chip heritability. BMC Medical Genomics 8(S3) https://doi.org/10.1186/1755-8794-8-S3-S3

Sunblad, LG and Andersson B (1995) No difference in frost hardiness between high and low altitude *Pinus sylvestris* (L.) offspring. Scand. J. For. Res. 10: 22-26.

Sutinen, M-L, Arora, R, Wisniewski, M, Ashworth, E, Strimbeck, R, Palta, J (2001) Mechanisms of Frost Survival and Freeze-Damage in Nature. *In* F.J. Bigras and S.J. Colombo (eds). Conifer cold hardiness. Kluwer Academic Publ., Dordecht. The Netherlands. 89-120. 10.1007/978-94-015-9650-3_4.

Swidrak I, Gruber A, Kofler W, Oberhuber W (2011) Effects of environmental conditions on onset of xylem growth in Pinus sylvestris under drought. Tree Physiol 31:483–493. https://doi.org/10.1093/treephys/tpr034

Tan, B (2018) Genomic selection and genome- wide association studies to dissect quantitative traits in forest trees. Doctoral dissertation, Umeå

Tan, B, Ingvarsson, PK (2019) Integrating genome-wide association mapping of additive and dominance genetic effects to improve genomic prediction accuracy in *Eucalyptus*. bioRxiv 841049; doi: https://doi.org/10.1101/841049

Vangestel C, Eckert AJ, Wegrzyn JL, et al.(2018) Linking phenotype, genotype and environment to unravel genetic components underlying cold hardiness in coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*). Tree Genet Genomes. https://doi.org/10.1007/s11295-017-1225-x

Wang J, Ding J, Tan B, et al.(2018) A major locus controls local adaptation and adaptive life history variation in a perennial plant. Genome Biol 19:. https://doi.org/10.1186/s13059-018-1444-y

Wittmann MJ, Bergland AO, Feldman MW, et al.(2017) Seasonally fluctuating selection can maintain polymorphism at many loci via segregation lift. Proc Natl Acad Sci U S A 114:E9932–E9941. https://doi.org/10.1073/pnas.1702994114

Williams PA, Allen CD, Macalady AK, et al.(2013) Temperature as a potent driver of regional forest drought stress and tree mortality. Nat Clim Chang 3:292–297. https://doi.org/10.1038/nclimate1693

Williams AP, Cook ER, Smerdon JE, et al.(2020) Erratum: Large contribution from anthropogenic warming to an emerging North American megadrought (American Association for the Advancement of Science. Science 370:314–318. https://doi.org/10.1126/SCIENCE.ABF3676

Yakovlev IA, Fossdal C-G, Johnsen Ø, et al.(2006) Analysis of gene expression during bud burst initiation in Norway spruce via ESTs from subtracted cDNA libraries. Tree Genet Genomes 2:39–52. https://doi.org/10.1007/s11295-005-0031-z

Zhou X, Stephens M (2014) Efficient multivariate linear mixed model algorithms for genome-wide association studies. Nat Methods 11:407–9. https://doi.org/10.1038/nmeth.2848

Zhou, X, Stephens, M (2012) Genome-wide efficient mixed-model analysis for association studies. Nature genetics. 44:821–4.

CHAPTER ONE TABLES AND FIGURES

TABLE 1: Description of traits used in this analysis. Measurements were conducted across all three gardens except for lammas growth and lethal drought survival. Lammas measurements were only taken at the low garden, and lethal drought at the low and high.

Trait Group	Trait	Time	Abbrev.	Description
Survival	Over winter	Fall 2017-Spring 2018	wint17-18	Survival (1) or mortality (0) between the fall and spring.
	Over winter	Fall 2018-Spring 2019	wint8-19	
	Growing season	Spring-Fall 2018	grow18	Survival (1) or mortality (0) between the spring and the fall.
	Growing season	Spring-Fall 2019	grow19	
	Overall	Fall 2019	surv19	Survival (1) or mortality (0) from time of planting to fall 2019.
	Lethal Drought	Spring-Fall 2020	drt20	Survival (1) or mortality (0) during the experimentally imposed lethal drought.
Spring Phenology	Bud Advancement	Spring 2018	budadv18	The difference in bud stage (0-4) between two spring measurement time points.
	Bud Advancement	Spring 2019	budadv19	
	Bud Burst Slope	Spring 2018	budslp18	The slope of the line between the change from first and second bud stage measurements relative to Julian day.
	Bud Burst Slope	Spring 2019	budslp19	
	Bud Burst Intercept	Spring 2018	budint18	The intercept was calculated using the equation of a line ($y=mx+b$) using the slope (m), bud stage (y), and Julian day(x).
	Bud Burst Intercept	Spring 2019	budint19	
Fall Phenology	Bud Variation	Fall 2018	budv18	Score of bud variation during dormancy initiation. Scores of 0-1 indicate active buds. Scores 2-3 indicate dormant/set buds. Needle length relative to full length needles varied within active and dormant buds.
	Bud Variation	Fall 2019	budv19	
	Bud Set	Fall 2018	set18	
	Bud Set	Fall 2019	set19	
Bud Injury	Bud Damage	Spring 2018	bdmgS18	Presence of a damaged bud (0) or normally/fully formed bud (1).
	Bud Damage	Fall 2018	bdmgF18	
	Bud Damage	Spring 2019	bdmgS19	
	Bud Damage	Fall 2019	bdmgF19	
Lammas	Lammas Growth	Fall 2019	lms19	Presence of lammas growth (1) or absence (0) at the end of the growing season.

TABLE 2: Multi-trait groupings for traits measured across time. Models were run for each grouping at each individual garden. N represents the number of individuals with phenotype data available for each analysis.

			Garden		
			Low	Inter.	High
Traits	**Year**	**Abbreviation**		**N**	
Bud set (set18, set19)	2018–19	BudSetAll_MT	190	199	191
Fall bud variation (budv18, budv19)	2018–19	BudVarAll_MT	-	-	191
Slope, intercept (budslp18, budint18)	2018	BudDev18_MT	194	201	193
Slope, intercept (budslp19, budint19)	2019	BudDev19_MT	188	197	188
Slope, intercept (budslp18, budslp19, budint18, budint19)	2018–19	BudDevAll_MT	188	197	187
Slope, intercept, bud advancement (budslp18, budint18, budadv18)	2018	SpringPhen18_MT	194	201	193
Slope, intercept, bud advancement (budslp19, budint19, budadv19)	2019	SpringPhen19_MT	-	197	187
Spring and fall damage (bdmgS18, bdmgF18, bdmgS19, bdmgF19)	2018–19	BudDamageAll_MT	191	199	170
Survival: (wint17-18, grow18, wint18-19, grow19)	2018–19	SeasonalSurvival_MT	*	*	194
Survival: (wint17-18, grow18, wint18-19, grow19, surv19)	2018–19	OverallSurvival_MT	*	199	*
Survival: (wint17-18, grow18, wint18-19, grow19, surv19, drt20)	2018–19	Overall+Drought_MT	189	*	*

* Due to convergence issues multi-trait survival analyses were different at each garden
- Poor model fit, was not included

TABLE 3: Multi-trait groupings for traits associated with seed source climate variables. Models were run for each grouping at each individual garden. N represents the number of individuals with phenotype data available for each analysis. CMD=Climatic moisture deficit, FFP=frost free period, TD=continentality, and Abbrev= the model name.

GARDEN	CMD			FFP			TD		
	Traits	**Abbrev**	**N**	**Traits**	**Abbrev**	**N**	**Traits**	**Abbrev**	**N**
Low	budadv18 budslp18 budint18 wint17-18	CMD_Lo_MT	194	–	–	–	surv19 budint18	TD_Lo_MT	194
Int.	budslp18 budadv18 budint18 budint19 bdmgS18	CMD_Int_MT	197	surv19 set18 bdmgS19 wint18-19	FFP_Int_MT	199	surv19 budint19 bdmgF18	TD_Int_MT	199
High	–	–	–	surv19 budint18 bdmgS18 bdmgF19 wint17-18 grow19	FFP_Hi_MT	188	surv19 set18 set19 bdmgS18	TD_Hi_MT	191

TABLE 4: Univariate genome-wide association analyses results. Univariate genome-wide association analyses (GWAS) were conducted for climate-related adaptive traits using GEMMA (Zhou and Stephens 2013). Separate GWAS were conducted for phenological and fitness related traits at each of the three garden locations. Chip heritability and its standard error are determined in GEMMA using REML estimates. Detected single nucleotide polymorphisms (SNPs) were significantly associated with each trait following a Benjamini-Hochberg false discovery correction. $\bar{x}$ represents the sample mean for both the minor allele frequency (MAF) and the beta values produced in GEMMA (β).

Trait	Garden Site (Elevation)	Measurement Time	h^2_chip	se (h^2)	#SNPs	MAF $\bar{x}$	MAF range	β $\bar{x}$	β range
lms19	Low	Fall 2019	0.999	0.001	34	0.065	0.031-0.128	0.253	0.174-0.405
wint17-18	Low	2017–18	0.353	0.295	15	0.056	0.028-0.121	-0.171	-0.225- -0.115
grow18	Low	2018	0.512	0.169	2	0.062	0.054-0.07	-0.152	-0.162- -0.141
wint18-19	Intermediate	2018–19	0.514	0.158	8	0.047	0.03-0.065	-0.208	-0.24- -0.172
grow18	Intermediate	2018	0.346	0.444	1	0.087		-0.087	
bdmgS18	Intermediate	Spring 2018	1.79×10^{-6}	0.116	5	0.066	0.03-0.124	-0.098	-0.14- -0.064
bdmgF18	Intermediate	Fall 2018	0.518	0.272	1	0.033		-0.115	
bdmgS19	Intermediate	Spring 2019	0.736	0.123	1	0.028		-0.295	
budadv19	High	Spring 2019	1.78×10^{-6}	0.634	3	0.033	0.026-0.044	0.205	0.161-0.247
grow19	High	2019	0.248	0.285	2	0.034	0.031-0.036	-0.095	-0.103- -0.087
surv19	High	Fall 2019	0.237	0.195	1	0.082		0.165	
drt20	High	2020	0.517	0.275	1	0.042		0.137	

TABLE 5: Multi-trait genome-wide association analyses (GWAS) were conducted for trait groupings formed from repeat measures and for traits significantly associated with seed source climate variables. Detected single nucleotide polymorphisms (SNPs) were significantly associated with each trait following a Benjamini-Hochberg false discovery correction. Climate moisture deficit (CMD), Frost Free Period (FFP), and Continentality (TD) were the three climate variables used to determine trait groupings.

Garden Site (Elevation)	Multi-Trait Grouping	Traits Included	#SNPs
Low	Traits Associated with Maternal CMD	budadv18, budslp18, budint18, wint17-18	14
Low	Bud Development 2018	budslp18 and budint18	1
Low	Bud Development 2019	budslp19 and budint19	1
Low	Bud Development All	budslp and budint for 2018 and 2019	1
Low	Spring Phenology 2018	budadv18, budslp18, budint18	10
Intermediate	Spring Phenology 2019	budadv19, budslp19, budint19	15
Intermediate	Traits Associated with Maternal FFP	surv19, set18, and bdmgS19, wint18-19	2
High	Traits Associated with Maternal TD	surv19, set18, set19, and bdmgS18	1

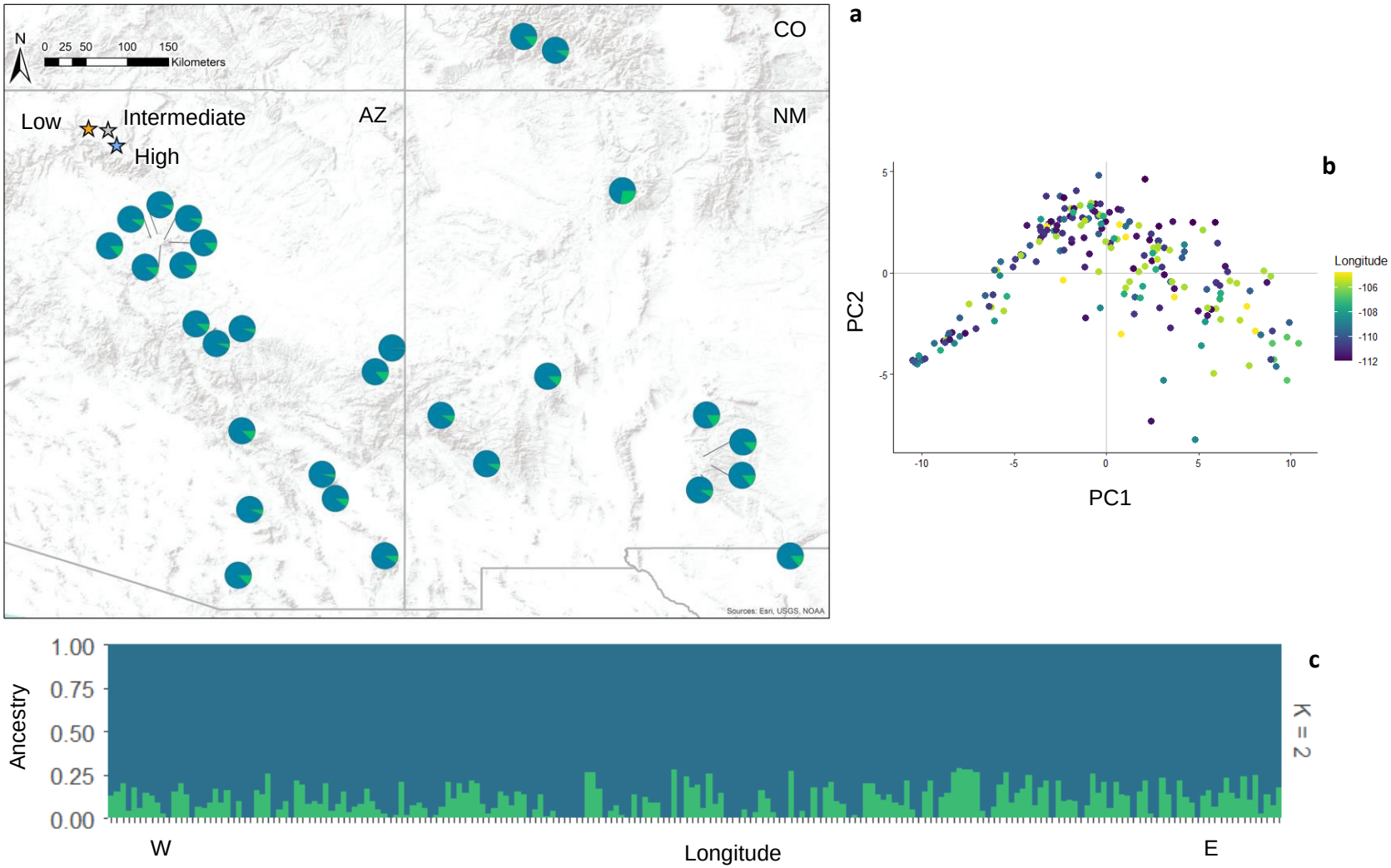

FIGURE 1: Map of sampling areas displaying average ancestry from population structure analyses in fastSTRUCTURE and location of the three common gardens. The location of the three common gardens are in the North Kaibab National Forest and are represented by stars (orange=low elevation, grey=intermediate elevation, and blue=high elevation) (a). Results from principal component analysis colored by longitude of sampled maternal trees (n=202) (b). Structure plot of all sampled maternal trees along a longitudinal gradient as determined from fastSTRUCTURE analysis where K=2 (c).

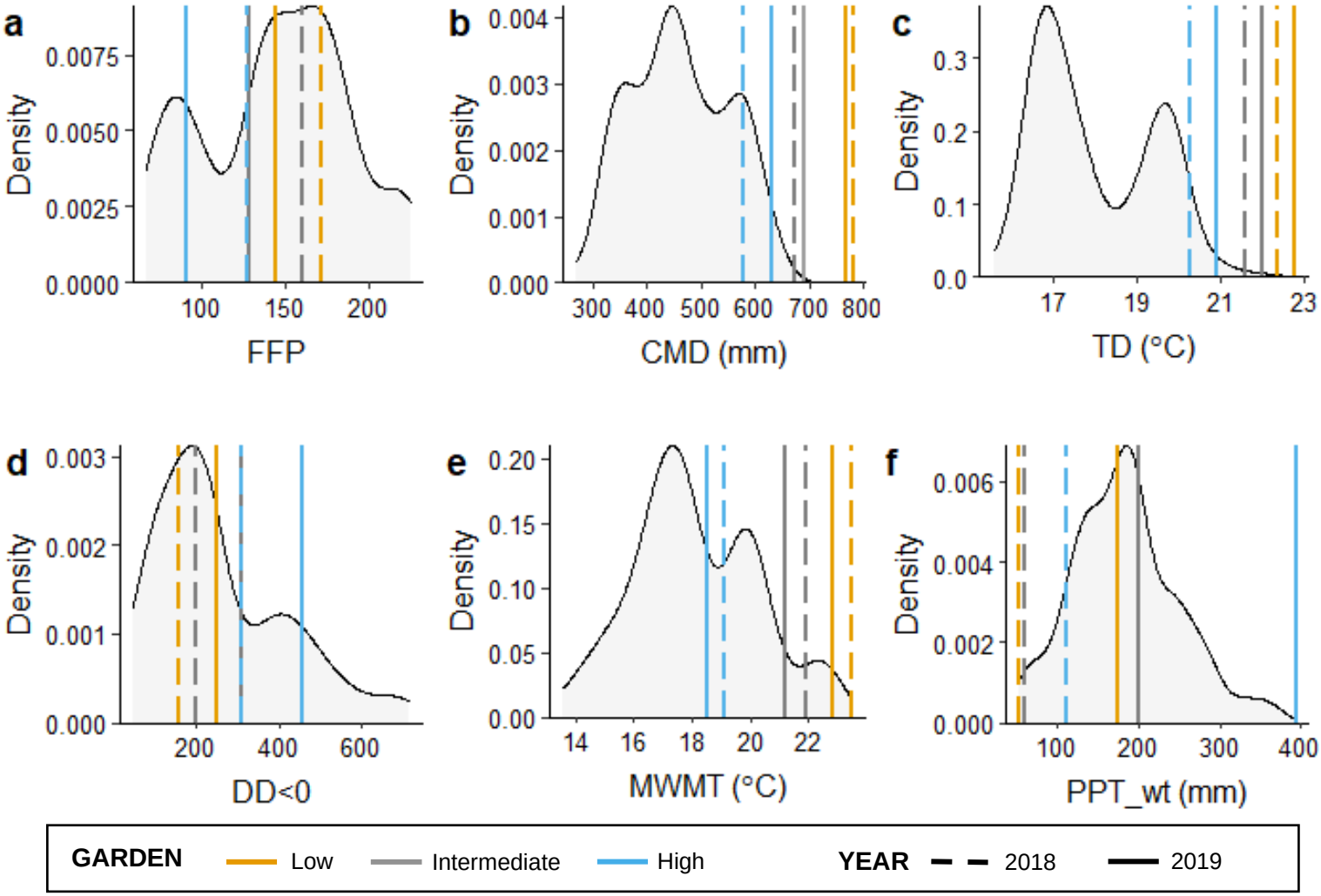

FIGURE 2: Distributions of maternal site (n=202) climate variables relative to garden climate values in 2018 and 2019. The vertical lines represent the 2018 (dashed) and 2019 (solid) climate values for the low (orange), the intermediate (gray), and the high (blue) elevation gardens. Winter precipitation (PPT_wt) for 2018 and 2019 was actually December of the previous year through February of that year. The other climate variables are frost free period (FFP), climate moisture deficit (CMD), continentality (TD), degree days below zero (DD<0), and mean warmest month temperature (MWMT).

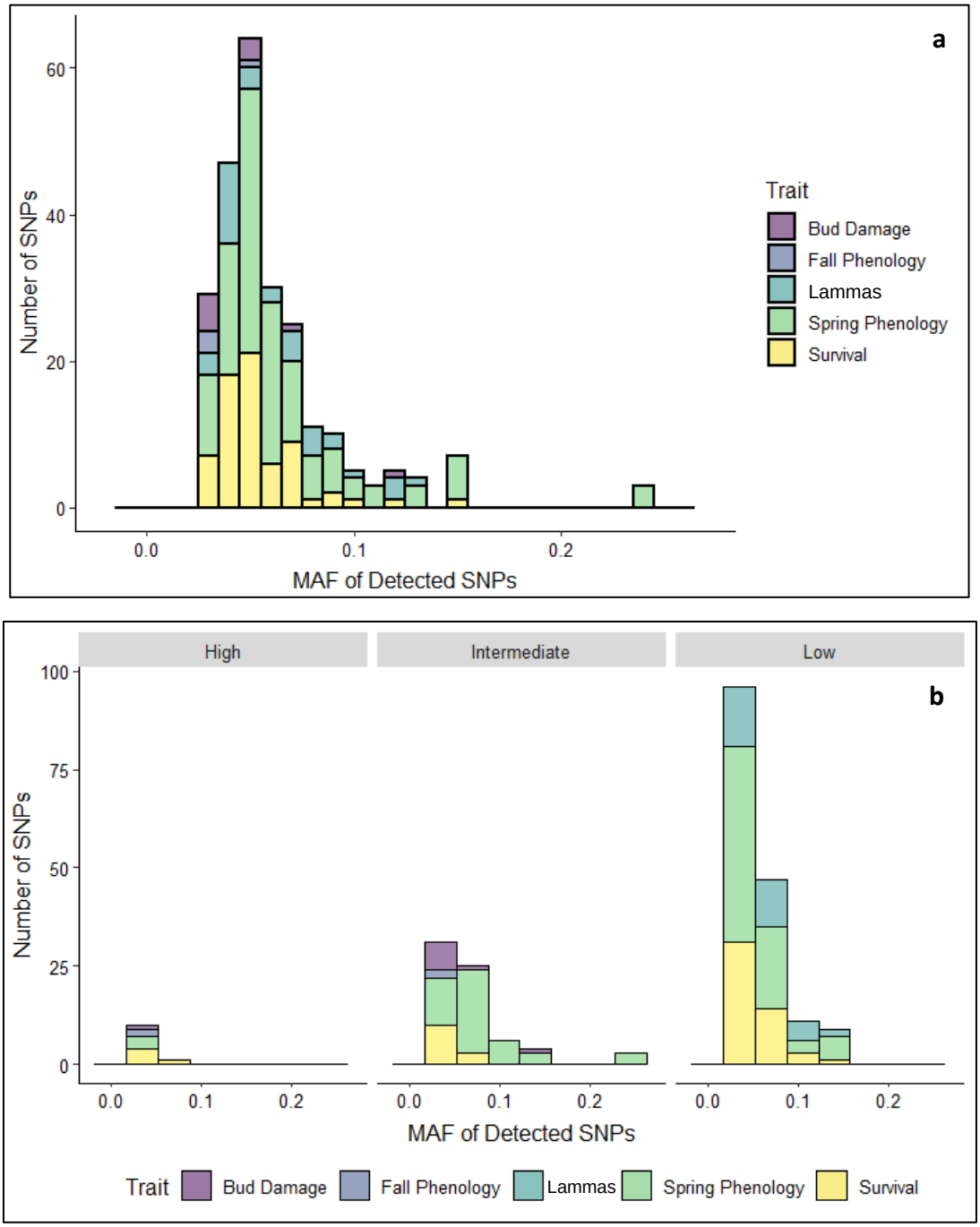

FIGURE 3: Minor allele frequencies for all significantly associated SNPs for phenological traits (Spring Phenology and Fall Phenology), Bud Damage, and all survival metrics (Survival). All minor allele frequencies are displayed together (a) and separated by detections at each garden (b).

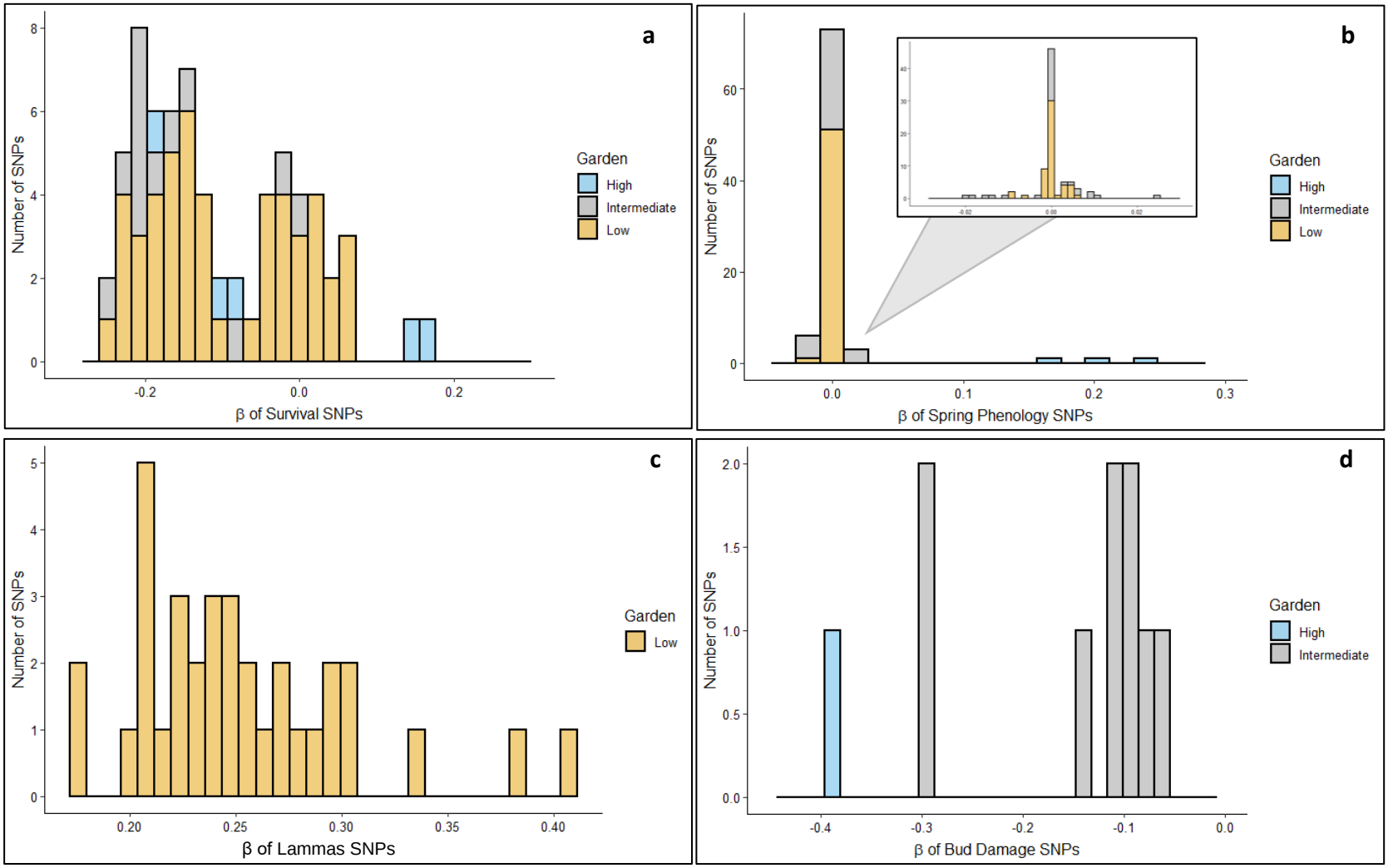

FIGURE 4: SNP effects on traits (β) for *Pinus strobiformis*. This figure contains histograms of β values estimated in GEMMA for trait groups that had a significant SNP detection including Survival (a), Spring Phenology (b), lammas growth (c), and Bud Damage (d). Histograms are colored by garden and fall phenology values were not included because only three SNPs were detected.

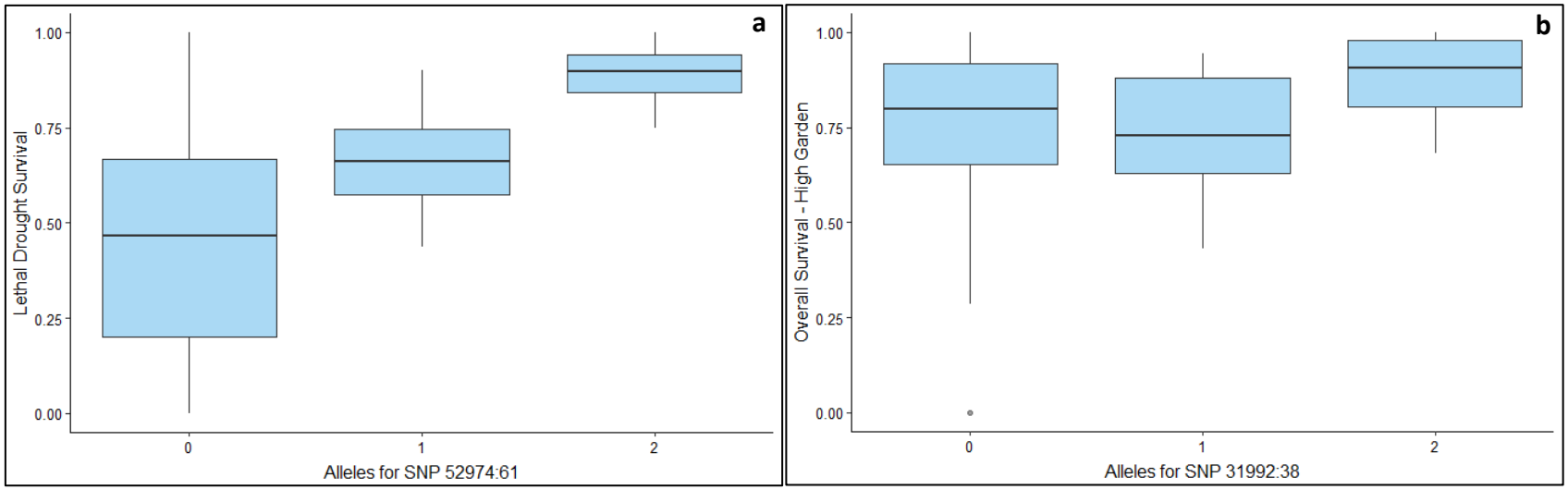

FIGURE 5: Phenotypic effect of the minor allele for two positive effect loci for survival. SNP 52974:61 for survival under lethal drought (drt20) (a) and 31992:38 for overall survival (surv19) (b) at the high elevation garden. The raw phenotypic values that were used to generate the maternal values are on the y axes. The number of minor alleles at each SNP for an individual maternal tree is on the x-axes. Individuals with a 0 are homozygous for the major or more common allele, individuals with a 1 are heterozygous, and individuals with a 2 are homozygous for the minor allele. Contig 31992 matched a nucleotide sequence length in two *Pinus taeda clones* (%ID =86.36, ALR= 44, *e-value* =2x10^{-6}).

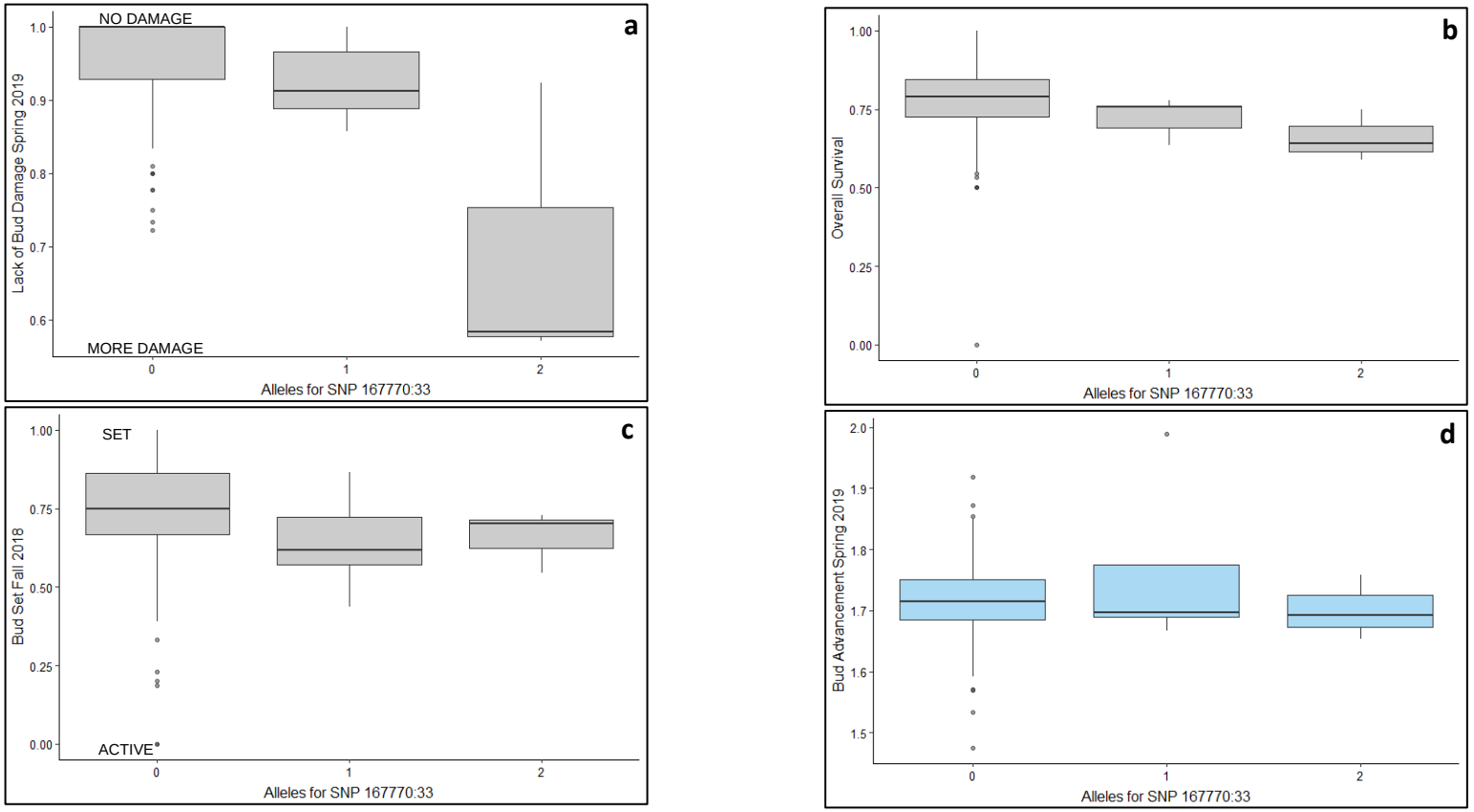

FIGURE 6: Phenotypic effect of the only SNP to be detected at multiple gardens, SNP 167770.33. SNP 167770.33 was detected for four traits at the intermediate garden (grey), winter survival 2018-2019 is not included in the visual. The phenotypic values for the associations at the intermediate garden are lack of bud damage spring 2019 (a) overall survival 2019 (b), and bud set 2018 (c). SNP 167770.33 was associated with spring bud advancement (budadv19) at the high elevation garden (d-blue). The number of minor alleles at each SNP for an individual maternal tree is on the x-axes. Individuals with a 0 are homozygous for the major or more common allele, individuals with a 1 are heterozygous, and individuals with a 2 are homozygous for the minor allele. Contig 167770 matched nucleotide sequences in give *Pinus taeda* clones including accessions: AC241265.2(%ID =88.06, ALR= 67, *e-value* =$3x10^{-17}$), AC241270.1 (%ID =80, ALR= 70, *e-value* =$9x10^{-11}$), AC241326.1 (%ID =74.1, ALR= 58, *e-value* =0.012), AC241281.1(%ID =75.9, ALR= 58, *e-value* =0.042), and JQ017079.1 (%ID =91.67, ALR= 24, *e-value* =0.042).

CHAPTER ONE SUPPLEMENTARY TABLES

TABLE S1: Locations for maternal sites and 30-year climate averages (1980-2010). Climate data was extracted from climateWNA (Haman et al. 2013). Tree ID= unique identifier for each tree at a collection site. Pop=unique identifier for a collection area. LAT=Latitude, LONG=Longitude, MAT=mean annual temperature (˚C), MAP=mean annual precipitation (mm), FFP=frost free period, TD=continentality (difference mean warmest month and mean coldest month temperatures in ˚C), CMD=climate moisture deficit (mm), DD_0=degree days below zero, PPT_wt=winter precipitation (mm), MWMT=mean warmest month temperature (˚C).

Tree ID	Pop	State	LAT	LONG	MAT	MAP	FFP	TD	CMD	DD_0	PPT_wt	MWMT
FLW80	FLW	AZ	31.68961	-110.886	14.5	723	219	16.8	626	45	168	22.9
FLW81	FLW	AZ	31.68985	-110.885	14.5	727	218	16.8	622	46	169	22.8
FLW652	FLW	AZ	31.69277	-110.884	14.4	734	217	16.8	611	47	170	22.7
FLW651	FLW	AZ	31.69295	-110.884	14.4	734	217	16.8	611	47	170	22.8
MAD655	MAD	AZ	31.71221	-110.857	13.9	785	212	16.6	550	52	175	22.2
MAD656	MAD	AZ	31.71221	-110.857	13.9	785	212	16.6	549	52	175	22.2
MAD657	MAD	AZ	31.71287	-110.857	14	781	213	16.6	552	52	174	22.3
MAD654	MAD	AZ	31.71312	-110.858	14	778	213	16.6	553	51	174	22.3
MAD87	MAD	AZ	31.71469	-110.861	14.2	763	216	16.7	570	49	170	22.5
MAD88	MAD	AZ	31.71473	-110.861	14.2	763	216	16.7	570	49	170	22.5
MAD86	MAD	AZ	31.71552	-110.862	14.3	755	217	16.7	580	48	169	22.6
MAD85	MAD	AZ	31.71556	-110.864	14.3	750	218	16.7	584	47	168	22.7
CHI932	CHI	AZ	31.91031	-109.272	10.5	737	145	17.4	557	136	163	19.2
CHI929	CHI	AZ	31.91183	-109.272	10.6	733	146	17.4	561	135	162	19.3
CHI930	CHI	AZ	31.91311	-109.27	10.7	726	148	17.4	573	131	160	19.4
CHI931	CHI	AZ	31.91363	-109.27	10.7	724	148	17.4	573	130	160	19.4

ONI234	ONI	AZ	31.92527	-109.26	11.4	677	156	17.5	640	109	147	20.2
ONI233	ONI	AZ	31.92616	-109.26	11.4	676	157	17.5	643	109	147	20.2
ONI232	ONI	AZ	31.92618	-109.261	11.4	677	156	17.5	641	109	148	20.2
SBI247	SBI	AZ	32.41306	-110.72	11.3	789	186	16.5	449	105	215	19.7
SBI246	SBI	AZ	32.41366	-110.719	11.3	787	186	16.6	453	103	214	19.8
SBI249	SBI	AZ	32.41392	-110.72	11.3	788	186	16.6	453	103	215	19.8
SBI245	SBI	AZ	32.41399	-110.719	11.3	786	187	16.6	454	103	214	19.8
SBI248	SBI	AZ	32.41431	-110.722	11.3	789	186	16.6	454	103	216	19.8
SPE237	SPE	AZ	32.41711	-110.74	11.1	806	184	16.5	449	108	224	19.6
SPE239	SPE	AZ	32.41753	-110.738	11.2	803	185	16.5	451	106	223	19.7
SPE236	SPE	AZ	32.41789	-110.74	11.2	806	185	16.5	450	107	224	19.7
SPE235	SPE	AZ	32.41914	-110.74	11.2	805	185	16.5	454	105	225	19.7
SCR244	SCR	AZ	32.44769	-110.785	11.5	831	192	16.2	454	93	246	20
SCR243	SCR	AZ	32.4479	-110.785	11.5	831	192	16.2	454	93	246	20
SCR242	SCR	AZ	32.44829	-110.784	11.6	831	192	16.2	454	93	247	20
TKY659	TKY	AZ	32.63181	-109.815	11.5	629	180	17.6	582	105	159	20.6
TKY658	TKY	AZ	32.63197	-109.815	11.5	627	180	17.7	587	104	159	20.6
TKY93	TKY	AZ	32.633	-109.815	11.5	630	180	17.6	583	104	160	20.6
TKY94	TKY	AZ	32.63333	-109.815	11.5	629	181	17.7	585	104	160	20.7
TKY95	TKY	AZ	32.6335	-109.815	11.5	630	181	17.7	584	104	160	20.7
SHA72	SHA	AZ	32.66052	-109.863	8.8	841	155	16.2	340	184	212	17.3
SHA76	SHA	AZ	32.66716	-109.864	8.5	858	153	16.1	329	194	217	17.1

GRA892	GRA	AZ	32.69621	-109.884	7.3	941	139	15.6	277	256	236	15.7
GRA98	GRA	AZ	32.69712	-109.88	7.1	953	137	15.6	270	266	239	15.5
RIG291	RIG	AZ	32.70448	-109.966	10.2	630	176	16.6	472	133	154	18.9
RIG293	RIG	AZ	32.70458	-109.966	10.2	630	176	16.6	470	134	154	18.8
RIG294	RIG	AZ	32.70465	-109.965	10.2	632	176	16.5	469	135	154	18.8
RIG295	RIG	AZ	32.70466	-109.965	10.2	632	176	16.5	469	135	154	18.8
RIG662	RIG	AZ	32.70476	-109.967	10.3	626	177	16.6	478	131	153	18.9
WEB46	WEB	AZ	32.71086	-109.925	8.8	832	157	16.2	339	186	208	17.3
WEB47	WEB	AZ	32.71261	-109.925	8.9	823	159	16.3	345	180	206	17.5
WEB49	WEB	AZ	32.71317	-109.924	8.9	828	159	16.3	345	180	208	17.5
WEB50	WEB	AZ	32.71404	-109.924	9	822	160	16.3	348	176	207	17.7
GLO928	GLO	AZ	33.28316	-110.829	12.2	725	188	18.7	618	98	250	21.9
GLO925	GLO	AZ	33.28372	-110.826	12.2	720	188	18.7	619	97	247	22
GLO927	GLO	AZ	33.28397	-110.827	12.1	722	188	18.7	617	98	248	21.9
GLO926	GLO	AZ	33.28431	-110.828	12.1	724	187	18.7	616	99	249	21.9
IST951	IST	AZ	33.87008	-109.4	5.6	711	78	17.7	432	443	200	14.9
IST952	IST	AZ	33.87091	-109.4	5.6	711	78	17.7	432	443	200	14.9
IST955	IST	AZ	33.87092	-109.398	5.6	711	78	17.7	432	442	200	14.9
IST953	IST	AZ	33.87142	-109.399	5.6	711	78	17.7	432	443	200	14.9
RUD99	RUD	AZ	33.98016	-109.364	5.2	724	74	17.6	383	488	190	14.4
RUD17	RUD	AZ	33.98061	-109.367	5.2	724	74	17.6	384	488	190	14.4
RUD16	RUD	AZ	33.98074	-109.369	5.2	724	74	17.6	384	488	190	14.4

RUD98	RUD	AZ	33.98084	-109.364	5.2	724	74	17.6	383	488	189	14.4
RUD82	RUD	AZ	33.98107	-109.366	5.2	724	74	17.6	383	489	189	14.4
MDW93	MDW	AZ	34.36896	-111.008	9.4	919	170	19.6	408	207	329	19.8
MDW94	MDW	AZ	34.36921	-111.006	9.4	919	170	19.6	406	208	330	19.8
MDW95	MDW	AZ	34.37	-111.007	9.4	919	170	19.6	405	209	330	19.8
MDW97	MDW	AZ	34.37086	-111.007	9.4	917	169	19.6	405	209	328	19.8
RAN83	RAN	AZ	34.39825	-110.999	9.1	855	160	19.7	442	227	294	19.6
RAN90	RAN	AZ	34.39889	-111	9.1	855	160	19.7	442	227	294	19.6
RAN88	RAN	AZ	34.3994	-110.999	9.1	853	159	19.8	442	227	293	19.6
RAN89	RAN	AZ	34.40001	-110.999	9.1	852	159	19.8	442	228	292	19.6
KNO66	KNO	AZ	34.40705	-111.113	9.5	894	167	19.7	388	203	280	20
KNO61	KNO	AZ	34.40812	-111.115	9.5	896	167	19.7	382	205	280	19.9
KNO63	KNO	AZ	34.40816	-111.114	9.5	895	167	19.7	382	205	280	19.9
MOG5	MOG	AZ	34.45029	-111.242	9.8	761	163	19.7	528	188	272	20.3
MOG4	MOG	AZ	34.45046	-111.243	9.8	760	163	19.7	530	188	272	20.3
MOG8	MOG	AZ	34.45095	-111.243	9.8	761	163	19.7	527	189	272	20.3
MOG6	MOG	AZ	34.452	-111.243	9.8	760	164	19.7	527	191	272	20.3
MOG14	MOG	AZ	34.45316	-111.243	9.8	759	164	19.7	525	192	272	20.3
ELD89	ELD	AZ	35.24726	-111.636	7.1	606	99	20.1	575	368	186	17.9
ELD90	ELD	AZ	35.24734	-111.636	7.1	606	99	20.1	575	368	186	17.9
ELD92	ELD	AZ	35.24758	-111.637	7.1	606	99	20.1	576	368	186	17.9
ELD91	ELD	AZ	35.24764	-111.636	7.1	607	99	20.1	574	369	186	17.9

ELD86	ELD	AZ	35.25233	-111.63	6.9	627	97	20	556	383	197	17.6
ELD267	ELD	AZ	35.25348	-111.632	6.9	628	97	20	556	384	197	17.6
OLD15	OLD	AZ	35.25486	-111.605	7.2	600	101	20.1	584	359	183	18
UEL641	UEL	AZ	35.26251	-111.605	7.2	607	100	20.1	579	365	187	17.9
UEL640	UEL	AZ	35.26328	-111.606	7.1	609	100	20	577	367	188	17.9
UEL638	UEL	AZ	35.26414	-111.606	7.1	611	100	20	575	369	189	17.8
UEL637	UEL	AZ	35.27453	-111.607	7	622	98	20	564	378	195	17.7
KAC170	KAC	AZ	35.31561	-111.707	4.9	773	76	19.3	411	568	257	15.2
KMD975	KMD	AZ	35.32323	-111.715	4.8	779	75	19.2	399	577	257	15
KMD592	KMD	AZ	35.32335	-111.714	4.7	784	75	19.1	395	582	259	14.9
SIS971	SIS	AZ	35.34121	-112.01	7.4	598	98	19.7	600	337	182	17.9
SIS967	SIS	AZ	35.34136	-112.008	7.3	600	97	19.7	597	341	183	17.9
SIS968	SIS	AZ	35.34136	-112.008	7.3	600	97	19.7	597	341	183	17.9
SIS969	SIS	AZ	35.34136	-112.008	7.3	600	97	19.7	597	341	183	17.9
SIS970	SIS	AZ	35.34145	-112.009	7.3	599	97	19.7	599	340	182	17.9
WLR987	WLR	AZ	35.35092	-111.62	5.3	767	82	19.7	451	538	281	15.7
WLR990	WLR	AZ	35.35132	-111.622	5.2	777	81	19.6	445	547	286	15.6
WLR989	WLR	AZ	35.35241	-111.622	5.2	777	82	19.6	445	547	286	15.6
WLR991	WLR	AZ	35.35677	-111.612	5.9	694	89	19.8	501	473	242	16.5
WLR988	WLR	AZ	35.35762	-111.613	5.9	697	88	19.8	500	476	244	16.4
BIS9	BIS	AZ	35.36298	-111.74	5.9	680	85	19	476	440	217	16.1
ABI159	ABI	AZ	35.36674	-111.673	3.6	934	67	18.9	322	717	363	13.6

ABI160	ABI	AZ	35.36822	-111.673	3.6	933	67	19	324	715	363	13.7
ABI158	ABI	AZ	35.36914	-111.673	3.7	926	68	19	329	706	359	13.7
ABI156	ABI	AZ	35.36979	-111.674	3.7	922	68	19	330	701	357	13.8
ATH3	ATH	AZ	35.3867	-111.675	5.2	769	81	19.4	434	535	273	15.5
KEL506	KEL	AZ	35.39733	-111.856	6.3	623	87	19.5	536	420	191	16.8
KEL508	KEL	AZ	35.39739	-111.856	6.3	623	87	19.5	536	419	191	16.8
KEL507	KEL	AZ	35.39746	-111.856	6.3	623	87	19.5	536	420	192	16.8
KEH502	KEH	AZ	35.40262	-111.85	6.1	633	85	19.4	516	434	195	16.5
KEN84	KEN	AZ	35.40262	-111.85	6.1	633	85	19.4	516	434	195	16.5
KEH501	KEH	AZ	35.40275	-111.851	6.2	633	86	19.4	516	433	195	16.6
KEN88	KEN	AZ	35.4029	-111.851	6.2	633	86	19.4	516	433	195	16.6
KEH503	KEH	AZ	35.40301	-111.85	6.1	634	85	19.3	516	434	195	16.5
KEH504	KEH	AZ	35.40354	-111.851	6.1	634	86	19.3	516	433	195	16.5
KEN87	KEN	AZ	35.40378	-111.851	6.1	635	86	19.3	515	433	195	16.5
VAL940	VAL	CO	37.42142	-107.537	5.6	698	90	22	433	593	166	17
VAL936	VAL	CO	37.47547	-107.549	4.4	764	74	20.2	341	663	188	15.2
VAL937	VAL	CO	37.47869	-107.549	4.4	768	73	20.1	337	666	189	15.1
VAL938	VAL	CO	37.47888	-107.545	4.6	759	75	20.3	351	651	187	15.4
VAL939	VAL	CO	37.47904	-107.545	4.5	759	75	20.3	351	652	187	15.3
SJM934	SJM	CO	37.51736	-107.735	6.2	721	115	21.1	370	497	176	17.3
SJM935	SJM	CO	37.51753	-107.735	6.2	721	115	21	370	497	176	17.3
SJM933	SJM	CO	37.51756	-107.735	6.2	721	115	21	370	497	176	17.3

SAC648	SAC	NM	32.7097	-105.742	9.7	685	166	17.3	431	174	123	18.2
SAC260	SAC	NM	32.71043	-105.744	9.7	685	167	17.3	431	174	123	18.2
SAC261	SAC	NM	32.71074	-105.742	9.6	687	166	17.3	425	176	123	18.1
SAC262	SAC	NM	32.71124	-105.741	9.6	689	166	17.2	425	177	124	18.1
SAC263	SAC	NM	32.71125	-105.741	9.6	689	166	17.2	425	177	124	18.1
SUN204	SUN	NM	32.7794	-105.814	9.7	714	177	16.8	378	166	134	17.9
SUN13	SUN	NM	32.78002	-105.814	9.7	715	176	16.8	377	167	134	17.8
SUN3	SUN	NM	32.78021	-105.813	9.7	717	176	16.8	375	168	134	17.8
SUN645	SUN	NM	32.78025	-105.812	9.6	720	176	16.7	371	170	135	17.8
SUN21	SUN	NM	32.7806	-105.814	9.7	715	177	16.8	378	167	134	17.9
SUN644	SUN	NM	32.78116	-105.814	9.7	715	177	16.8	378	167	134	17.9
SUN4	SUN	NM	32.78127	-105.813	9.6	719	176	16.8	372	169	135	17.8
PEA137	PEA	NM	32.92119	-108.142	10.4	708	170	18	494	147	186	19.8
PEA135	PEA	NM	32.92163	-108.143	10.4	708	170	18	494	147	186	19.7
PEA136	PEA	NM	32.92293	-108.142	10.5	705	170	18	498	146	185	19.8
SIG134	SIG	NM	32.92568	-108.165	10.2	725	168	18	473	156	191	19.5
SIG133	SIG	NM	32.92581	-108.165	10.2	725	168	18	474	156	191	19.5
SIG131	SIG	NM	32.92665	-108.165	10.2	723	168	18	476	155	190	19.5
SIX642	SIX	NM	32.97108	-105.583	8.9	629	141	16.9	506	198	101	17.4
SIX285	SIX	NM	32.97166	-105.583	8.9	629	141	16.9	506	198	102	17.4
SIX43	SIX	NM	32.97209	-105.583	8.9	629	141	16.9	506	198	101	17.4
SIX286	SIX	NM	32.97292	-105.583	8.9	629	141	16.9	506	197	101	17.4

SIX696	SIX	NM	32.97292	-105.583	8.9	629	141	16.9	506	197	101	17.4
SIX287	SIX	NM	32.97295	-105.582	8.9	628	141	16.9	507	197	101	17.4
BRA201	BRA	NM	32.97762	-105.717	7.8	724	138	16.7	340	255	137	16.1
BRA23	BRA	NM	32.9779	-105.72	7.9	723	139	16.7	343	253	137	16.1
BRA53	BRA	NM	32.97814	-105.712	7.8	725	137	16.7	337	258	137	16
BRA21	BRA	NM	32.97821	-105.712	7.8	725	137	16.7	337	258	137	16
BRA56	BRA	NM	32.97826	-105.711	7.8	725	137	16.6	337	258	137	16
BRA10	BRA	NM	32.97828	-105.721	7.9	723	139	16.7	344	252	137	16.2
BRA26	BRA	NM	32.97838	-105.72	7.9	723	138	16.7	342	253	137	16.1
BRA8	BRA	NM	32.97852	-105.721	7.9	723	139	16.7	344	253	137	16.2
BRA7	BRA	NM	32.97852	-105.721	7.9	723	139	16.7	344	253	137	16.1
POT628	POT	NM	33.02134	-105.755	8.4	704	146	16.8	372	227	137	16.7
POT630	POT	NM	33.0256	-105.744	8.3	708	144	16.8	367	233	137	16.6
POT631	POT	NM	33.02582	-105.743	8.3	708	144	16.8	367	233	137	16.6
POT629	POT	NM	33.02593	-105.742	8.2	707	144	16.8	366	233	137	16.6
MUS127	MUS	NM	33.04988	-105.647	8.5	666	136	16.9	443	220	112	16.9
MUS129	MUS	NM	33.05	-105.645	8.5	665	136	16.9	447	220	112	16.9
MUS126	MUS	NM	33.0503	-105.647	8.5	667	136	16.9	441	220	113	16.9
MUS125	MUS	NM	33.05058	-105.648	8.5	667	137	16.9	441	221	113	16.9
MUS128	MUS	NM	33.05124	-105.646	8.5	666	136	16.9	443	220	112	16.9
COO224	COO	NM	33.05823	-105.634	8.6	656	135	16.9	469	214	108	17.1
COO220	COO	NM	33.05889	-105.635	8.6	657	135	16.9	467	214	108	17.1

COO221	COO	NM	33.0589	-105.634	8.6	656	135	16.9	469	214	108	17.1
COO222	COO	NM	33.05894	-105.633	8.6	656	135	16.9	469	214	108	17.1
COO223	COO	NM	33.05959	-105.634	8.6	657	135	16.9	468	214	108	17.1
FRO145	FRO	NM	33.45021	-108.657	7.9	898	138	17.5	321	269	244	16.9
FRO144	FRO	NM	33.45032	-108.657	7.9	898	138	17.5	321	269	244	16.9
FRO142	FRO	NM	33.45061	-108.654	7.9	896	138	17.5	323	269	243	16.9
FRO143	FRO	NM	33.4507	-108.656	7.9	898	138	17.5	321	269	244	16.9
FRO141	FRO	NM	33.45087	-108.655	7.9	897	138	17.5	321	269	244	16.9
BEA140	BEA	NM	33.45392	-108.642	7.9	890	137	17.6	332	268	241	16.9
BEA138	BEA	NM	33.45448	-108.643	7.9	889	137	17.6	333	268	241	17
BEA139	BEA	NM	33.45514	-108.643	7.9	887	137	17.6	333	268	240	17
BON626	BON	NM	33.45574	-105.751	9	704	144	17	427	199	125	17.5
BON625	BON	NM	33.45586	-105.751	9	704	144	17	429	198	125	17.5
BON627	BON	NM	33.45625	-105.751	9	703	144	17	429	199	125	17.5
WIT739	WIT	NM	33.8784	-107.485	9.3	466	169	18.5	572	215	57	18.5
WIT977	WIT	NM	33.87964	-107.487	9.3	465	169	18.5	569	216	57	18.5
WIT87	WIT	NM	33.87998	-107.486	9.3	465	169	18.5	573	215	57	18.5
WIT96	WIT	NM	33.88003	-107.486	9.3	465	169	18.5	573	215	57	18.5
WIT976	WIT	NM	33.88023	-107.486	9.3	465	169	18.5	572	215	57	18.5
ROR980	ROR	NM	35.90357	-106.67	7.1	598	128	19.8	451	385	134	17.5
ROR979	ROR	NM	35.90379	-106.669	7.1	599	128	19.7	450	384	135	17.5
ROR981	ROR	NM	35.90379	-106.669	7.1	599	128	19.7	450	384	135	17.5

ROR982	ROR	NM	35.90405	-106.669	7.1	599	128	19.8	451	385	134	17.5
ROR983	ROR	NM	35.90421	-106.668	7.1	600	128	19.7	450	385	135	17.5
BCY956	BCY	TX	31.91762	-104.827	12.4	586	223	17.1	474	87	69	20.6
BCY962	BCY	TX	31.91695	-104.824	12.6	577	224	17.2	497	83	68	20.8
BCY963	BCY	TX	31.91574	-104.825	12.5	578	224	17.1	494	84	68	20.8
GUMO958	GUMO	TX	31.91661	-104.843	12	609	222	16.9	423	94	72	20.1
GUMO960	GUMO	TX	31.91734	-104.844	12	609	222	16.9	420	95	72	20.1
GUMO961	GUMO	TX	31.91794	-104.843	12	609	222	16.9	419	95	72	20.1
GUMO959	GUMO	TX	31.91818	-104.842	12	609	222	16.9	418	95	72	20.1
GUMO957	GUMO	TX	31.91841	-104.841	12	609	222	16.9	418	95	72	20.1

TABLE S2: Garden and maternal site climate summaries. The Range and Mean for select 30-year climate averages (1980-2010) across all 202 Maternal Sites are listed. The 30 year-climate averages (1980-2010) and the climate variable measurements for the two years of our study, 2018 and 2019, are listed for each of the three gardens along with their elevation (m).

Climate Variable	Maternal Site		Low Garden (2057m)			Intermediate Garden (2276m)			High Garden (2688m)		
	Range	Mean	30yr.	2018	2019	30 yr.	2018	2019	30yr.	2018	2019
MAP (mm)	465:953	717	416	379	476	498	445	570	688	569	844
MAT (˚C)	3.6:14.5	8.8	10.3	11.4	10	9.1	10.2	8.7	6.8	7.9	6.5
FFP	67:224	224	157	171	144	143	160	129	106	127	91
TD (˚C)	15.6:22	18	21.6	22.4	22.8	20.8	21.6	22	19.6	20.3	20.9
CMD (mm)	270:643	462	727	781	768	610	674	692	514	578	633
DD_0 (days)	45:717	24	189	155	250	238	197	309	375	310	458
PPT_wt (mm)	57:363	183	109	52	157	127	59	180	249	108	369
MWMT (˚C)	13.6:22.9	18.1	22.1	23.5	22.8	20.5	21.9	21.2	17.8	19.1	18.5

TABLE S3: Climate transfer distances from maternal site to the three gardens. Transfer distance was calculated using the following equation: *maternal climate value - garden climate value*. At each garden the average transfer distance (Mean) and the minimum and maximum (Min:Max) transfer distances for all 202 maternal sites for each garden climate are reported. The percent negative (% neg) at each garden is the percentage of maternal families whose maternal climate value is less than the gardens. For example, at the low garden 0% of the maternal sites receive less MAP (mean annual precipitation), and 100% of families have lower MWMT (mean warmest month temperature).

Climate Variable	Low Garden (2057m)			Intermediate Garden (2276m)			High Garden (2688m)		
	Mean	**Min:Max**	**% neg**	**Mean**	**Min:Max**	**% neg**	**Mean**	**Min:Max**	**% neg**
MAP (mm)	300.8	+49:+537	0%	218.8	-33:+455	2%	28.8	-223:+265	38%
MAT (˚C)	-1.47	-6.7:+4.2	75%	-0.27	-5.5:+5.4	56%	2.03	-3.2:+7.7	20%
FFP	-12.9	-90:+67	56%	1.12	-76:+81	48%	38.12	-39:+118	27%
TD (˚C)	-3.6	-6:+0.4	99.5%	-2.8	-5.2:+1.2	98%	-1.6	-4:+2.4	78%
CMD (mm)	-265	-457:-84	100%	-148	-340:+33	95%	-52	-244:+129	68%
DD_0 (days)	69.9	-144:+528	39%	20.9	-193:+479	60%	-116.1	-330:+342	76%
PPT_wt (mm)	74.2	-52:+254	12%	56.2	-70:+236	18%	-65.8	-192:+114	86%
MWMT (˚C)	-3.98	-8.5:+0.8	94%	-2.38	-6.9:+2.4	88%	0.32	-4.2:+5.1	49%

TABLE S4: Temperature and precipitation values at the gardens for overwinter and growing seasons from 2017-2019. the three gardens during the first winter (Winter17-18) from November 2017-February 2018, second winter (Winter18-19) from November 2018-February 2019, first growing season (Growing18) in 2018, and second growing season (Growing19) in 2019. The monthly average (Temp-avg), minimum (Temp-min-avg), and maximum (Temp-max-avg) are reported for all three gardens during each time period. The overall maximum (Temp-max), and overall minimum temperatures (Temp-min) during each period are included. The monthly average precipitation (Precip-avg) and total precipitation (Precip. Total) during each time period are reported.

Climate Variable	Low Garden (2057m)				Intermediate Garden (2276m)				High Garden (2688m)			
	Winter 17-18	Winter 18-19	Growing 18	Growing 19	Winter 17-18	Winter 18-19	Growing 18	Growing 19	Winter 17-18	Winter 18-19	Growing 18	Growing 19
Temp-avg (˚C)	4.4	1.1	186	16.9	3.45	0.3	17.1	15.3	1.7	-1.5	14.5	12.8
Temp-min-avg (˚C)	-2.8	-5	10.7	9.4	-3.5	-5.7	9.4	8	-5.3	-7.4	6.8	5.4
Temp-min (˚C)	-5.2	-7.6	2.9	2.5	-6.2	-8.5	1.8	1.4	-8	-10.4	-0.4	-0.8
Temp-max-avg (˚C)	11.4	7.1	26.4	24.3	10.4	6.2	24.8	22.7	8.6	4.4	22.4	20.3
Temp-max (˚C)	15.8	11.6	31.1	30.8	14.6	10.5	29.5	29.1	12.8	8.3	26.7	26.5
Precip.-avg (mm)	13.2	43.5	29.8	24.8	15	50	34.8	31.2	27.8	98.8	36.8	32.3
Precip. Total (mm)	53	174	179	149	60	200	209	187	111	395	221	194

TABLE S5: Maternal value relationships with climate variables for multi-trait grouping determination. The table includes results from generalized linear mixed effects models run in *glmmTMB* for maternal climate variables (Climate Var) and a maternal value (Trait). Only the traits with a significant relationship are included in the table below. The following model parameters were included, the estimate of the slope (Estimate), the standard error of the estimate (SE) and the p-value.

Garden	Climate Var	Trait Group	Trait	Estimate	SE	*p*-value
Low	CMD	Spring Bud Advancement	budadv18	-3.35E-05	1.38E-05	0.015
Low	CMD	Spring Int	budint18	5.12E-05	2.54E-05	0.043
Low	CMD	Spring Slope	budslp18	-7.56E-07	3.36E-07	0.024
Low	CMD	Survival	wint17-18	-3.00E-04	1.50E-04	0.034
Int	CMD	Bud Damage	bdmgS18	1.49E-04	8.57E-05	0.082
Int	CMD	Spring Bud Advancement	budadv18	-1.00E-04	4.28E-05	0.019
Int	CMD	Spring Int	budint18	2.28E-04	1.04E-04	0.028
Int	CMD	Spring Int	budint19	-1.72E-04	9.21E-05	0.062
Int	CMD	Spring Slope	budslp18	-3.05E-06	1.35E-06	0.024
Low	FFP	Bud Damage[n*]	bdmgS19	-4.45E-04	1.30E-04	0.001
Low	FFP	Lammas [n*]	lms19	-0.002	4.85E-04	2.48E-04
Low	FFP	Survival [n*]	grow19	-0.001	0.001	0.093
Low	FFP	Survival [n*]	wint18-19	-4.81E-04	2.44E-04	0.048
Int	FFP	Bud Damage [n*]	bdmgF18	-3.42E-04	1.66E-04	0.040

Int	FFP	Bud Damage	bdmgS19	-0.001	3.58E-04	0.005
Int	FFP	Bud Set	set18	-3.54E-04	9.88E-05	3.32E-04
Int	FFP	Spring Slope [n*]	budslp18	-6.28E-06	2.82E-06	0.026
Int	FFP	Survival	surv19	-1.84E-04	7.27E-05	0.017
Int	FFP	Survival	wint18-19	-0.001	3.49E-04	0.035
High	FFP	Bud Damage	bdmgF18	-0.002	3.64E-04	7.22E-10
High	FFP	Bud Damage	bdmgF19	-0.002	0.001	1.34E-05
High	FFP	Bud Damage	bdmgS18	-0.007	0.001	1.18E-10
High	FFP	Bud Damage	bdmgS19	-0.002	4.61E-04	2.48E-04
High	FFP	Spring Bud Advancement	budadv18	1.84E-04	1.05E-04	0.080
High	FFP	Spring Bud Advancement	budadv19	3.21E-04	7.71E-05	3.15E-05
High	FFP	Spring Int	budint18	-0.001	0.001	0.030
High	FFP	Spring Int [n*]	budint19	-0.003	0.001	0.000
High	FFP	Spring Slope [n*]	budslp18	6.40E-06	3.65E-06	0.079
High	FFP	Spring Slope	budslp19	1.57E-05	3.78E-06	3.19E-05
High	FFP	Survival	grow19	-2.69E-04	1.26E-04	0.032
High	FFP	Survival	surv19	-0.002	3.98E-04	1.24E-05
High	FFP	Survival	wint17-18	-0.003	0.001	1.19E-05

Low	TD	Spring Int	budint18	0.003	0.002	0.036
Low	TD	Survival	wint18-19	0.014	0.008	0.054
Int	TD	Bud Damage	bdmgF18	0.011	0.005	0.039
Int	TD	Bud Damage [n*]	bdmgS19	0.033	0.011	0.002
Int	TD	Spring Int	budint18	0.016	0.007	0.017
Int	TD	Survival	surv19	0.006	0.002	0.018
High	TD	Bud Damage	bdmgS18	0.208	0.032	0.000
High	TD	Bud Set	set18	0.019	0.009	0.042
High	TD	Bud Set	set19	-0.021	0.009	0.022
High	TD	Survival [n*]	drt20	0.018	0.007	0.014
High	TD	Survival	surv19	0.056	0.013	8.91E-06
High	TD	Survival [n*]	wint18-19	0.007	0.003	0.045

[n*] Trait was not include in the final multi-trait GWAS analysis due to convergence issues within GEMMA.

TABLE S6: Gene hits for BLASTn searches in North American conifer gene space. This table includes percent identity (%ID), alignment length region (ALR) and expectation value (*e-value*) for North American conifers *Pinus lambertiana*, *Pinus taeda*, *Pseudotsuga menziesii*. The *Pinus strobiformis* 80 base pair contig region associated with the gene hit is listed. The trait or trait grouping that had a significant SNP detection on the contig used is also listed.

Species	**Description**	**Accession**	**Contig**	**Trait or Group**	**%ID**	**ALR**	***e-value***
P. lambertiana	hypothetical protein (2_3756_03) gene, 3'UTR	JQ262601.1	52217	FFP_Int_MT	95.2	21	0.009
P. lambertiana	hypothetical protein (CL1029Contig1_01) gene	JQ263324.1	72802	BudDevAll_MT	88	25	0.033
P. taeda	hypothetical protein (0_1078_01) gene	JQ015486.1	112465	SpringPhen19_MT	91.67	24	0.042
P. menziesii	CN640419.1 heat shock protein 70 kDa gene	EU865077.1	21871	SpringPhen19_MT	90.91	22	0.033
P. menziesii	ES420771.1 anaphase promoting complex/cyclsome protein gene	EU865518.1	235570	Lammas	85.7	28	0.009
P. menziesii	Pm_CL1692Contig1 Zn-finger containing protein gene	EU866114.1	69504	Lammas	100	18	0.009

TABLE S7: SNPs detected in different analyses or located on the same contig region. See Figure 6 for phenotypic effects of SNP 167770:33. MAF=minor allele frequency, β= beta effect of the SNP on the maternal value, ±= the standard error of the beta value.

Model Abbreviation	Trait	Garden	Contig	Position	MAF	β	±
bdmgS19	bdmgS19	Int	167770	33	0.028	-0.295	0.053
FFP_Int_MT	surv19	Int		33	0.028	-0.028	0.012
	wint18-19			33	0.028	-0.137	0.054
	set18			33	0.028	-0.016	0.016
	bdmgS19			33	0.028	-0.290	0.053
budadv19	budadv19	Hi		33	0.028	0.207	0.041
lms19	lms19	Lo	45856	47	0.045	0.267	0.062
BudDev18_MT	budslp18			75	0.072	-2.22E-04	7.10E-05
	budint18				0.072	0.010	5.45E-03
lms19	lms19	Lo	49636	28	0.076	0.206	0.052
				54	0.076	0.206	0.052
lms19	lms19	Lo	5309	16	0.042	0.234	0.059
				28	0.042	0.234	0.059
bdmgS18	bdmgS18	Int	80350	18	0.052	-0.098	0.022
				35	0.05	-0.103	0.023

TABLE S8: Univariate results for all gardens and traits. In this table we report the chip heritability (h^2_chip) estimated in GEMMA, the number of significantly associated SNPs detected in each analysis (SNPs) the genetic variance (vg) and the environmental (ve) estimated by GEMMA using the REML method.

Garden	Trait Group	Trait	h^2_chip	se (h^2_chip)	SNPs	vg	ve
Low	Bud Damage	bdmgF18	0.340	0.225	0	5.77E-02	2.00E-02
Low		bdmgS19	0.317	0.229	0	1.11E-02	4.23E-03
Int		bdmgF18	0.518	0.272	1	3.06E-02	5.10E-03
Int		bdmgF19	0.163	0.306	0	6.84E-04	6.27E-04
Int		bdmgS18	1.79E-06	0.116	5	1.23E-07	1.23E-02
Int		bdmgS19	0.736	0.123	1	2.05E-01	1.31E-02
High		bdmgF18	1.79E-06	0.106	0	4.61E-06	4.61E-01
High		bdmgF19	0.242	0.219	0	1.11E-01	6.23E-02
High		bdmgS18	0.426	0.198	0	2.47E-01	5.93E-02
High		bdmgS19	1.77E-06	0.197	0	4.51E-11	4.51E-06
Low	Bud Set	set18	1.78E-06	0.199	0	1.38E-07	1.38E-02
Int		set18	0.754	0.133	0	1.63E-02	9.50E-04
Int		set19	0.455	0.231	0	3.82E-02	8.20E-03
High		set18	1.78E-06	0.302	0	3.14E-07	3.14E-02
High		set19	4.28E-02	0.127	0	9.39E-04	3.75E-03
Low	Fall Bud Variation	budv18	1.78E-06	0.160	0	1.88E-08	1.88E-03
Low		budv19	1.78E-06	0.000	0	6.02E-21	6.02E-16
Int		budv18	0.826	0.093	0	1.03E-04	3.90E-06
Int		budv19	0.486	0.242	0	9.41E-03	1.78E-03
High		budv18	1.78E-06	0.095	0	6.37E-08	6.37E-03
High		budv19	1.79E-06	0.235	0	5.70E-07	5.70E-02
Low	Spring Bud Advancement	budadv18	1.79E-06	0.038	0	3.03E-09	3.03E-04
Low		budadv19	0.242	0.826	0	1.62E-03	9.01E-04
Int		budadv18	1.79E-06	0.226	0	3.10E-08	3.10E-03
Int		budadv19	0.267	0.175	0	1.22E-03	5.98E-04
High		budadv18	1.78E-06	0.203	0	2.30E-08	2.30E-03
High		budadv19	1.78E-06	0.634	3	3.07E-07	3.07E-02
Low	Bud Slope	budslp18	1.79E-06	0.055	0	1.79E-12	1.79E-07
Low		budslp19	0.242	0.826	0	9.16E-07	5.11E-07
Int		budslp18	1.79E-06	0.124	0	3.08E-11	3.08E-06
Int		budslp19	0.267	0.175	0	6.92E-07	3.39E-07

High		budslp18	1.78E-06	0.148	0	5.53E-11	5.53E-06
High		budslp19	1.78E-06	0.605	0	3.99E-08	3.99E-03
Low	Bud Intercept	budint18	1.79E-06	0.073	0	1.02E-08	1.02E-03
Low		budint19	1.78E-06	0.255	0	2.22E-07	2.22E-02
Int		budint18	1.79E-07	0.101	0	1.83E-07	1.83E-02
Int		budint19	0.156	0.132	0	1.18E-02	1.14E-02
High		budint18	7.45E-02	0.129	0	1.39E-02	3.08E-02
High		budint19	1.78E-06	0.114	0	4.84E-11	4.84E-06
Low	Survival	wint17-18	0.353	0.295	15	0.073	0.024
Low		wint18-19	0.120	0.158	0	0.014	0.018
Low		surv19	5.57E-03	0.078	0	0.002	0.069
Low		drt20	1.78E-06	0.161	0	1.99E-07	0.020
Low		grow18	0.512	0.169	2	0.073	0.012
Low		grow19	1.78E-06	0.149	0	9.02E-07	0.090
Int		wint17-18	1.000	2.91E-04	0	-5.68E-11	-5.68E-16
Int		wint18-19	0.514	0.158	6	0.131	0.022
Int		surv19	0.210	0.369	0	0.003	0.002
Int		grow18	0.346	0.444	1	0.024	0.008
Int		grow19	0.654	0.133	0	0.075	0.007
High		wint17-18	0.485	0.189	0	0.388	0.074
High		wint18-19	0.704	0.135	0	0.018	0.001
High		surv19	0.237	0.195	1	0.084	0.049
High		drt20	0.517	0.275	1	0.057	0.009
High		grow18	0.019	0.084	0	0.003	0.023
High		grow19	0.248	0.285	2	0.008	0.004
Low	Lammas	lms19	0.9999	0.0001	34	5.8E-01	5.8E-06

TABLE S9: SNPs affecting survival, bud damage, and fall phenology detected in multi-trait analyses. The SNPs below were detected in the multi-trait analyses for trait groupings associated with frost free period (FFP) at the intermediate site, and continentality (TD) at the high elevation site. The effect of each SNP on the maternal value (β) and the standard error for that effect (se) are reported.

	FFP (Intermediate)				**TD (High)**	
	167770:33		**52217:22**		**47561:20**	
Trait	**β**	**se(β)**	**β**	**se(β)**	**β**	**se(β)**
surv19	-0.028	0.012	0.007	0.017	-0.183	0.068
set18	-0.016	0.016	-0.021	0.014	0.102	0.047
set19	–	–	–	–	0.007	0.017
bdmgS18	–	–	–	–	-0.4	0.085
bdmgS19	-0.29	0.053	-0.092	0.051	–	–
wint18-19	-0.137	0.054	-0.216	0.046	–	–

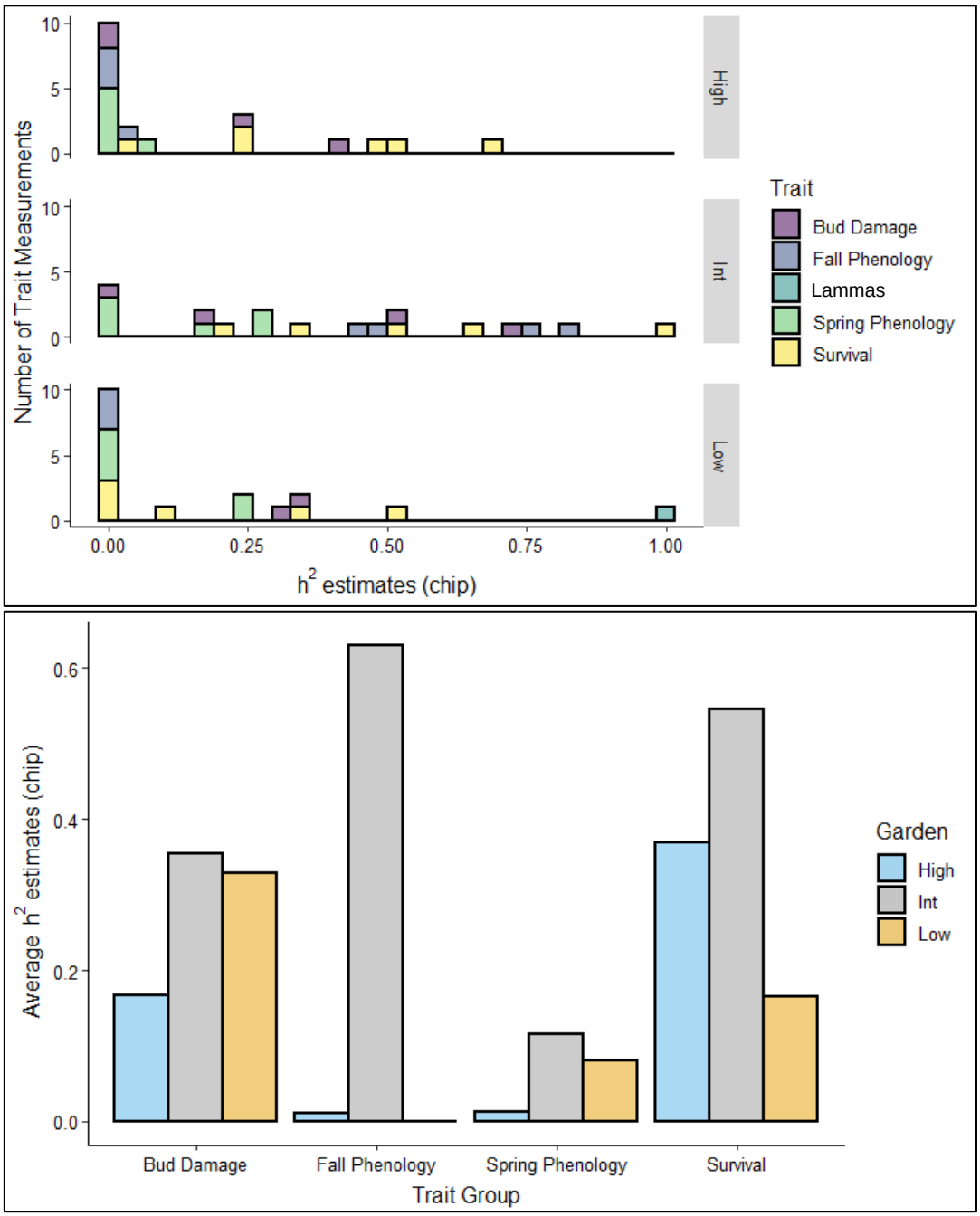

FIGURE S1: Heritability estimates by trait group across gardens. Chip heritability estimates were calculated in GEMMA using the REML method on the 13,255 SNPs that passed filtering steps and a minor allele frequency cut off of 0.05. The top figure displays histograms for chip heritabilities at each garden colored by trait group. The bottom figure displays trait group heritabilities color coded by garden.

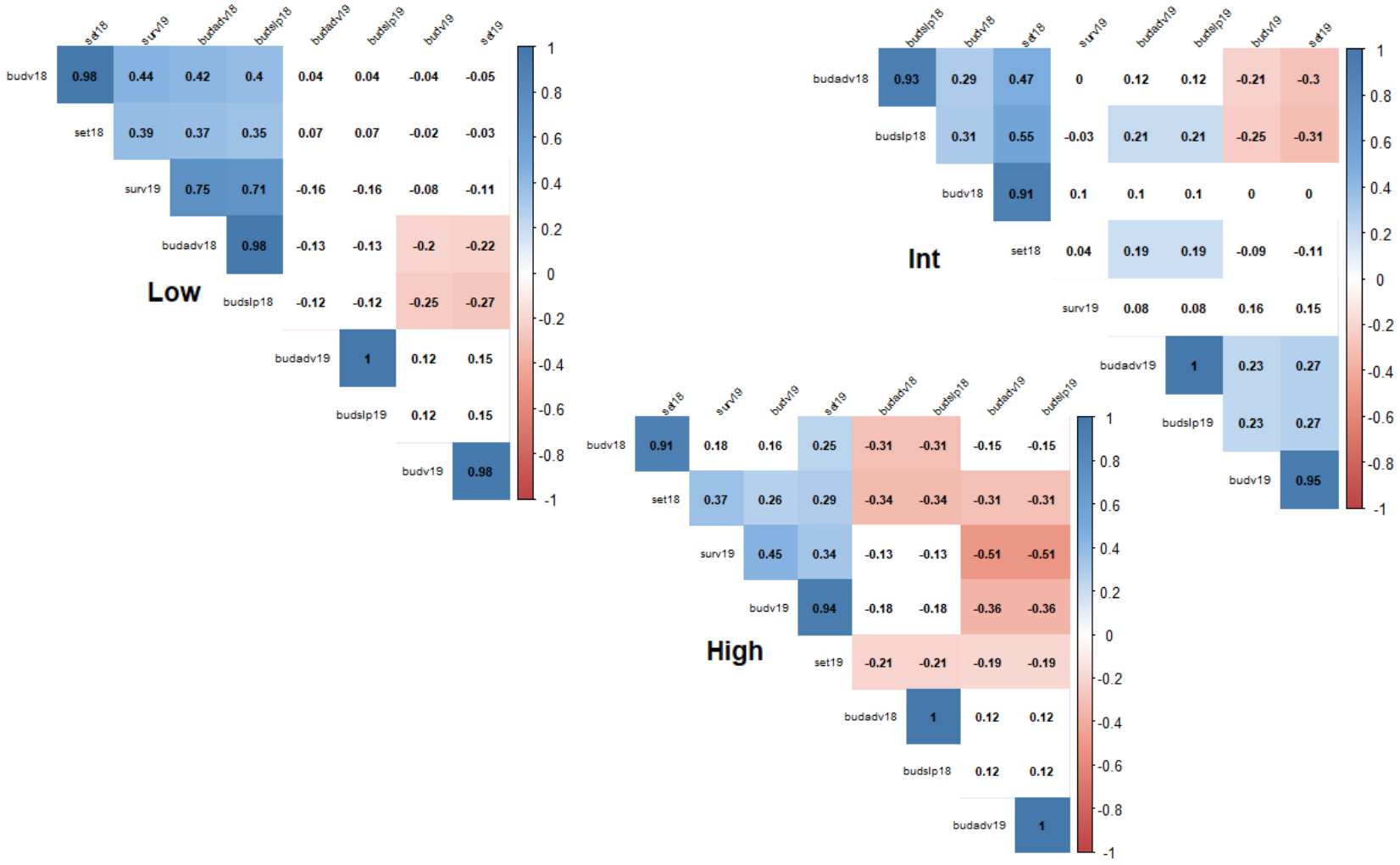

FIGURE S2: Correlation matrices for maternal tree trait averages at each garden. Red indicates a significant negative relationship whereas blue represent significant positive relationships. See Table 1 for trait abbreviations.

CHAPTER TWO

Fitness consequences and allele distribution of phenology related polymorphisms in *Pinus strobiformis*

INTRODUCTION

Phenological, or seasonally specific, responses to environmental cues have been a major focus for forest researchers for over a century because phenology mediates the trade-off between cold hardiness, drought avoidance, and growth (Aitken and Hannerz 2001). Prolonged growing seasons or early growth initiation may result in damage to buds responsible for shoot elongation, roots, xylem tissue, and needles. Overnight freezing temperatures followed by high light intensity can damage needles and reduce photosynthesis (Kozlowski et al. 1991), while drought during the growing season may limit carbohydrate reserves needed to develop cold tolerance. Damage related to freezing temperatures regularly coincide with water limitation and tissue dehydration, so separating their impact on tree damage is challenging (Sutinen et al. 2001). Rapid shifts in temperature and precipitation related to anthropogenic climate change threaten to decouple evolved responses to environmental stimuli resulting in maladaptive phenology and increased mortality (Choat et al. 2018, Chmura et al. 2011, Poulus 2014, Bailey and Harrington 2016, Klein 2015, Anderegg et al. 2016). Understanding phenological responses to novel conditions and the genetic contributors to phenology can aid forest managers in identifying vulnerable populations and populations with substantial genetic variation for phenological plasticity.

High effective population sizes in conifers suggest that genetic drift and allelic differentiation should be low across populations. However, when phenotypic expression conferring fitness differs across environments, we anticipate that selection will alter allele frequencies and lead to local adaptation (Schuster et al. 1989). The strength of the selection on particular phenotypes and underlying alleles may drive differentiation and specialization within and across populations. Species on heterogenous landscapes may contain specialist populations

that have locally adapted alleles or generalist populations with lots of plasticity (Frank et al. 2017). Highly specialized local adaptation can be detrimental to survival under climate change so identifying species and trait specific patterns of local adaptation is a critical climate mitigation step. Alleles that confer a competitive advantage in one environment may be maladaptive under novel conditions imposed by climate change by decoupling gene-phenotype relationships (Hänninen et al.2001).

Variation in quantitative traits for wild tree populations often follow a clinal pattern, that reflect specific ecological pressures along a climatic gradient (Alberto et al. 2013). However, the degree of local adaptation in trees is highly variable and comparison across species can be challenging. For example, Norway spruce that inhabit variable high elevation climates exhibit strong signals of specialized local adaptation. Conversely, the co-occurring silver fir has moderate to weak relationships with seed source climate and exhibits less influence of local adaptation (Frank et al. 2017). Local adaptation can also differ among traits for a particular species. *Pseudotsuga menziesii* exhibits local adaptation for bud flush, but not bud set (Acevedo-Rodriguez et al. 2006), and inland populations show strong relationships between growth potential and freezing temperatures (Rehfeldt et al. 2014). Limited genetic variability for phenological responses in coastal populations of Douglas-Fir (St. Clair and Howe 2007) and Norway spruce (Frank et al. 2017) may limit their capacity to adapt to climate change. Conversely, species, like *Pinus contorta*, with high levels of site specific and range-wide climate variability maintain high levels of within and across population genetic diversity that may buffer species against climate (Aitken and Hannerz 2001). High levels of gene flow and selection under historic climate conditions suggest that conifers may possess more variability in responsiveness to diverse climate cues than observed in their current distribution (Rosenblad et al. 2019,

Hänninen et al. 2001). Therefore, it is important to understand the genetic architecture of standing variation under different environments (Fournier-Level et al. 2011).

Common gardens have enabled forest researchers to identify geographic variation in trait expression along continuous environmental gradients, and physiological or phenological trade-offs between growth and cold tolerance (Aiken and Bemmels 2016). Common gardens are regularly paired with genomic approaches like genome wide association studies to detect genetic signatures of adaptive trait variation (Ingvarsson and Street 2011). Genome-wide association studies (GWAS) detect loci that contribute to variation in traits. While genome wide association studies have successfully identified loci and gene regions for a variety of conifers, their application for forest conservation is often limited by the inherent complexity of large conifer genomes and lack of replicability of results across growing conditions (Cortés et al. 2020). The climate and geographic context of loci detected by GWAS can improve interpretation of the factors that maintain quantitative genetic variation. Several studies utilizing this approach illuminate potential drivers of evolution in natural settings by connecting alleles with known fitness consequences to climate gradients, enabling researchers to detect genetic signatures of local adaptation. In one such study on *Picea sitchensis* alleles delaying bud set were discovered in higher frequencies in warmer climates (Lobo 2011). A common garden experiment on *Arabidopsis* used four geographically distinct growing conditions to understand the relationship among locally adapted climatic gradients and fitness by measuring survival and lifetime fruit production (Fournier-Level et al. 2011). Alleles associated with increased fitness (survival and fruit production) were associated with cool seasonal temperatures in Germany, Spain, and Finland, whereas alleles that decreased survival and fruit production were correlated with precipitation in the coolest part of year. Ultimately the distribution of alleles that increased

survival were limited by temperature in *Arabidopsis*. These studies demonstrate the utility of combining genomic signatures of plant performance under different growing conditions with range wide climate variables to discover climate or geographic gradients that illuminate selective pressure on adaptive traits.

Southwestern white pine (*Pinus strobiformis*) is a high elevation conifer threatened by predicted increasing temperatures and altered precipitation regimes (Seager and Vecchi 2010, Shirk et al. 2018) and an invasive fungal pathogen (*Cronartium ribicola*) (Conklin 2004). *P. strobiformis* contribute to biodiversity in mixed conifer forests by providing food resources for birds and mammals (Mattson and Arundel 2013, Samano and Tomback 2003). The bulk of the species range is found in the Sierra Madre Occidental in Mexico and it is scattered throughout Arizona, New Mexico on mountains and in canyons with small populations in Colorado and Texas (Looney and Waring 2013). Northern populations on average experience colder winters with more snow, but far less annual precipitation than the southern populations in Mexico (Moler 2020). Recent molecular analyses identified the extent of hybrid zone primarily located in New Mexico and Arizona, between *P. strobiformis* and the more northerly distributed *Pinus flexilis* (Menon et al. 2018). *P. strobiformis'* disjunct and isolated distribution across the American Southwest make this species particularly vulnerable to climate change. Species distribution models predict that suitable habitat for *P. strobiformis* will decrease across their range and shift to higher latitudes and altitudes (Shirk et al. 2018). Studying the distribution of genetic markers linked to survival and phenology under particular climate conditions will broaden our understanding of the evolved responses to climate cues in *P. strobiformis*.

This paper builds upon a previous GWAS that detected loci related to phenology, bud damage, and survival for 202 families of *P. strobiformis* across three common gardens that span

three distinct ecological communities in northern Arizona (Swenson 2021). Here we used both phenotypic and genomic data to answer the following questions: Q1) How do phenological traits vary across temporal and spatial scales and what is the relationship between phenological responses and overall survival across distinct growing conditions? Q2) Do alleles linked to survival and phenological traits confer a garden-specific advantage, and how are these alleles distributed across *P. strobiformis* range? To answer Q1 we will investigate how spring phenology, fall phenology, and bud damage vary across years and gardens and how these traits influence survival. We hypothesize H1a) that all traits will vary across years and gardens due to the unique climate pressures at each garden and variable climate observed across growing years. H1b) Maternal trees with increased bud damage will have higher mortality because a damaged bud is indicative of maladaptive cues and may signal additional constraints related to cold temperatures. And H1c) relationships for spring and fall phenology with survival will be specific to each growing environment because warmer temperatures at the lower sites may support longer periods of growth, whereas colder temperatures and snow accumulation may reduce periods of growth.

To answer Q2 we will first determine if particular genetic variants, or minor alleles, detected in the GWAS have a clear positive (favorable) or negative (unfavorable) effect on survival at the gardens. We will then investigate possible relationships for favorable and unfavorable minor alleles with seed source maternal climate variables to detect patterns of allelic distribution and signatures of evolutionary forces. We will also investigate the relationship between minor alleles associated with spring phenology that are not directly tied to garden specific survival with seed source climate variables. We hypothesize (H2a) that the strength of the relationship between favorable or unfavorable minor alleles with garden specific survival will

vary by garden. Secondly, we hypothesize (H2b) that favorable and unfavorable alleles will show clinal patterns with seed source temperature related climate variables indicating local adaptation of minor alleles to particular climate conditions. Specifically, maternal trees with more favorable minor alleles will be from colder (shorter frost-free period) and more variable (higher continentality) seed source sites because the garden conditions are more similar to their home ranges. Maternal trees with more unfavorable minor alleles will be from areas with longer growing seasons and less temperature variability because the large shifts in temperature at the gardens may result in cold stress injuries.

METHODS

Common Garden Establishment and Phenotypes

Pinus strobiformis seeds were collected from 202 individual trees spanning 50 populations across the US range of *P. strobiformis* in Colorado, Arizona, New Mexico, and Texas and planted in three common gardens (Figure 1, Table S1). From 2015-2017 up to 25 seedlings per maternal tree were planted at three Southwest Experimental Garden Array (SEGA) sites in the Kaibab National Forest, Arizona, USA. Each garden contained raised-bed boxes with 100 individual seedlings planted in a random planting design in 10x10 rows. The random planting design was implemented to reduce the need to account for block effects related to box microclimates in subsequent analyses. Trees were irrigated from April-October during each growing season. Specific watering amounts can be found in Bucholz et al. (2020). The three common gardens are distributed along an elevational and climatic gradient spanning the climate conditions experienced at maternal seed source locations and those at the margins (Figure 2). However, substantial annual variation in temperature and precipitation occurred across the two growing seasons from fall 2017 through fall 2019. Notably, the 2018-2019 winter was 2°C

colder than the 2017-2018 winter, and 121-284mm more precipitation fell in the 2018-2019 winter compared to the previous across all gardens. The 2018 growing season was ~2°C warmer and received ~30mm more precipitation than the second growing season.

Trait measurements were collected throughout two growing seasons from October 2017-October of 2019 (Table 1). Spring phenology, or bud burst, data were collected twice each year at each garden during 2018 and 2019. Bud stage was scored from 0-4 representing bud development from dormant (0) through bud elongation (2), needle emergence (3), and needle elongation (4). This scoring system is modified from Goodrich and colleagues (2016) with personal contributions from Bucholz, Moler, and Whipple. For each individual tree, bud stage advancement (budadv) was recorded as the change in bud score between the two measurement points, regardless of time, and bud burst slope (budslp) was calculated from the relationship between bud score and the Julian measurement day. Using these two components of spring phenology may help detect differences in the rate of bud development and the number of stages advanced during a measurement window with and without reference to time. Fall bud dormancy initiation phenology was recorded in the fall of 2018 and 2019 on two scales, bud set (set) and fall bud variation (budv). Fall bud variation on a 0-3 scale, encapsulates the nuance and variation in bud formation, color, and needle length at the end of the growing season. Scores of 0 and 1 were considered active by presence of a green bud whereas scores of 2 or 3 were considered dormant. Further distinctions within active (0,1) and dormant buds (2, 3) was based on variation in needle length relative to full length needles. Higher scores represented new full-length needles whereas lower scores had underdeveloped or short needles. The second metric, binary bud set is the condensed version of fall bud variation that focuses exclusively on whether the bud is active

(0) or dormant (1). Using both metrics enables us to capture more variation in dormancy initiation by accounting for bud and needle morphological changes.

Other bud and shoot growth characteristics were recorded during spring and fall phenology measurements including bud injury and lammas growth. Bud damage (bdmg) was determined when a tree had obviously produced a bud, the shoot began to elongate, but the bud/shoot failed to fully develop. Damaged buds were crispy, brown, and brittle, characteristic of damage related to cold temperatures (Sutinen et al. 2001). Bud damage represents a phenological mismatch with growing environment conditions and possible damage in response to rapid drops in temperature. A score of 0 indicates a damaged bud and a score of 1 indicates a healthy bud. Presence of lammas growth (lms19) at the low elevation garden was recorded as a 1 in the fall of 2019 and absence was recorded as a 0. Lammas growth is a secondary flush in one growing season (Kaya et al. 1994) that may provide individuals, especially seedlings, with a competitive advantage (Howe et al. 2003) by elongating the growing season (Goto et al. 2017). Overall survival (surv19) was recorded in the fall of 2019 following two growing seasons. An average of all individuals from a maternal family was calculated for each trait and measurement time period for use in statistical analysis.

Trait variation and relationships with survival

To determine if the observed annual variation in temperature and precipitation at the garden sites affected phenology we ran single factor ANOVAs for maternal average spring and fall phenology across years. For bud damage maternal averages, we used a two-way ANOVA to compare bud damage across seasons and years. To determine if traits differed by growing environment ANOVAs were employed to compare each trait at each measurement time across gardens. A Tukey's Test for post-hoc analyses was used to identify site specific differences in

trait values using the base package *stats* in R. To assess relationships between different phenological measurements we calculated and visualized Pairwise Pearson correlation coefficients (Figure S1). Generalized linear mixed effects models in program R using *glmmTMB* (Brooks et al. 2017) were used to determine which phenological events were related to overall mortality at each garden through two steps. The first step was to determine which measurement times for spring phenology, fall phenology, and bud damage had significant relationships with overall survival, and which trait measurement time explained the greatest amount of the variation in survival. Each trait was included as a fixed effect with survival as the response in separate models. The measurement time for each trait (spring phenology, fall phenology, and bud damage) with the lowest Akaike Information Criterion (AIC) (Bozdogan 1987) value was selected for use in step two.

In step two, additive models were constructed using all combinations of the traits and measurement times identified in step one to determine which suite of phenological events explained the most variation in survival. The model with the lowest AIC or, in cases where ΔAIC was less than two, the most parsimonious model was selected. Analysis was conducted for values at each garden separately. Covariates and random effects were excluded from the analyses for several reasons: 1) the random planting design implemented across gardens should reduce the need to account for block or microclimate effects, and 2) models that included random blocking effects with the raw mostly binary data, instead of family averages, failed to converge. While we are aware that blocking and planting years affect phenotype expression, this analysis is intended to identify general trends in annual variability and provide a framework for applying phenotypic relationships to bolster the ecological and evolutionary relevance of GWAS results.

SNP groupings and minor allele distributions

A total of 103 single nucleotide polymorphisms, SNPs, were detected in a GWAS that used univariate and multivariate models from GEMMA (Zhou and Stephens 2012) for phenological traits and survival in *P. strobiformis* (Swenson 2021). During filtering steps discussed in Swenson (2021) two alleles were retained at each SNP. The major allele was considered the one at a higher frequency across the 202 maternal trees used in that analysis and the lower frequency allele was considered the minor allele. Low coverage and ascertainment bias surely affect the conclusions that can be drawn in this analysis (Han et al. 2015, Namroud et al. 2008), however our genomic sampling effort using two restriction enzymes (Peterson et al. 2012) should provide even coverage across *P. strobiformis* genome and provide a good representation of the genomic space (Menon et al. 2018, Parchman 2018) .In the GWAS alleles significantly associated with traits had variable effects across growing conditions. This prompted further investigation into the direct relationships between survival, phenological traits, alleles, and maternal site climate variables.

To determine how alleles with similar effects on survival under particular growing conditions were distributed across *P. strobiformis* US range we grouped minor alleles into garden specific groups. Groupings of minor alleles were based on the relationships among survival and phenological traits from the previous section (Q1) and the β effects of the minor alleles on phenotypes detected in GEMMA (Swenson 2021). Two groupings of minor alleles, favorable and unfavorable, were established for each garden for a total of six allele groupings (Table 4). A minor allele was grouped at a particular garden based on the following criteria: 1) Minor alleles were considered favorable if they increased the likelihood of survival (positive β) or unfavorable if they increased mortality (negative β); 2) Minor alleles detected for phenological traits were grouped as favorable or unfavorable based on the relationships with

survival discovered from the analysis for Q1 (Table 2). For example, if there was a positive relationship between bud burst and survival at one garden then those minor alleles would be considered favorable. If there was a negative relationship between bud burst and survival at another garden the same minor allele would be considered unfavorable. This establishes a garden-specific set of minor alleles that are connected to survival. 3) If there was no effect of a trait on survival then the associated minor alleles were not included. 4) If a trait was not measured at a garden the associated minor alleles were not included (i.e., lammas growth). 5) Minor alleles detected from multi-trait groupings with contradictory or unclear effects were not grouped. Two additional groupings were included, one for all minor alleles associated with spring phenology regardless of the relationship to survival, and two, a group of unfavorable minor alleles that included lammas growth, for a total of eight groupings. The spring phenology allele group was created to see if spring phenology alleles alone demonstrated a clinal relationship with climate variables. Since lammas growth was only recorded at the low elevation garden the lammas growth alleles were included as a separate group so that cross garden allele groups contained the same traits.

Distinguishing alleles as favorable from GWAS results is common (e.g., De La Torre et al. 2019, Su et al. 2019, Lipka et al. 2013, Xiao et al. 2017, Bartholomé et al. 2016, Porth et al. 2013), however most distinguish favorable alleles as those that increase the expression of a trait of interest without reference to survival. In this study, favorable and unfavorable alleles were tied to site specific survival. Additionally, alleles can be considered unfavorable or deleterious if the effects of an allele on fitness are directly established (Pyhäjärvi et al. 2020), as we have done. However, our interpretation of unfavorable and favorable alleles should not be considered globally for *P. strobiformis* as these putatively beneficial or harmful alleles were detected under

specific growing conditions. Alleles with strong effects in a novel environment, such as our gardens, may be neutral under native climate conditions (Fournier-Level et al. 2011).

Genotype matrices in PLINK '012' format were used to sum minor alleles across loci for each maternal tree. At a particular locus an individual maternal tree may have either a 0, 1, or 2. An individual who is homozygous for the major, more common, allele has a 0, whereas individuals that are heterozygous for the minor allele have a 1, and homozygous for the minor allele a 2. For each allele grouping the total number of minor alleles for each maternal tree was calculated. The final minor allele counts were used in generalized linear mixed effects models (*glmmTMB*) to determine if minor allele counts were associated with maternal seed source climate variables or site-specific survival. In models that included survival and minor allele counts, survival was used as the response variable, and minor allele counts as the predictor. In the models that included climate variables and minor allele counts, minor allele counts were the response variable, and climate variables were the predictor variable. Thus, the minor allele counts serve both as a dependent and independent variable depending on the biological relationship. The climate variables used in this analysis were previously identified as important variables for describing *P. strobiformis* ecological niche space and genetic divergence history (Shirk et al. 2018, Menon et al. 2018) (Figure 2). Specifically, we included climate moisture deficit (CMD), precipitation in winter (PPT_wt), frost free period (FFP), degree days below zero (DD_0), mean warmest month temperature (MWMT), and continentality (TD) as fixed effects and minor allele counts for each grouping as the response. The geographic distribution of these alleles was visually assessed using ArcGIS to determine if favorable or unfavorable alleles were concentrated locally or distributed across the species range encompassed in this study.

RESULTS

Annual variation in phenology

2018 was warmer (Figure 2) and bud development occurred more quickly across all three gardens than in 2019 (Figure 3, Table S1). This was especially true at the high elevation garden where the difference in average bud stage advancement between 2018 ($\bar{x}$ =1.74±0.025) and 2019 ($\bar{x}$= 1.23± 0.022) was more than two times greater than the two lower gardens (Table S1). More families entered dormancy earlier in 2018 ($\bar{x}$ =0.73±0.001) than in 2019 ($\bar{x}$= 0.48± 0.01) (p=<2x10^{-16}, CI: -0.29, -0.22), with approximately 25% more trees already dormant at the measurement time in 2018 than in 2019. However, fall phenological traits were not significantly different between 2018 and 2019 at the high (budv: p=0.86, CI: -0.07, 0.08; set: p=0.09, CI: -0.07, 0.006), and low elevation garden (budv:p=0.64, CI: -0.06, 0.1; set: p=0.55, CI: -0.03, 0.05), (Figure 4, Table S1). Bud damage occurred more in 2018 than in 2019 across all gardens (Table S3). At the high elevation garden there was a significant difference between bud damage across seasons (p=1.38x10^{-4}) and years (p=2.53x10^{-4}) with greater bud damage in 2018 ($\bar{x}$ =0.71±0.01) than 2019 ($\bar{x}$ =0.88 ± 0.01) and more damage in spring ($\bar{x}$ =0.77±0.01) than in the fall ($\bar{x}$ =0.82±0.01). Bud damage was greatest in the spring of 2018 at the low ($\bar{x}$ =0.84±0.01) and intermediate elevation garden ($\bar{x}$ =0.94±0.01) compared to other measurement times (Table S3). Bud damage was significantly greater in 2018 than 2019 at the low (p= 3.92x10^{-13}) and intermediate gardens (p= 0.039). Bud damage was also greater in the spring at the intermediate garden (p=0.024), but there was no difference in seasons at the low elevation garden (p=0.616) (Table S2).

Garden variation in phenology

Spring bud burst occurred earlier at the two lower gardens (Figure 3), but bud stage advancement was greatest at the high elevation garden in both 2018 ($\bar{x}$ =1.7±0.03) and 2019 ($\bar{x}$ =1.23 ± 0.02). Bud stage advancement was slowest at the intermediate garden in both 2018 ($\bar{x}$ = 0.1.23±0.03) and 2019 ($\bar{x}$ =1.04 ± 0.02) (Table S1). While spring phenology metrics between the two lower gardens and the high elevation garden were significantly different in 2018 (budadv & budslp: p= <2x10^{-16}) and 2019 (budadv: p=6.25x10^{-10}, budslp: <2x10^{-16}) the difference in both the rate of bud development (bud slope) and bud stage advancement was more pronounced in 2018 than 2019. For example, the difference in bud stage advancement in 2018 between the intermediate and high elevation garden was -0.5, whereas in 2019 it was -0.18. This pattern held true for the low and high elevation garden as well. Bud damage differed across gardens for every measurement time (bdmgS18: p = <2x10^{-16}, bdmgF18: p = <2x10^{-16}, bdmgS19: p = 1.39x10$^{-10,}$ bdmgF19: p = 9.28x10^{-11}) (Figure 5), and the most damage occurred at the high elevation site in spring 2018 with an average of ~35% individuals experiencing bud damage ($\bar{x}$ =0.65±0.02). The least amount of bud damage was present in the fall of 2019, with the least bud damage at the intermediate garden ($\bar{x}$ =0.96±0.004). Across gardens bud set in 2018 was very similar, and most individuals had entered dormancy at the time of the measurement (low: $\bar{x}$ =0.65 ± 0.02, int: $\bar{x}$ =0.73 ± 0.01, high: $\bar{x}$ =0.69 ± 0.01) (Table S1). However, in 2019 individuals at the intermediate garden remained active longer ($\bar{x}$ =0.48 ± 0.01) than the other two gardens (low: $\bar{x}$ =0.67 ± 0.01, high: $\bar{x}$ =0.65 ± 0.02). Bud set did not significantly differ between the high and low elevation gardens in either 2018 (p=0.19, CI: -0.08, 0.01) or 2019 (p= 0.81, CI: -0.03, 0.06).

Relationship of phenology to survival

We compared models to determine which phenological measurements best explained survival at each garden. Producing a successful bud without damage increased survival at all three gardens (Figure 6). Greater bud stage advancement increased survival at the low site, but decreased survival at the high (Figure 7), and early bud set moderately increased survival at all three gardens. The measurement time explaining the most variation in survival differed by garden (Table 3) The largest effect on survival at the low elevation garden was spring 2019 bud damage (bdmgS19: $\beta=0.57\pm0.11$) with maternal families with less damage experiencing higher survival (Figure 6). Greater spring 2018 bud stage advancement also had a large effect on survival at the low elevation site (budadv18: $\beta=0.31\pm0.03$) (Figure 7) and entering dormancy earlier in 2018 weakly improved survival (budv18: $\beta=0.07 \pm 0.03$). Increased survival at the intermediate garden was best described by a lack of bud damage in spring 2019 (bdmdgS19: $\beta=0.34\pm0.1$) (Figure 6) and a weak effect of earlier bud set in 2019 (set19: $\beta=0.09\pm0.04$). No spring phenology traits were associated with survival at the intermediate garden. Like the other gardens, survival at the high elevation garden increased with less bud damage (bdmgF18: $\beta=0.55\pm0.05$) (Figure 7), however bud damage in the fall of 2018 explained more variation in survival at the high elevation garden. Survival at the high elevation garden was more strongly associated with slower bud burst (budadv19: $\beta=-0.09\pm0.03$) (Figure 7) and entering dormancy earlier (budv19 $\beta=0.07\pm0.03$) in 2019 than in 2018. These relationships highlight a yearly and garden specific relationship between phenology and survival.

Determination of favorable and unfavorable minor alleles

We used the relationships between phenological metrics and garden specific survival and allelic effects on trait values from Swenson (2021) to inform the distinction between alleles with

favorable and unfavorable effects under the novel conditions imposed in the experimental gardens. More minor alleles were grouped as unfavorable than favorable at each garden (Table 4), because the majority of minor alleles had a negative effect on traits. The low and high elevation garden contained similar number of SNPs with unfavorable minor alleles, 48 and 52 respectively, and SNPs with favorable minor alleles, 22 and 17, respectively. The intermediate garden had the fewest number of SNPs with favorable, 8, or unfavorable minor alleles, 39, because spring phenology was not associated with survival at this garden. SNPs associated with bud damage and bud set contained unfavorable minor alleles that increased the likelihood of mortality (Swenson 2021). A second unfavorable minor allele grouping was created at the low elevation garden to investigate additional relationships related to lammas growth. The 34 SNPs associated with lammas contained unfavorable minor alleles because presence of lammas growth decreased survival ($p=0.01$, $\beta = -0.63 \pm 0.03$, CI, -1.11, -0.15). The total number of SNPs in the spring phenology grouping was 37. Minor alleles in this grouping were not given a distinction of favorable nor unfavorable because we wanted to investigate climate gradients for alleles related to phenology alone. Most maternal trees had low minor allele counts for all groups (Figure S2). The exception to this is for unfavorable minor allele counts at both the low and high elevation gardens two maternal trees had more than 20 minor alleles. Minor allele counts were highest for the unfavorable group at the low elevation garden that included lammas. One maternal tree had over 60 minor alleles (Figure S2).

Minor allele counts and survival

We investigated the relationships between unfavorable, favorable, and spring phenology minor allele counts and garden specific survival and discovered that at all three gardens maternal trees with a greater number of unfavorable minor alleles had greater mortality. While this is

expected given that alleles affecting survival were included in the unfavorable allele groups, the results shed light on the magnitude of the effect of the alleles on garden specific survival. Specifically, at the low elevation garden on average, for every 10 minor alleles a maternal tree had survival decreased by 10% (p=3.7e-05, β = –0.01 ± 0.003, CI, -0.02, -0.007) (Figure 8). The relationship between a greater number of minor alleles and decreased survival was significant at the intermediate (p=0.002, β = –0.006 ± 0.002, CI, -0.01, -0.002), and high (p=0.007, β = –0.007 ± 0.003, CI, -0.01, -0.002) elevation gardens but less pronounced (Figure 8). Maternal trees with more minor alleles for spring phenology were also associated with more mortality at the high (β = –0.007 ± 0.003, CI, -0.01, 7.9e-05) and low gardens (β = –0.01 ± 0.004, CI, -0.02, -0.004), but not the intermediate. Favorable allele groupings were not significantly associated with survival at any garden.

Minor allele counts and source climate

We investigated the relationship between minor allele counts and seed source climate variables and discovered that maternal trees with more minor alleles detected at the gardens for unfavorable, favorable, and spring phenology allele groups were significantly associated with drier maternal site conditions (Figure 9 & S3, Table S4). Specifically, maternal trees with more minor alleles were from seed source sites with less winter precipitation (Figure S3) for unfavorable alleles at all three gardens and favorable alleles at the low and intermediate (Table S4). Maternal trees with a greater number of minor alleles were from areas with higher climate moisture deficit (Figure 9) for unfavorable alleles across all three gardens, and favorable alleles at the high elevation (Table S4). Additionally, higher spring phenology minor allele counts were associated with low maternal site winter precipitation (p=0.004, β = - 0.01 ± 0.004 CI: -0.02,-0.004) and higher climate moisture deficit (p=0.007, β = 0.08 ± 0.003, CI: 0.002, 0.014). A

greater number of unfavorable minor alleles, including those for lammas growth, was almost significantly related to greater maternal continentality (TD) (p=0.0504, $\beta = 0.82 \pm 0.42$ CI: -0.001,1.64) (Table S4, Figure S4) at the low elevation site. None of the other climate variables included were significantly associated with minor allele counts.

DISCUSSION

In this study we investigated the annual variation and variation across growing conditions in phenological traits and their relationship with survival. As hypothesized most phenological traits varied across the two study years and across gardens, except for fall phenology, which demonstrated little variation between the high and low elevation gardens. Additionally, we detected a consistent pattern where maternal families that experienced greater bud damage also had lower survival. Spring bud burst phenology had opposing relationships with survival at the two extreme gardens, demonstrating that early growth in *P. strobiformis* responds to climatic shifts in context dependent ways.

We utilized the garden specific relationships between phenology and survival to generate assemblies of minor alleles associated with unfavorable and favorable effects on fitness. These assemblies enabled us to identify potential climatic drivers of selection on seasonal phenology and survival. Contrary to our predictions, *P. strobiformis* maternal trees with more minor alleles were from seed source sites with less winter precipitation and greater climate moisture deficit for favorable, unfavorable, and spring phenology minor allele counts. This pattern suggests that *P. strobiformis* may have retained low frequency alleles along moisture availability gradients across the US range via a variety of evolutionary mechanisms. Specifically minor alleles may have been maintained at low frequencies due to large population sizes and high levels of within-site climate variability, local adaptation to seasonal moisture and early season growth, and prevailing

westerly wind patterns in the region may have influenced west to east gene flow patterns. Additionally, stressful environments may have reduced overall allelic and phenotypic diversity because they experience strong climate-related selection.

TEMPORAL AND ENVIRONMENTAL VARIATION

As predicted, spring phenological traits and bud damage varied across years and gardens, however, bud set did not vary across all gardens and environments. Seasonal phenology for *P. strobiformis* families generally developed earlier in the spring at the two lower elevation gardens, but more rapidly at the high elevation garden. Buds remained active longer at the intermediate garden, and dormancy varied little between the low and high elevation gardens. At each garden buds developed earlier and advanced stages more quickly in the spring of 2018 than 2019. The high level of divergence between growing conditions is in line with other green house and common garden studies in *P. strobiformis* that detected environment specific effects on phenotypes (Goodrich et al. 2016, Bucholz et al. 2020, DaBell 2017, Moler 2020). Our study is also in line with tree studies that demonstrate environmental and interannual plasticity in spring phenology, but little variation in dormancy initiation (Alberto et al. 2013).

Spring Phenology Variation

Increased snow and colder temperatures over the 2018-2019 winter likely drive the phenological delay in 2019 (Figure 2). Limited access to our sites from heavy snowfall in the spring of 2019 delayed our sampling and may confound the differences detected between years. The annual difference, however, revealed interesting patterns in early season phenological development. The early season measurements in 2018 (March-May) had much greater variation in bud developmental stage compared to later season measurements collected in 2019 (May-July). This finding is in line with results from with experimental warming treatments that

detected greater variability in bud stage advancement following warm early springs and more uniform spring phenology following cool springs (Montgomery et al. 2020). In this study pine species demonstrated larger shifts in bud burst date in response to spring temperatures compared to other conifers and deciduous trees. Additionally, phenology displays highly variable interannual patterns for *P. sylvestris* grown within, at the edge, and beyond the limits of their current distribution (Duputié et al. 2015).The only spring phenology trait that did not differ across gardens was bud stage advancement in 2018 between the low and intermediate gardens. However, another metric of spring phenology in 2018, bud slope, differed significantly between the same gardens. This implies that while the number of bud stages advanced was the same between gardens, the low elevation garden initiated growth earlier (Figure 3). Highly variable temperatures across the gardens and fewer degree days below zero in 2018 (Figure 2) may have triggered sporadic and highly variable onset of growth.

Bud Set Variation

While latitudinal and temperature related clines exist for bud set in different tree species (Alberto et al. 2013), low plasticity for bud set is common in tree species due to a high level of genetic control and reliance on cues from photoperiod (Aitken and Bemmels 2016). In line with these observations, bud set in our study did not differ between years, nor did it vary between the high and low elevation gardens for some measurements. The lack of bud set variation between the high and low garden may indicate that selection favors early physiological shut down.

Bud Damage Variation

We detected strong annual and environmental differences in bud damage susceptibility. Most bud damage was detected in the spring of 2018 following a dry and warm (+3˚C) winter that could have challenged plants cold and desiccation tolerance. Warm and dry winters may

impose drought stress that deplete carbohydrate reserves, while simultaneously increasing evaporative demand imposing a complex network of climatic pressures on seedlings (Kozlowski and Pallardy 2002). In a previous study using overlapping populations, *P. strobiformis* seedlings at the low elevation garden experienced greater water limitations evident in low stomatal conductance and low leaf mass per area in the summer of 2018 (Bucholz et al. 2020). Stomatal closure is a water use efficiency response to protect against desiccation, however, this behavior can reduce carbohydrate reserves needed for developing cold hardiness. Additionally, large leaf mass per area is linked to cold tolerance in trees (Jankowski et al. 2017). Therefore, low growing season stomatal conductance and low leaf mass per area at the low elevation garden suggests that individuals may have difficulty developing sufficient cold hardiness to avoid injury at this site.

PHENOLOGY AND SURVIVAL

Cold injury that results in mortality or damage to reproduction can limit species distribution (Aitkens and Hannerz 2001). Accordingly, to test the fitness consequences of traits related to cold hardiness and response to seasonal fluctuations we examined the relationships between survival and spring bud development, fall bud set, and bud damage. Our study highlights the trade-offs between early season growth and cold damage and the context dependent effect of phenology on overall survival. As predicted, the diverse conditions imposed by annual variation and growing environment resulted in variable relationships between phenology and survival. Bud damage and fall phenology were in the top model explaining survival at all gardens, however spring phenology did not significantly affect survival at the intermediate garden. Bud damage had the greatest effect on survival across all gardens (Figure 6) and spring phenology had variable impacts on survival at the low and high elevation gardens (Figure 7). More rapid spring phenology at the low elevation garden strongly increased survival

(β=0.31±0.03), whereas slow spring phenology increased survival at the high elevation garden (β=-0.08 ±0.04) (Figure 7). Opposing relationships for spring phenology and survival at the high and low elevation gardens further highlight the influence of context-specific cues triggering adaptive responses in phenology.

Our common gardens are near the northern edge of the species range and are situated at the climate margins in historic source site averages for most maternal families (Figure 2). During this two-year study continentality (TD) and climate moisture deficit (CMD) at all gardens exceeded the 30-year climate averages for most maternal sites. Our detection of relationships between spring phenology and survival at the edge of the species distribution enables us to conclude that *P. strobiformis* exhibits plastic responses, with fitness consequences, to context-dependent conditions that may be beneficial beyond the species current climate envelope (Duputié et al. 2015, Andersson and Federkov 2004). Plasticity and risk-taking strategies in spring phenology may enable *P. strobiformis* to become more prominent in mixed conifer forests and adjacent unburned aspen stands (Cocke et al. 2005), however, competition with other mixed conifer species, especially Douglas Fir (Sakulich and Taylor 2007), may limit the benefit of this plasticity. The earlier phenological development in response to temperature at the low elevation garden could suggest that if moisture is not limited, downslope movement into warmer climates may be possible.

While spring phenology demonstrated plastic relationships with survival, fall phenology had a consistent relationship, where early setting families had higher survival. This conservative and consistent pattern may suggest that bud set is genetically constrained to reduce the likelihood of cold related damage (Montgomery et al. 2020) and photoperiod may more greatly influence bud set in *P. strobiformis* than temperature (Alberto et al. 2013). Accordingly, strong selective

pressure for cold hardiness may have limited genetic variation for dormancy signaling and observable plasticity (Bansal et al. 2015). Despite the lack of observable plasticity, the influence of fall phenology on survival is supported by its presence in the top model explaining survival at each site (Table 3). Additionally, logistical limitations affected our sampling strategy and enabled us to collect bud stage only once during the month of October and may have reduced our capacity to detect phenotypic variation. We expected *P. strobiformis* populations to express more variability in fall phenology than observed in this study considering continentality and latitude often play a role in dormancy and cold hardiness (Andersson and Fedorkov 2004), high levels of genetic differentiation between populations for bud set were detected in 23 conifers, and altitudinal and latitudinal clines exist for many widespread conifers (Alberto et al. 2013).

Our study demonstrates that bud damage increases the risk of mortality in *P. strobiformis* seedlings in seasonally specific patterns. Damage in the fall at the two lower and warmer gardens explained more variation in mortality, whereas spring bud damage explained more variation in mortality at the high elevation garden. Seasonally specific impacts of frost damage have also occurred in other tree species. More northerly, colder, distributed *Pinus contorta* populations experience greater spring frost damage, whereas more southerly, warmer, populations experience more fall frost damage (Montwé et al. 2018). Additionally, in *Populus fremontii* frost damage occurring during spring bud development was associated with mortality (Grady et al. 2013).

MINOR ALLELE DISTRIBUTION AND FITNESS RELATIONSHIPS

Genomic approaches in conifers enable detection of loci related to local adaptation and fitness related traits (Lind et al. 2018). However, genotype-environment association studies often lack phenotypic and fitness consequences of detected loci (Rellstab et al. 2015, Namroud et al. 2008) and GWAS studies detect loci that are context-dependent and often lack evidence for

evolutionary mechanisms influencing genetic divergence (Lind et al. 2018, Villemereuil et al. 2016). To overcome these limitations, we combined phenological relationships with survival, minor alleles detected in a GWAS, and maternal climate variables to investigate potential mechanisms influencing *P. strobiformis* seasonal phenology and survival. We detected direct relationships between unfavorable minor alleles and survival, and the distribution of favorable, unfavorable, and phenological minor alleles along precipitation gradients. While the patterns among allele groups, survival and climate gradients are significant, overinterpretation of the species wide relevance of adaptive or fitness related alleles should be avoided (Barrett and Hoekstra 2011). Thus, the alleles deemed unfavorable or favorable in this study are referenced only in relation to the specific growing conditions in this study, and all *post-hoc* interpretations should be viewed within this context.

Minor allele counts and survival

Maternal families that had more unfavorable minor alleles had reduced survival across all three gardens (Figure 8). A higher number of spring phenology minor alleles at the high and low elevation gardens were associated with reduced survival as well. However, a greater number of favorable minor alleles did not affect survival (Table S4). While we expected to observe a significant relationship between higher unfavorable minor allele counts and reduced survival, we did not expect to see a similar pattern with spring phenology minor allele counts. The negative effect of spring phenology minor allele counts on survival links range wide genetic variation for phenology to fitness. This pattern suggests selection favoring the major, common, alleles for spring phenology across a range of growing conditions.

Minor allele counts and climate gradients

We anticipated minor allele distributions to be significantly correlated with seed source temperature variables due to the high level of temperature variability imposed at the three common gardens and the range of temperature variability across *P. strobiformis* US range (Figure 2). However, we discovered that unfavorable, favorable, and spring phenology minor allele counts were significantly correlated with moisture availability (Figure 9, S3, and S4; Table S5). Consistently, a greater number of minor alleles were found in regions with low winter precipitation and high climate moisture deficit. Periods of climate moisture deficit are particularly pronounced for populations in northern Arizona in spring and early summer (Moler 2020) (Figure S5). These populations may need to utilize winter and early spring precipitation for growth prior to a dry period. Throughout southern Arizona and much of New Mexico, however, CMD is higher in the late winter and early spring, but low in the summer. In these populations delaying growth until the summer may be more favorable. This pattern is consistent with specific growth responses to local climate observed in maritime and continental populations of *Pseudotsuga menziesii* and *Pinus monticola* (Aitken and Hannerz 2001). Coastal populations of *P. menziesii,* specifically, initiates bud burst earlier to avoid shoot elongation during the driest part of the year (Aitken and Hannerz 2001).

Several evolutionary mechanisms may explain the observed relationships between minor allele counts and precipitation variables. Considering conifers have high effective population sizes (Schuster et al. 1989) and *P. strobiformis* have high levels of modern gene flow (Menon et al. 2018) we would expect overall allelic differentiation resulting from genetic drift to be low and any observed genetic differences across geographic or climate gradients would result from selection under local environments (González-Martínez et al. 2006). Thus, local selective

pressures for seasonal precipitation and early season growth may partially explain the genetic differences for phenological traits and survival in *P. strobiformis.*

Under stabilizing selection theory, a larger number of minor alleles associated with phenotypes should be detected under growing conditions that are similar to seed source locations (Josephs et al. 2017). Under this assumption the individual populations nearest the garden sites would have experienced selection towards an optimum phenotype that differs from other populations. Thus, the populations nearest the gardens would possess different allele frequencies for these local adapted phenotypes. The maternal sites, in our study, nearest the north rim gardens have, on average, high winter precipitation and high CMD. Therefore, the high number of minor alleles associated with high CMD may reflect stabilizing selection (Figure 9), whereas the high number of minor alleles associated with less winter precipitation does not, at first glance, fall in line with stabilizing selection theory (Figure S3). However, winter precipitation at the three gardens in 2018 was lower than the 30-year averages for most maternal source sites, and higher than most maternal source sites in 2019 (Figure 2). If the minor alleles detected across the three gardens were a product of phenotypic expression related to the dry winter of 2018 our results may align with stabilizing selection theory. Given that trait values in both 2018 and 2019 influenced overall survival a lag effect may have impacted phenotypic expression and the associated alleles.

Genetic differences along precipitation gradients may also result from prevailing westerly wind patterns that could influence the direction of gene flow. This explanation was brought to light by a correlation between longitude and winter precipitation (-0.73) for US populations of *P. strobiformis*. Generally, maternal sites in New Mexico have less winter precipitation than maternal sites in Arizona (Figure S5). Accumulation of less common alleles in more eastern

populations could occur through immigration if pollen dispersal follows westerlies and gene flow from eastern to western populations is limited. Genetic differentiation along an east-west divide was observed for *P. strobiformis* in their Mexican range (Moreno-Letelier and Pinero et al. 2009). However, in that study the genetic structure of populations in southern Arizona were not significantly different than populations near the Texas border. This could imply that genetic differentiation along an east-west gradient may not exist for populations of *P. strobiformis* in the US. Additionally, unidirectional gene flow related to wind patterns has not been widely observed in North American conifers. For example, in a *P. menziesii* hybrid zone gene flow was bi-directional and not limited by regional wind patterns (Gugger 2010). Additionally, the distribution patterns for *Pinus flexilis* haplotypes in the southwestern US reveal patterns of gene flow in northeast and northwest trajectories (Mitton et al. 2000).

In our study minor alleles with unfavorable and favorable effects at a particular garden and those detected for spring phenology were present throughout *P. strobiformis* US range including high and low counts in neighboring populations. Thus, high within-site climate variability accompanied by large population sizes may better explain the retention of lower frequency allele variants than unidirectional gene flow. Range wide distribution of minor alleles associated with climate gradients is evident in *Pinus taeda* populations (De La Torre et al. 2019) and high within site genetic variation is attributed to highly variable local climate conditions for *Pinus contorta* (Aitken and Hannerz 2001). Rare alleles thus may be maintained locally and responses to selection may be population or site specific (Josephs et al. 2017, Josephs et al. 2019). Similar environmental pressures in different populations may select different beneficial or unfavorable alleles (Hancock et al. 2011). Future studies should consider comparing Fst and Qst values to better interpret the cause of genetic divergence along this climate gradient.

CONCLUSIONS

In this study we detected phenological responses to annual and environmental differences in abiotic conditions. Warmer conditions resulted in earlier spring bud development, however, the number of bud stages advanced at the high elevation garden was greater than the lower sites. This demonstrates that shorter growing seasons may prompt delayed but rapid growth responses. However, trees that advanced fewer bud stages during the measurement period had greater survival at the high elevation site, whereas advancing more bud stages at the low elevation garden was associated with greater survival. Bud damage and delayed initiation of dormancy decreased survival across all gardens. Our results highlight the context dependent fitness consequences of phenological variation.

We utilized patterns of phenological relationships with survival and loci effect estimates to generate environment specific assemblies of minor alleles and extend the utility of SNPs detected in a GWAS. These assemblies enabled us to identify links among genetic variability, phenological expression and fitness. Specifically, maternal trees with more unfavorable or phenological minor alleles had lower survival. While detecting selection and adaptive alleles using next generation sequencing may benefit from site frequency analysis the three lines of evidence used in this study including phenotype relationships with survival, effect estimates of loci, and the site specific relationship between minor allele counts and survival at least partially confirm that alleles deemed unfavorable negatively affected seedling survival.

These assemblies also enabled us to identify genetic divergence along precipitation gradients across *P. strobiformis* US range. *P. strobiformis* families with more favorable, unfavorable or phenological minor alleles were from seed source sites with less winter precipitation and more climate moisture deficit. This pattern enabled us to determine that

selective pressure on early season growth, local adaptation within highly variable maternal sites, and patterns of gene flow may explain how low frequency, rare alleles, are maintained in wild conifer populations.

Our study contributes to a rapidly growing body of knowledge the highlights the importance of seasonal precipitation and moisture deficit for *P. strobiformis.* Previous studies have detected the influence of precipitation gradients on *P. strobiformis* seasonal growth (Goodrich et al. 2016), seedling emergence (Moler et al. 2021) reproductive morphology (Leal-Sáenz et al. 2020), hybridization history (Menon et al. 2018), drought physiology (Bucholz et al. 2020), geographic distribution (Shirk et al. 2018), and genetic divergence (Moreno-Letelier and Pinero 2009). Future research should address the role hybridization history of *Pinus strobiformis* has on divergent variation in genetic structure, survival, phenology, and climate gradients.

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LITERATURE CITED

Acevedo-Rodriguez, R, Vargas-Hernandez, JJ, Lopez-Upton, J, and Velazquez Mendoza, J (2006) Effect of geographic origin and nutrition on shoot phenology of Mexican Douglas-Fir (*Pseudotsuga* sp.) seedlings. Agrociencia. 40(1):125-137

Aitken SN, Bemmels JB (2016) Time to get moving: assisted gene flow of forest trees. Evol Appl 9:271–290. https://doi.org/10.1111/eva.12293

Aitken, SN, Hannerz M (2001) Genecology and Gene Resource Management Strategies for Conifer Cold Hardiness. *In* F.J. Bigras and S.J. Colombo (eds). Conifer cold hardiness. Kluwer Academic Publ., Dordecht. The Netherlands. 23–53. https://doi.org/10.1007/978-94-015-9650-3_2

Aitken SN, Yeaman S, Holliday JA, et al.(2008) Adaptation, migration or extirpation: climate change outcomes for tree populations. Evol Appl 1:95–111. https://doi.org/10.1111/j.1752-4571.2007.00013.x

Alberto FJ, Aitken SN, Alía R, et al.(2013) Potential for evolutionary responses to climate change - evidence from tree populations. Glob Chang Biol 19:1645–1661. https://doi.org/10.1111/gcb.12181

Anderegg WRL, Klein T, Bartlett M, et al.(2016) Meta-analysis reveals that hydraulic traits explain cross-species patterns of drought-induced tree mortality across the globe. Proc Natl Acad Sci U S A 113:5024–5029. https://doi.org/10.1073/pnas.1525678113

Andersson B, Fedorkov A (2004) Longitudinal differences in Scots pine frost hardiness. Silvae Genet 53:76–80. https://doi.org/10.1515/sg-2004-0014

Bailey JD, Harrington CA (2006) Temperature regulation of bud-burst phenology within and among years in a young Douglas-fir (*Pseudotsuga menziesii*) plantation in western Washington, USA. Tree Physiol 26:421–430. https://doi.org/10.1093/treephys/26.4.421

Barrett RDH, Hoekstra HE (2011) Molecular spandrels: Tests of adaptation at the genetic level. Nat Rev Genet 12:767–780. https://doi.org/10.1038/nrg3015

Benito Garzón M, Alía R, Robson TM, Zavala MA (2011) Intra-specific variability and plasticity influence potential tree species distributions under climate change. Glob Ecol Biogeogr 20:766–778. https://doi.org/10.1111/j.1466-8238.2010.00646.x

Bonan GB (2008) Forests and climate change: Forcings, feedbacks, and the climate benefits of forests. Science. 320:1444–1449

Bozdogan H (1987). Model selection and Akaike's Information Criterion (AIC): The general theory and its analytical extensions. Psychometrika, 52(3): 345–370. https://doi.org/10.1007/BF02294361

Brooks ME, Kristensen K, van Benthem KJ, et al.(2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. R J 9:378–400. https://doi.org/10.32614/rj-2017-066

Bucholz E (2020) Early growth, water relations and survival: common garden studies of *Pinus strobiformis* under climate change. Doctoral dissertation, Northern Arizona University

Bucholz, BR, Waring, KM, Kolb, TE Swenson, JK, and Whipple, AV (2020) Water relations and drought response of *Pinus strobiformis*. Canadian Journal of Forest Research. 50(9): 905-916. https://doi.org/10.1139/cjfr-2019-0423

Chmura DJ, Anderson PD, Howe GT, et al.(2011) Forest responses to climate change in the northwestern United States: Ecophysiological foundations for adaptive management. For Ecol Manage 261:1121–1142. https://doi.org/10.1016/J.FORECO.2010.12.040

Choat B, Brodribb TJ, Brodersen CR, et al.(2018) Triggers of tree mortality under drought. Nature 558:531–539. https://doi.org/10.1038/s41586-018-0240-x

Cocke AE, Fule PZ, Crouse JE (2005) Forest Change on a Steep Mountain Gradient after Extended Fire Exclusion: San Francisco Peaks, Arizona, USA. Brit Ecol Soc 42:814–823. https://doi.org/10.1111/j.1365-2664.2005.01077.x

Conklin, DA (2004) Development of white pine blister rust outbreak in New Mexico. USDA Forest Service, 17. Southwestern Region, R3-04-01.

DaBell J (2017). *Pinus strobiformis* response to an elevational gradient and correlations with source climate. MSc thesis, Northern Arizona University

De La Torre AR, Puiu D, Crepeau MW, et al.(2019) Genomic architecture of complex traits in loblolly pine. New Phytol 221:1789–1801. https://doi.org/10.1111/nph.15535

de Villemereuil P, Gaggiotti OE, Mouterde M, Till-Bottraud I (2016) Common garden experiments in the genomic era: new perspectives and opportunities. Heredity 116:249–54. https://doi.org/10.1038/hdy.2015.93

Duputié A, Rutschmann A, Ronce O, Chuine I. (2015) Phenological plasticity will not help all species adapt to climate change. Glob Chang Biol, 8:3062-73. https://doi.org/10.1111/gcb.12914

Foley JA, Asner GP, Costa MH, et al.(2007) Amazonia revealed: forest degradation and loss of ecosystem goods and services in the Amazon Basin. Front Ecol Environ 5:25–32. https://doi.org/10.1890/1540-9295

Fournier-Level A, Korte A, Cooper MD, et al.(2011) A map of local adaptation in *Arabidopsis thaliana*. Science 334:86–89. https://doi.org/10.1126/science.1209271

Frank A, Sperisen C, Howe GT, et al.(2017) Distinct genecological patterns in seedlings of Norway spruce and silver fir from a mountainous landscape. Ecology 98:211–227. https://doi.org/10.1002/ecy.1632

Gamfeldt L, Snäll T, Bagchi R, et al.(2013) Higher levels of multiple ecosystem services are found in forests with more tree species. Nat Commun 4:1340. https://doi.org/10.1038/ncomms2328

Garfin G, Eischeid J, Lenart M, et al.(2010) Downscaling Climate Projections in Topographically Diverse Landscapes of the Colorado Plateau in the Arid Southwestern United States: Proceedings of the 9th Biennial Conference on Colo. 21–44

González-Martínez SC, Krutovsky K V., Neale DB (2006) Forest-tree population genomics and adaptive evolution. New Phytol 170:227–238. https://doi.org/10.1111/j.1469-8137.2006.01686.x

Goodrich BA, Waring KM, Kolb TE (2016) Genetic variation in *Pinus strobiformis* growth and drought tolerance from southwestern US populations. Tree Physiol 36:1219–1235. https://doi.org/10.1093/treephys/tpw052

Goodrich BA, Waring KM, Auty D, Sánchez Meador AJ (2018) Interactions of management and white pine blister rust on *Pinus strobiformis* regeneration abundance in southwestern USA. For An Int J For Res 91:492–505. https://doi.org/10.1093/forestry/cpy009

Grady KC, Kolb TE, Ikeda DH, Whitham TG (2015) A bridge too far: Cold and pathogen constraints to assisted migration of riparian forests. Restor Ecol 23:811–820. https://doi.org/10.1111/rec.12245

Gugger PF (2010) Phylogeography of Douglas-fir: Testing hypotheses from the fossil record. PhD dissertation, University of Minnesota.

Han E, Sinsheimer JS, Novembre J (2015) Fast and accurate site frequency spectrum estimation from low coverage sequence data. Bioinformatics 31:720–727. https://doi.org/10.1093/bioinformatics/btu725

Hancock AM, Witonsky DB, Alkorta-Aranburu G, et al.(2011) Adaptations to Climate-Mediated Selective Pressures in Humans. PLoS Genet 7(4) 1-16. https://doi.org/10.1371/journal.pgen.1001375

Hänninen H., E. Beuker, Ø. Johnsen, I. Leinonen, M. Murray, L. Sheppard and T. Skrφppa. (2001) Impacts of climate change on cold hardiness of conifers. *In* Conifer Cold Hardiness. F.J. Bigras and S.J. Colombo (eds). Kluwer Academic Publishers, Dordrecht. 305–333

Ingvarsson PK, Street NR (2011) Association genetics of complex traits in plants. New Phytol 189:909–922. https://doi.org/10.1111/j.1469-8137.2010.03593.x

Mitton, JB, Kreiser, BR, and Latta, RG (2000) Glacial refugia of limber pine (*Pinus flexilis* James) inferred from the population structure of mitochondrial DNA. Molec Ecol 9: 91–97

Jankowski A, Wyka TP, Żytkowiak R, et al.(2017) Cold adaptation drives variability in needle structure and anatomy in *Pinus sylvestris* L. along a 1,900 km temperate–boreal transect. Funct Ecol 31:2212–2223. https://doi.org/10.1111/1365-2435.12946

Jordan, R, Hoffmann, AA, Dillon, SK, and Prober, SM (2017). Evidence of genomic adaptation to climate in *Eucalyptus microcarpa:* Implications for adaptive potential to projected climate change. Molecular Ecology, 26(21), 6002–6020. https://doi.org/10.1111/mec.14341

Josephs EB, Berg JJ, Ross-Ibarra J, Coop G (2019) Detecting adaptive differentiation in structured populations with genomic data and common gardens. Genetics 211(3): 989-1004 https://doi.org/10.1534/genetics.118.301786

Josephs EB, Stinchcombe JR, Wright SI (2017) What can genome-wide association studies tell us about the evolutionary forces maintaining genetic variation for quantitative traits? New Phytol 214:21–33. https://doi.org/10.1111/nph.14410

Klein T (2015) Drought-induced tree mortality: from discrete observations to comprehensive research Commentary. Tree Physiol 35:225–228. https://doi.org/10.1093/treephys/tpv029

Kozlowski, TT, Kramer, PJ and Pallardy SG (1991) The Physiological Ecology of Woody Plants. Academic Press, San Diego, CA.

Kozlowski ATT, Pallardy SG, Url S, Pallardy SG (2002) Acclimation and Adaptive Responses of Woody Plants to Environmental Stresses. Bot Rev 68(2) 68:270–334

Leal-Sáenz A, Waring KM, Menon M, et al.(2020) Morphological differences in *Pinus strobiformis* across latitudinal and elevational gradients. Front Plant Sci11:1–16. https://doi.org/10.3389/fpls.2020.559697

Lind BM, Menon M, Bolte CE, et al.(2018) The genomics of local adaptation in trees: are we out of the woods yet? Tree Genet Genomes 14: 29 https://doi.org/10.1007/s11295-017-1224-y

Lobo N (2011) Clinal variation at putatively adaptive polymorphisms in mature populations of sitka spruce (*Picea sitchensis*). MSc thesis, University of British Columbia.

Looney CE, Waring KM (2013) *Pinus strobiformis* (southwestern white pine) stand dynamics, regeneration, and disturbance ecology: A review. For Ecol Manage 287:90–102. https://doi.org/10.1016/J.FORECO.2012.09.020

Martin, J, Looker, N, Hoylman, Z, Jencso, K, & Hu, J (2018). Differential use of winter precipitation by upper and lower elevation Douglas fir in the Northern Rockies. Global change biology, 24(12), 5607-5621.

Mattson, D.J. and Arundel, T.A. (2013) Consumption of seeds of south- western white pine (*Pinus strobiformis*) by black bear (*Ursus americanus*). Southwest. Naturalist 58: 243-245

Menon M (2020) Interspecific gene flow potentiates adaptive evolution in a hybrid zone formed between *Pinus strobiformis* and *Pinus flexilis.* Doctoral dissertation, Virginia Commonwealth University

Menon M, Bagley JC, Friedline CJ, et al.(2018) The role of hybridization during ecological divergence of southwestern white pine (*Pinus strobiformis*) and limber pine (*P. flexilis*). Mol Ecol 27:1245–1260. https://doi.org/10.1111/mec.14505

Moler, ERV (2020) Climate change studies of Southwestern White Pine: Phenotypic and life history trait responses to temperature manipulations during seed development and seedling growth. Doctoral dissertation, Northern Arizona University

Moler E, Kolb T, Brady A, et al.(2020) Developmental stage during experimentally elevated temperature moderates pine seedling phenotypic and demographic responses.

Moler ERV., Page G, Flores-Renteria L, Garms CG, Hull, JB, Cooper, HF, Swenson, JK, Perks, S,Waring, KM, and Whipple, AV (2021) A method for experimental warming of developing tree seeds with a common garden demonstration of seedling responses. Plant Methods 17, 1 https://doi.org/10.1186/s13007-020-00700-7

Montgomery RA, Rice KE, Stefanski A, et al.(2020) Phenological responses of temperate and boreal trees to warming depend on ambient spring temperatures, leaf habit, and geographic range. Proc Natl Acad Sci USA 117:10397–10405. https://doi.org/10.1073/pnas.1917508117

Montwé D, Isaac-Renton M, Hamann A, Spiecker H (2018) Cold adaptation recorded in tree rings highlights risks associated with climate change and assisted migration. Nat Commun 9:1–7. https://doi.org/10.1038/s41467-018-04039-5

Moreno-Letelier A, Piñero D (2009) Phylogeographic structure of *Pinus strobiformis* Engelm. across the Chihuahuan Desert filter-barrier. J Biogeogr 36:121–131. https://doi.org/10.1111/j.1365-2699.2008.02001.x

Namroud MC, Beaulieu J, Juge N, et al.(2008) Scanning the genome for gene single nucleotide polymorphisms involved in adaptive population differentiation in white spruce. Mol Ecol 17:3599–3613. https://doi.org/10.1111/j.1365-294X.2008.03840.x

Parchman TL, Gompert Z, Mudge J, et al.(2012) Genome-wide association genetics of an adaptive trait in lodgepole pine. Mol Ecol 21:2991–3005. https://doi.org/10.1111/j.1365-294X.2012.05513.x

Parchman TL, Jahner JP, Uckele KA, et al.(2018) RADseq approaches and applications for forest tree genetics. Tree Genet Genomes 14:39. https://doi.org/10.1007/s11295-018-1251-3

Penczykowski RM, Connolly BM, Barton BT (2017) Winter is changing: Trophic interactions under altered snow regimes. Food Webs 13:80–91. https://doi.org/10.1016/j.fooweb.2017.02.006

Peterson BK, Weber JN, Kay EH, et al.(2012) Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS One 7:. https://doi.org/10.1371/journal.pone.0037135

Poulos HM (2014) Tree mortality from a global-change type drought coupled with a short-duration freezing event in a Southwestern piñon-juniper woodland, USA. https://doi.org/10.7287/peerj.preprints.237v1

Rehfeldt GE, Leites LP, St Clair BJ, et al.(2014) Comparative genetic responses to climate in the varieties of Pinus ponderosa and *Pseudotsuga menziesii*: Clines in growth potential. For Ecol Manage 324:138–146. https://doi.org/10.1016/j.foreco.2014.02.041

Rellstab C, Gugerli F, Eckert AJ, et al.(2015) A practical guide to environmental association analysis in landscape genomics. Mol Ecol 24:4348–4370. https://doi.org/10.1111/mec.13322

Rosenblad KC, Perret DL, Sax DF (2019) Niche syndromes reveal climate-driven extinction threat to island endemic conifers. Nat Clim Chang 9:627–631. https://doi.org/10.1038/s41558-019-0530-9

Sáenz-Romero, C, Kremer, A, Nagy, L, Újvári-Jármay, É, Ducousso, A, Kóczán-Horváth, A, Mátyás, C (2019) Common garden comparisons confirm inherited differences in sensitivity to climate change between forest tree species. PeerJ, (1), 1–28. https://doi.org/10.7717/peerj.6213

Sakulich, J, Taylor, AH, (2007) Fire regimes and forest structure in a sky island mixed conifer forest, Guadalupe Mountains National Park, Texas, USA. For. Ecol. Manage. 241, 62–73.

Samano, S, and Tomback, DF, (2003) Cone opening phenology, seed dispersal, and seed predation in southwestern white pine (*Pinus strobiformis*) in southern Colorado. Ecoscience 10, 319–326. https://doi.org/10.1080/11956860.2003.11682780

Schuster, WS., Alles, DL, & Mitton, JB (1989) Gene Flow in Limber Pine: Evidence from Pollination Phenology and Genetic Differentiation Along an Elevational Transect American Journal of Botany, 76(9), 1395–1403. https://www.jstor.org/stable/2444563

Seager R, Vecchi GA (2010) Greenhouse warming and the 21st century hydroclimate of southwestern North America. Proc Natl Acad Sci USA https://doi.org/10.1073/pnas.0910856107

Shirk AJ, Cushman SA, Waring KM, et al.(2018) Southwestern white pine (*Pinus strobiformis*) species distribution models project a large range shift and contraction due to regional

climatic changes. For Ecol Manage 411:176–186. https://doi.org/10.1016/j.foreco.2018.01.025

St. Clair J, Howe GT (2007) Genetic maladaptation of coastal Douglas-fir seedlings to future climates. Glob Chang Biol 13:1441–1454. https://doi.org/10.1111/j.1365-2486.2007.01385.x

Sutinen, M-L, Arora, R, Wisniewski, M, Ashworth, E, Strimbeck, R, Palta, J. (2001) Mechanisms of Frost Survival and Freeze-Damage in Nature. *In* F.J. Bigras and S.J. Colombo (eds). Conifer cold hardiness. Kluwer Academic Publ., Dordecht. The Netherlands. 89-120. 10.1007/978-94-015-9650-3_4.

Swenson (2021) Chapter one: Detection of environment specific alleles for phenology and survival at the edge of a conifer's climate niche. MSc thesis, Northern Arizona University

Winkler DE, Butz RJ, Germino MJ, et al.(2018) Snowmelt Timing Regulates Community Composition, Phenology, and Physiological Performance of Alpine Plants. Front Plant Sci 9:1140. https://doi.org/10.3389/fpls.2018.01140

Zhou, X, Stephens, M (2012) Genome-wide efficient mixed-model analysis for association studies. Nature genetics. 44:821–4.

CHAPTER TWO TABLES AND FIGURES

TABLE 1: Description of traits used in this analysis. Measurements were conducted across all three gardens except for lammas growth. Lammas measurements were only taken at the low elevation garden.

Trait Group	Trait	Time	Abbreviation	Description
Survival	Overall	Fall 2019	surv19	Survival (1) or mortality (0) from time of planting to fall 2019.
Spring Phenology	Bud Advancement	Spring 2018	budadv18	The difference in bud stage (0-4) between two spring measurement times.
	Bud Advancement	Spring 2019	budadv19	
	Bud Burst Slope	Spring 2018	budslp18	The slope of the line between the change from first and second bud stage measurements relative to Julian day.
	Bud Burst Slope	Spring 2019	budslp19	
Fall Phenology	Bud Variation	Fall 2018	budv18	Active buds (0-1) or dormant/set buds (2-3). Needle length relative to full length needles varied within active and dormant buds.
	Bud Variation	Fall 2019	budv19	
	Bud Set	Fall 2018	set18	Bud set or dormant (1) or active (0).
	Bud Set	Fall 2019	set19	
Bud Injury	Bud Damage	Spring 2018	bdmgS18	Presence of a damaged bud (0) or normally/fully formed bud (1).
	Bud Damage	Fall 2018	bdmgF18	
	Bud Damage	Spring 2019	bdmgS19	
	Bud Damage	Fall 2019	bdmgF19	
Lammas	Lammas Growth	Fall 2019	lms19	Presence of lammas growth (1) or absence (0) at the end of the growing season.

TABLE 2: Results from generalized linear mixed effects models for phenological traits and survival for *Pinus strobiformis* seedlings at each garden. A "+" indicates a positive relationship, and a "–" indicates a negative relationship with survival. The direction of effects of spring phenology (bud burst), fall phenology (bud set), bud damage, and lammas on garden specific seedling survival were used to group alleles as favorable or unfavorable at each garden. See Table 3 for the top model at each garden. Higher values for spring phenology represent early bud burst, and high values for bud set represent earlier bud set. Higher values of trait "bud damage" represent buds that fully formed or lacked bud damage, whereas lower values represent more bud damage. Here a positive relationship means trees with fully formed buds were more likely to survive than those that were damaged.

Garden	**Trait**			
	Early Bud Burst	Early Bud Set	Lack of Bud Damage	Lammas
Low	+	+	+	–
Intermediate	no relationship	+	+	NA
High	–	+	+	NA

TABLE 3: Results from generalized linear mixed effects models of *Pinus strobiformis* seedling survival at three common garden sites. The top model for each garden is displayed. The variables considered for the top model were for spring phenology, fall phenology, and bud damage measurements across two years (2018-2019). β represents the slope estimate from the model for each parameter, SE(β) is the standard error of the slope, *p-value* is based on a Z-score (α=0.05),, and the 95% confidence intervals were calculated using program R base *stats* package.

Garden	**Top Model**		**β**	**SE(β)**	***p-value***		**95% confidence interval**	
Low	surv19~ bdmgS19 + budadv18+ budv18	(Intercept)	-0.40	0.09	1.14E-05	***	-0.59	-0.22
		bdmgS19	0.57	0.11	2.16E-07	***	0.36	0.79
		budadv18	0.31	0.03	< 2e-16	***	0.25	0.37
		budv18	0.07	0.03	0.0148	*	0.01	0.12
Intermediate	surv19~ bdmgS19 + set19	(Intercept)	0.41	0.10	2.59E-05	***	0.22	0.61
		bdmgS19	0.34	0.10	7.06E-04	***	0.14	0.53
		set19	0.09	0.04	2.23E-02	*	0.01	0.17
High	surv19~ bdmgF18 + budadv19 + budv19	(Intercept)	0.32	0.08	3.48E-05	***	0.17	0.48
		bdmgF18	0.55	0.05	< 2e-16	***	0.46	0.65
		budadv19	-0.09	0.03	6.07E-03	**	-0.15	-0.02
		budv19	0.06	0.02	4.53E-03	**	0.02	0.10

TABLE 4: Number of SNPs included in each minor allele grouping by trait. Minor alleles that were associated with survival, bud burst, bud damage, and lammas were grouped as favorable or unfavorable based on their β effect (see Swenson 2021) and relationship with garden specific survival. The SNPs in each grouping were used to calculate the minor allele counts at each garden. Some SNPs were detected for multiple traits but were only included once in each SNP grouping. Therefore, the sum of SNPs across traits will not add up to the total number (Total #) of SNPs used in analysis.

Minor Allele Grouping	**Garden**	**Number of SNPs for each Trait**					**Total #**
		Survival	Bud Burst	Bud Set	Bud Damage	Lammas	
Unfavorable	$Low_{(Lammas)}$	33	17	3	9	34	**82**
	Low	33	17	3	9	0	**48**
	Intermediate	33	0	3	9	0	**39**
	High	33	22	3	9	0	**52**
Favorable	Low	8	20	0	0	0	**22**
	Intermediate	8	6*	0	0	0	**8**
	High	8	15	0	0	0	**17**
Spring Phenology	All	0	37	0	0	0	**37**

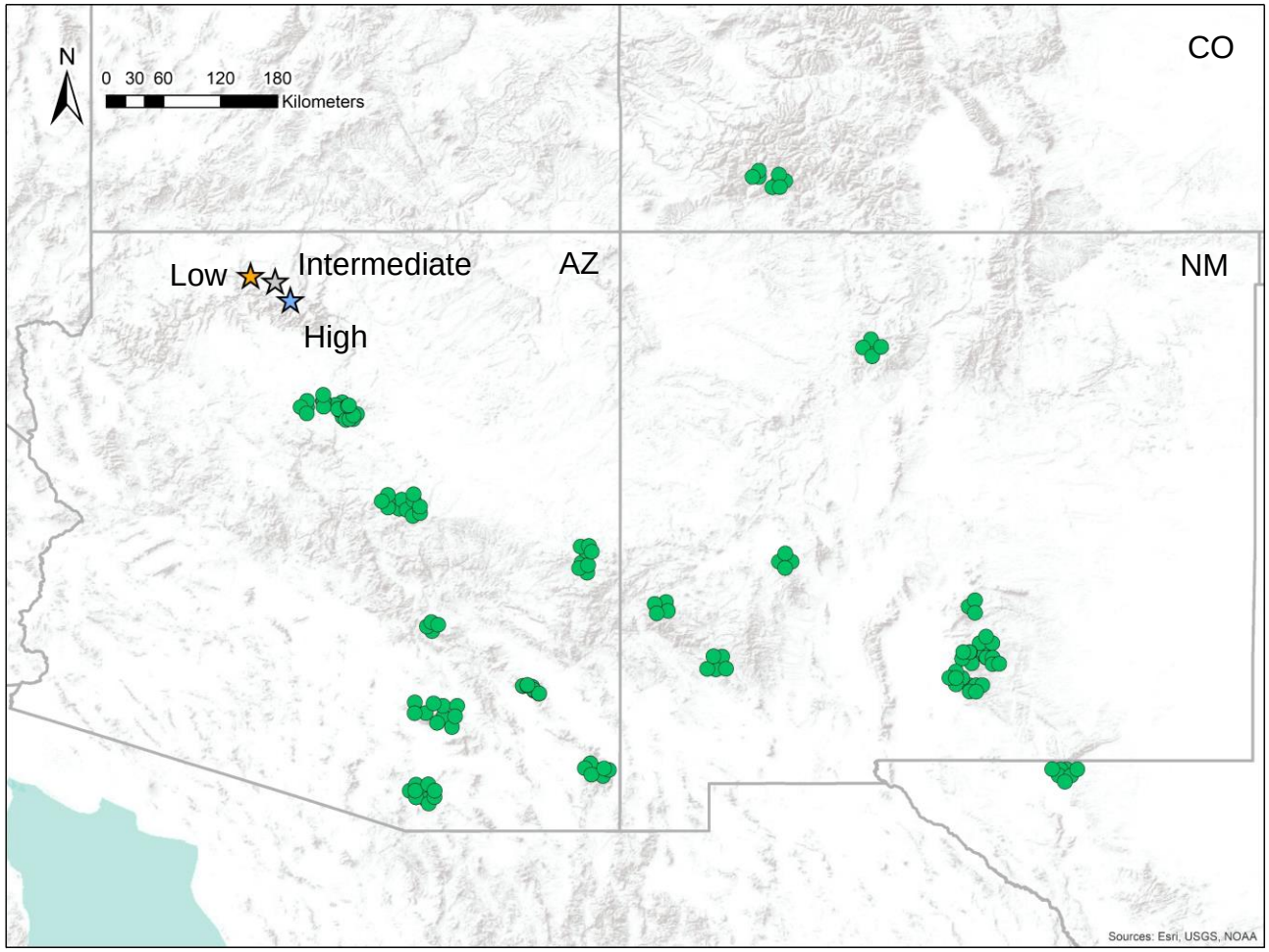

FIGURE 1: Location of maternal seed source sites (n=202) for *Pinus Strobiformis* and location of the three common gardens. Stars represent the three common gardens in the North Kaibab National Forest (orange=low elevation, grey=intermediate, and blue=high). Trees represent locations of maternal seed sources throughout the US range (n=202).

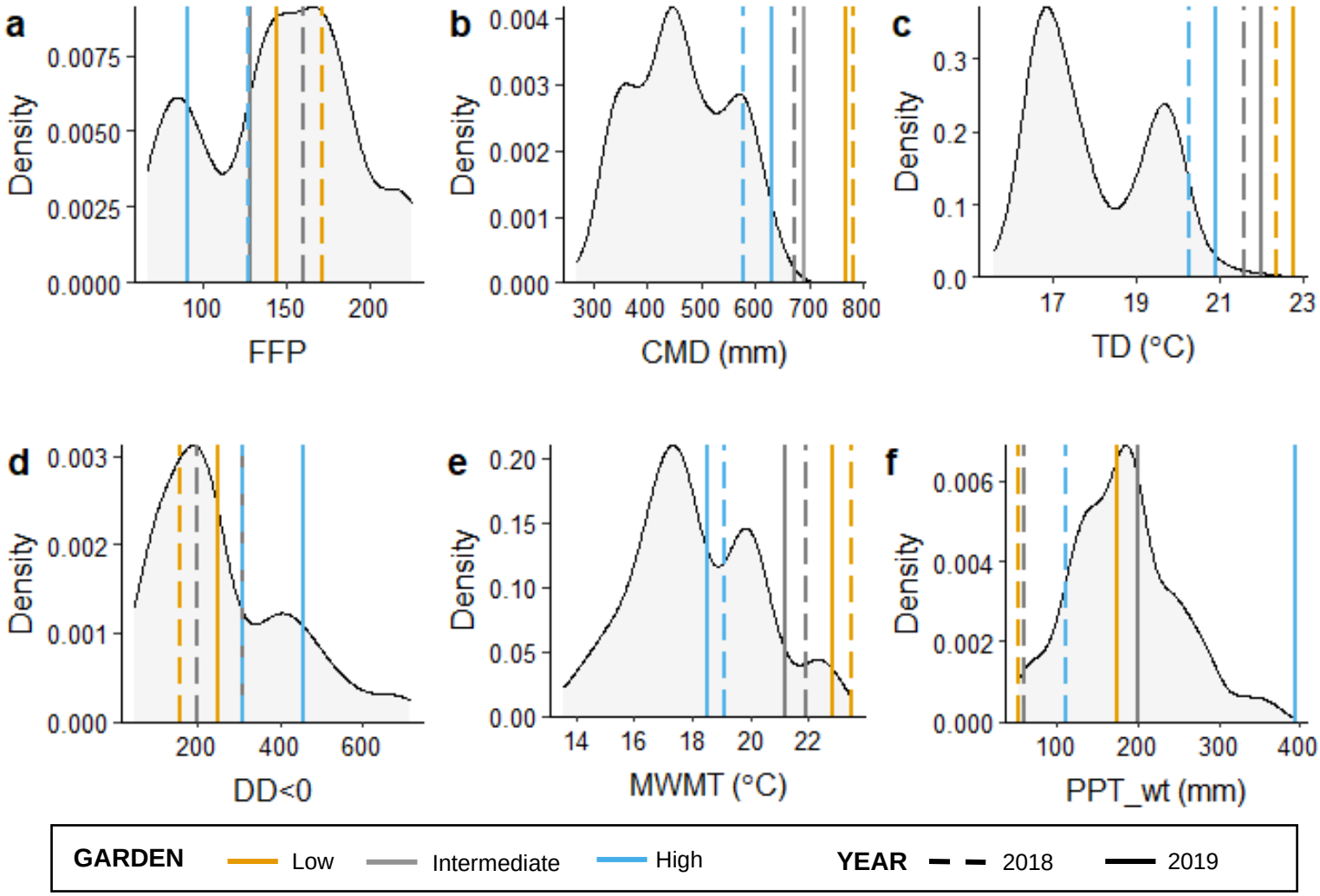

FIGURE 2: Distributions of maternal site (n=202) climate variables relative to garden climate values in 2018 and 2019. The vertical lines represent the 2018 (dashed) and 2019 (solid) climate values for the low (orange), the intermediate (gray), and the high (blue) elevation gardens. Winter precipitation (PPT_wt) for 2018 and 2019 was actually December of the previous year through February of that year. The other climate variables frost free period (FFP), climate moisture deficit (CMD), continentality (TD), degree days below zero (DD<0), and mean warmest month temperature.

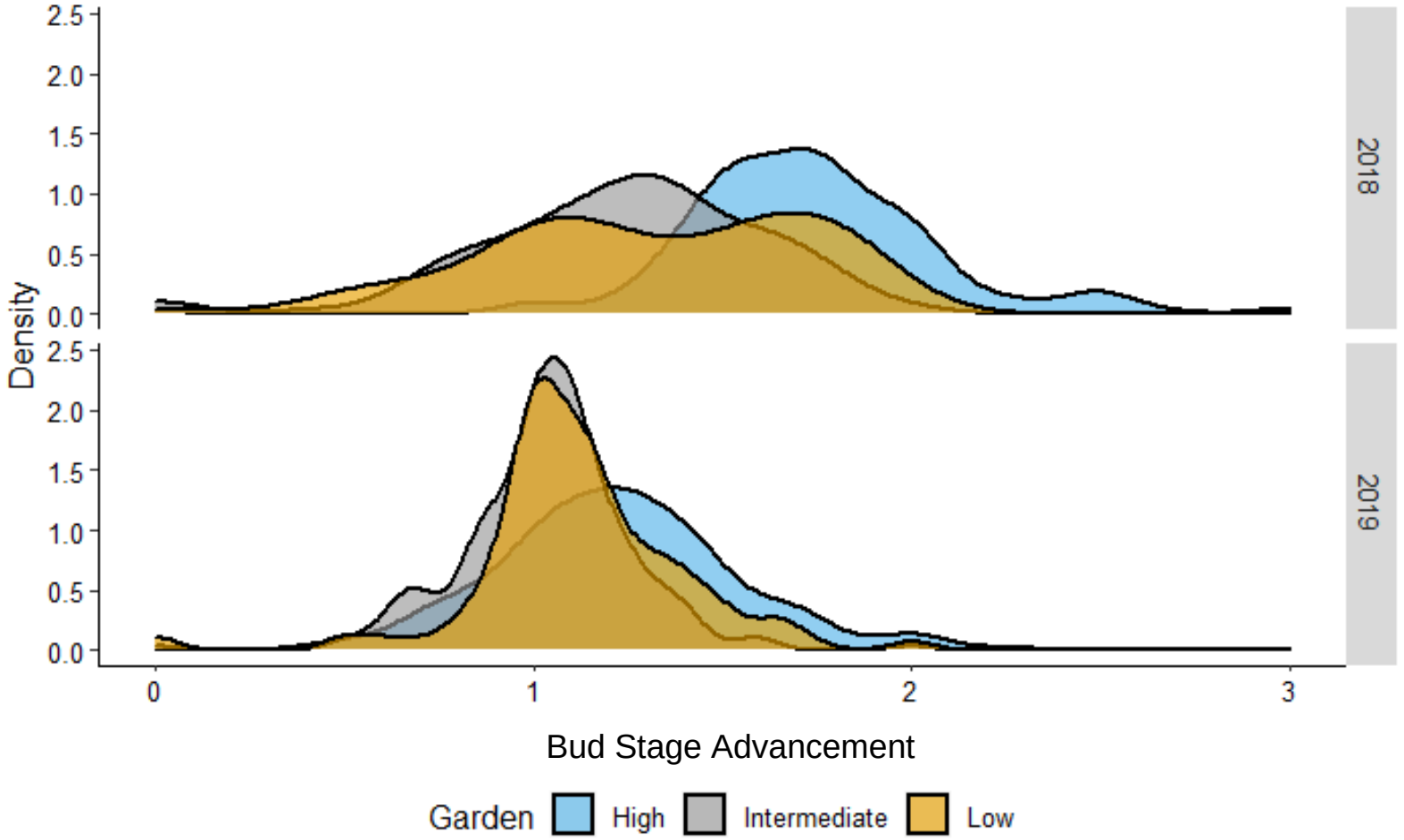

FIGURE 3: Spring phenology bud stage advancement distributions by garden in 2018 and 2019. Spring phenology bud stage advancement is the raw difference between the first and second bud stage measurements recorded at each garden during the growing season of 2018 and 2019 on *Pinus strobiformis* seedlings. Bud stage advancement represents the number of bud stages advanced during the measurement time frame.

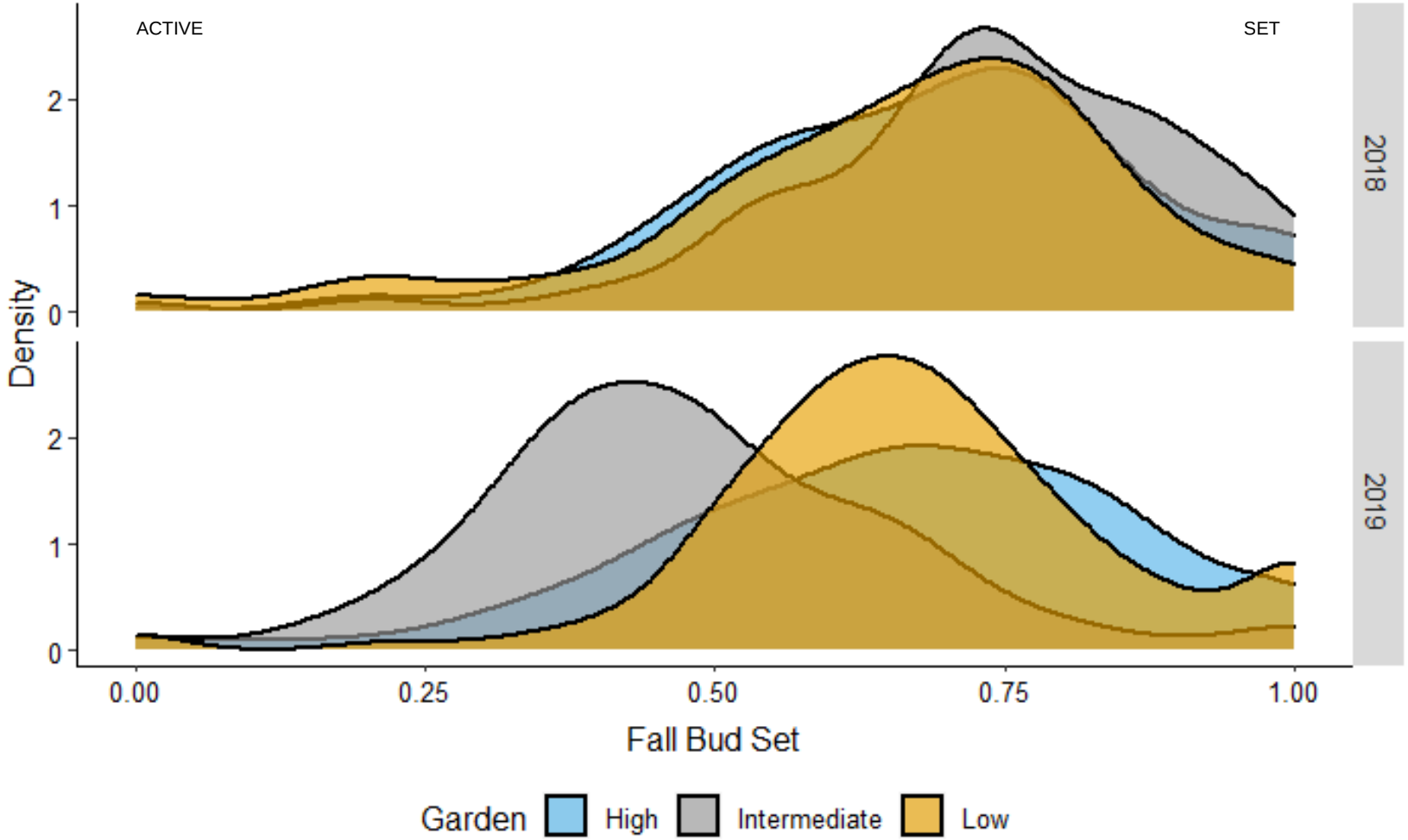

FIGURE 4: Fall phenology bud set distributions by garden in 2018 and 2019. Fall phenology bud set was measured once at each garden in 2018 and 2019 on *Pinus strobiformis* seedlings. The average bud set stage (0=active, 1=dormant/set) at each measurement time and garden was calculated for each maternal tree (n=202) and is displayed here. A second measurement that encapsulates the variation in bud dormancy and needle length was also included in the study (budv) but is not displayed.

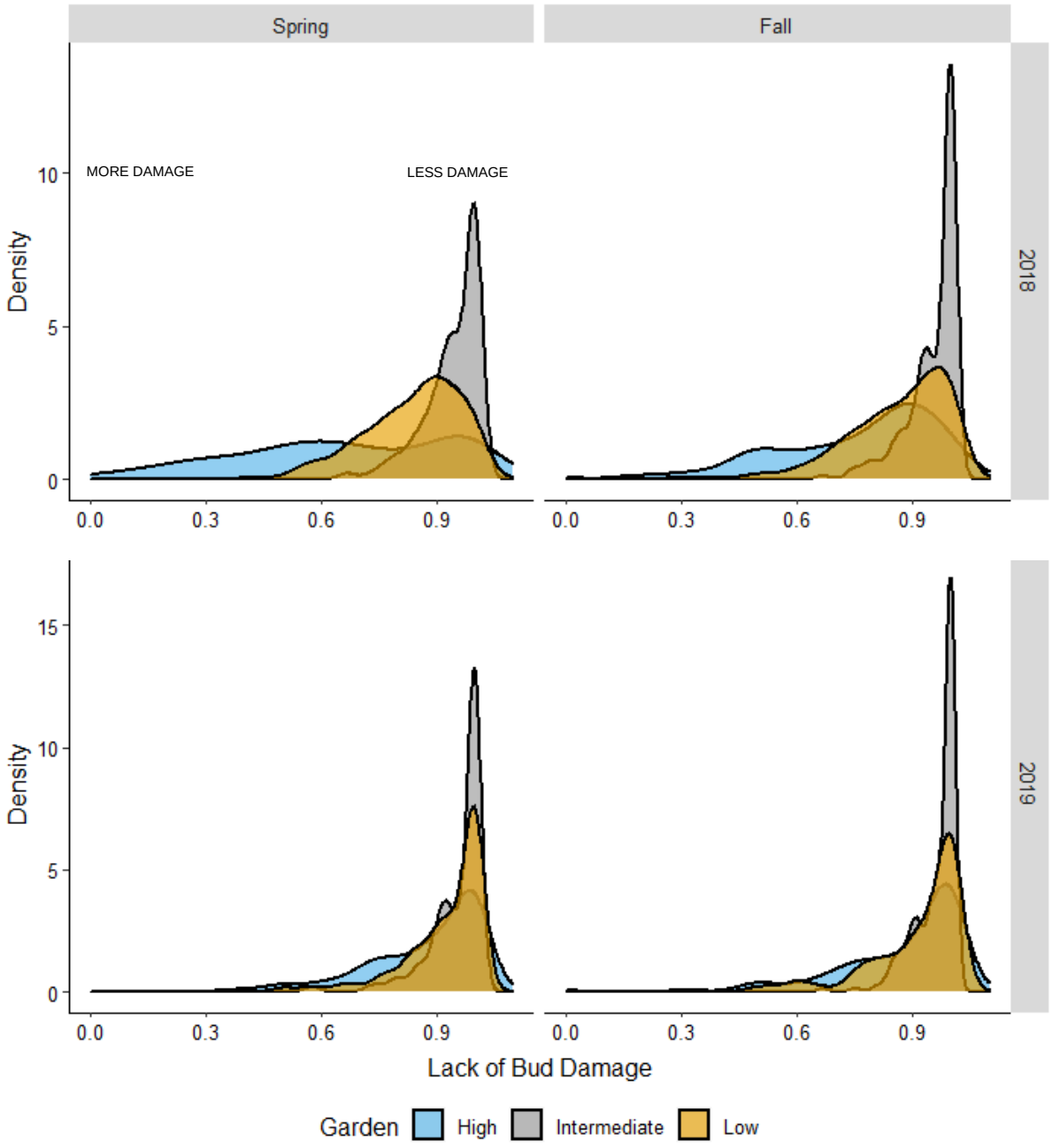

FIGURE 5: Lack of bud damage distributions by garden and season in 2018 and 2019. Bud damage (0) was measured in the spring and fall at each garden in 2018 and 2019 on *Pinus strobiformis* seedlings. The average lack of bud damage (0=damage, 1= no damage/fully formed bud) at each measurement time and garden was calculated for each maternal tree (n=202) and is displayed here.

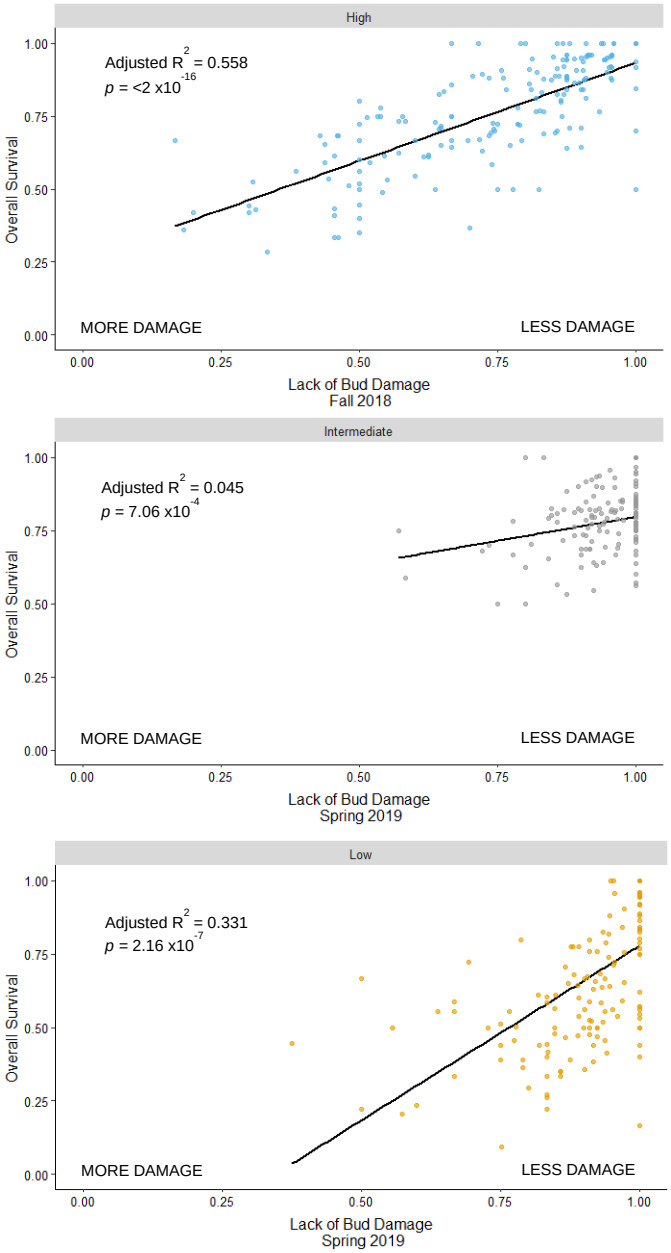

FIGURE 6: The bud damage measurement time that most influenced *P. strobiformis* seedlings overall survival at each garden. Lack of bud damage increased survival at all gardens. Higher values on the x-axis represent trees that were not damaged, and lower values represent more damage. On the y axis higher values represent greater survival, and lower values represent greater mortality. At the low ($\beta = 0.57 \pm 0.11$, CI: 0.36,0.79) and intermediate ($\beta = 0.34 \pm 0.1$, CI: 0.22, 0.61) garden spring 2019 bud damage explained the most variation in survival, whereas fall 2018 bud damage explained the most variation at the high elevation garden ($\beta = 0.55 \pm 0.05$, CI: 0.46, 0.65). Results are from the full top model at each garden (Table 3).

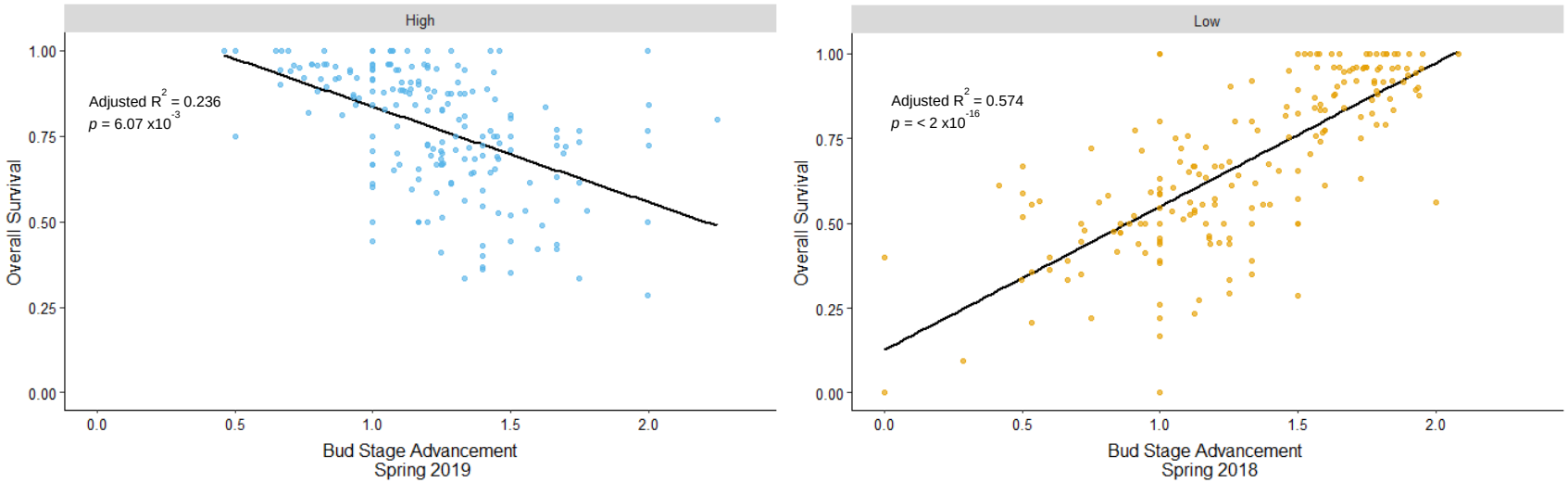

FIGURE 7: The spring phenology measurement that most influenced overall survival at the high and low elevation gardens. Higher values on the x-axis represent trees that advanced more bud stages, and lower values represent trees that grew more slowly or advanced fewer bud stages. On the y axis, higher values represent greater survival, and lower values represent greater mortality. There was no significant relationship between spring phenology traits and survival at the intermediate garden, not displayed here. At the low elevation garden 2018 bud stage advancement in 2018 increased survival ($\beta = 0.31 \pm 0.03$, CI: 0.25, 0.37) and spring 2019 bud stage advancement at the high elevation garden had a negative effect on survival ($\beta = -0.09 \pm 0.3$, CI: -0.15, -0.02). Results are from the full top model at each garden (Table 3).

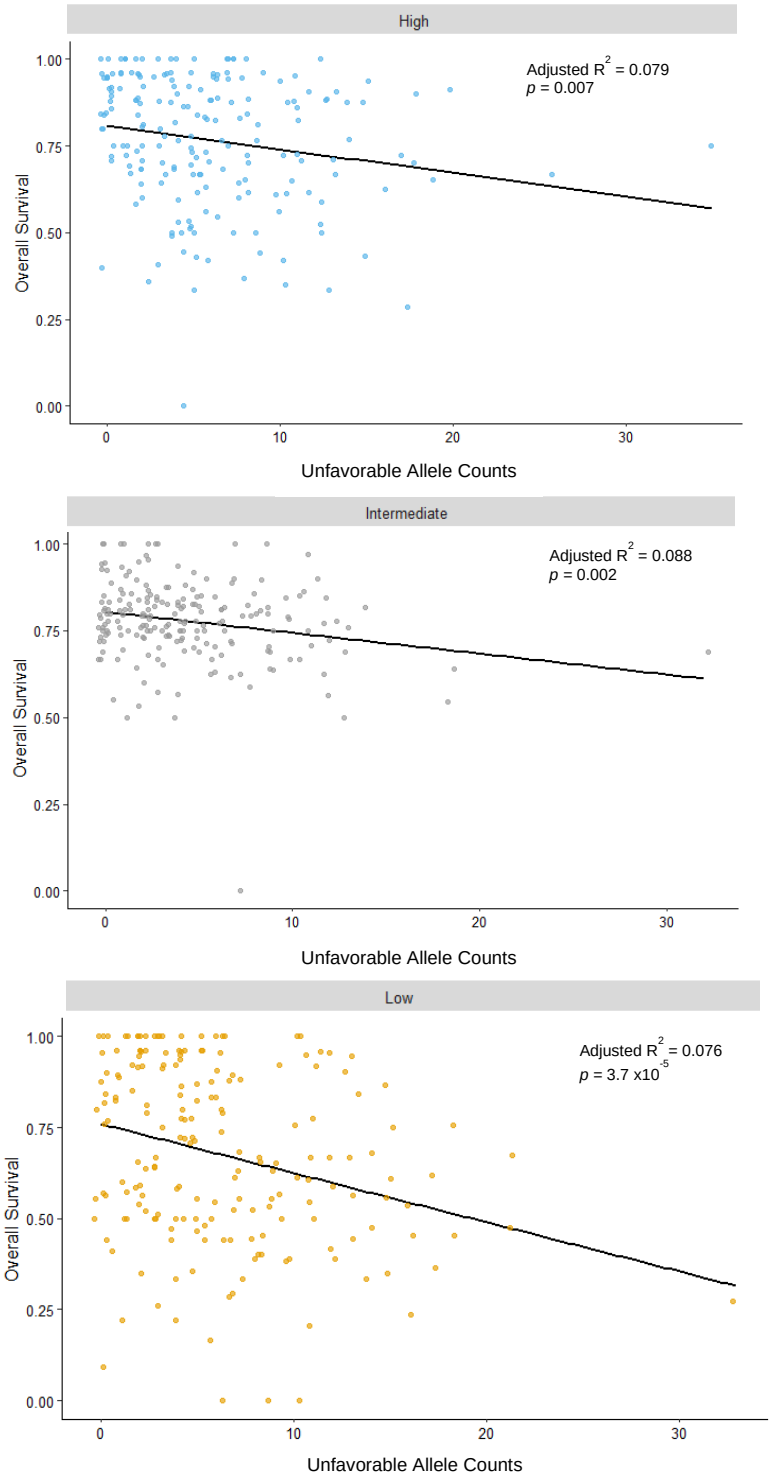

FIGURE 8: Minor allele counts and survival across gardens. In all situations having fewer rare alleles increased survival. The y-axis represents the overall survival for each individual garden. The y-axis represents the number of minor or rare allele counts for SNPs grouped as unfavorable for each garden or for spring phenology across all gardens. For generalized linear mixed effects model results see Table S4.

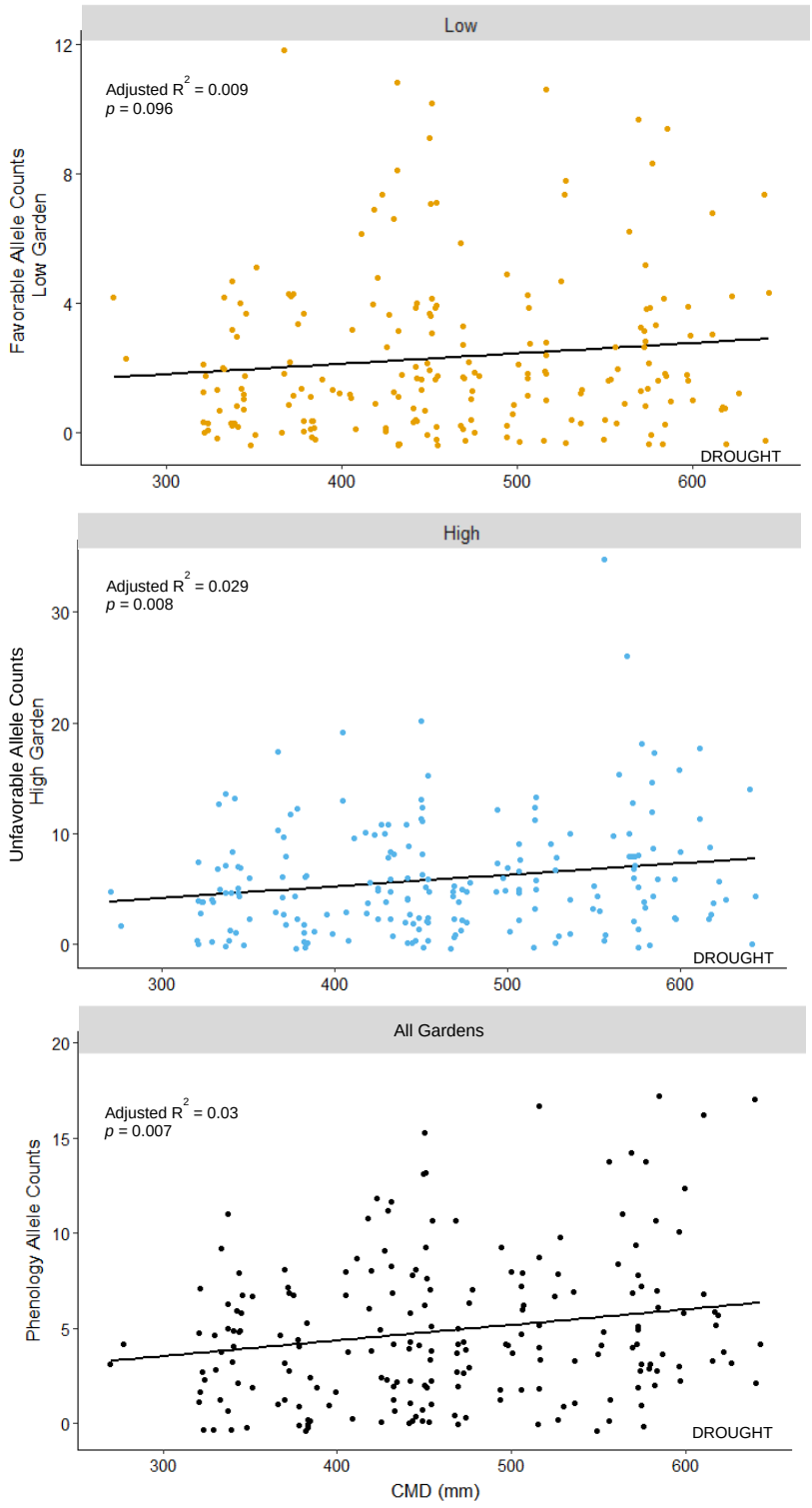

FIGURE 9 Relationships between *P. strobiformis* rare allele counts and seed source climate moisture deficit. Maternal trees with more minor alleles experience more climate moisture deficit in their home range. This relationship was true for minor allele counts across all three gardens for unfavorable and favorable minor alleles (see Table S2 for full model details). A higher number of minor alleles for spring phenology was significantly related to more seed source climate moisture deficit (p=0.007, β= 0.008 ±0.003).

CHAPTER TWO SUPPLEMENTARY FIGURES

TABLE S1: Results from ANOVA models for *P. strobiformis* seedlings comparing annual variation in phenological traits between 2018 and 2019. The table includes spring and fall phenology traits, annual averages ($\bar{x}$), standard error (SE($\bar{x}$))**,** *p-values* indicating significant differences based on an F-test (α=0.05), and the 95% confidence intervals were calculated using program R base stats package.

Garden	**Trait Group**	**Trait**	**2018**		**2019**		***p-value***		**95% CI**	
			$\bar{x}$	**SE($\bar{x}$)**	$\bar{x}$	**SE($\bar{x}$)**				
Low	Spring Phenology	budadv	1.302	0.031	1.119	0.020	9.45E-08	***	-0.270	-0.127
Low	Spring Phenology	budslp	0.032	0.001	0.027	0.000	6.00E-10	***	-0.007	-0.004
Low	Fall Phenology	budv	2.259	0.030	2.281	0.026	0.636		-0.060	0.098
Low	Fall Phenology	set	0.652	0.015	0.668	0.013	0.547		-0.027	0.051
Intermediate	Spring Phenology	budadv	1.228	0.027	1.038	0.016	7.65E-10	***	-0.256	-0.135
Intermediate	Spring Phenology	budslp	0.040	0.001	0.025	0.000	<2e-16	***	-0.018	-0.013
Intermediate	Fall Phenology	budv	2.246	0.021	1.855	0.023	<2e-16	***	-0.448	-0.325
Intermediate	Fall Phenology	set	0.734	0.012	0.478	0.013	<2e-16	***	-0.291	-0.222
High	Spring Phenology	budadv	1.737	0.025	1.226	0.022	<2e-16	***	-0.584	-0.451
High	Spring Phenology	budslp	0.054	0.001	0.044	0.001	<2e-16	***	-0.013	-0.009
High	Fall Phenology	budv	2.158	0.024	2.165	0.030	0.864		-0.070	0.083
High	Fall Phenology	set	0.685	0.013	0.654	0.015	0.0919	.	-0.074	0.006

TABLE S2: Results from two-way ANOVA models for *P. strobiformis* seedlings comparing annual and seasonal variation in bud damage. The table includes the general model results, see TABLE S3 for Tukey post-hoc analyses. Group includes Year (2018 and 2019) and Season (Spring or Fall), Df are the degrees of freedom, and the *p-value* is based on an F-test (α=0.05).

Garden	**Group**	**Trait**	**Df**	**F value**	***p-value***
Low	Year	Bud Damage	1	54.582	3.92E-13
Low	Season	Bud Damage	1	0.252	0.616
Low	Year:Season	Bud Damage	1	2.516	0.113
Intermediate	Year	Bud Damage	1	4.298	0.039
Intermediate	Season	Bud Damage	1	5.120	0.024
Intermediate	Year:Season	Bud Damage	1	0.345	0.557
High	Year	Bud Damage	1	142.910	< 2e-16
High	Season	Bud Damage	1	14.670	1.38E-04
High	Year:Season	Bud Damage	1	13.520	2.53E-04

TABLE S3: Results from Tukey post-hoc analysis of the two-way ANOVA models (Table S2) for *P. strobiformis* seedlings comparing annual and seasonal variation in bud damage. The table includes only the comparisons across years for either spring or fall because those were of biological interest. The table includes spring and fall bud damage annual averages ($\bar{x}$), standard error (SE($\bar{x}$)), *p-adj* indicates significant differences for just the pair selected based on an F-test (α=0.05), and the 95% confidence intervals were calculated using program R base stats package.

Garden	**Pair**	**2018**		**2019**		**Difference**	**95 % CI**		**p adj**
		$\bar{x}$	**SE($\bar{x}$)**	$\bar{x}$	**SE($\bar{x}$)**	**(*2019-2018*)**			
Low	2019:Fall-2018:Fall	0.859	0.010	0.912	0.009	0.053	0.020	0.086	2.8E-04
Low	2019:Spring-2018:Spring	0.840	0.009	0.922	0.008	0.082	0.049	0.115	0
Intermediate	2019:Fall-2018:Fall	0.957	0.005	0.964	0.004	0.007	-0.010	0.024	0.723
Intermediate	2019:Spring-2018:Spring	0.943	0.005	0.956	0.005	0.012	-0.005	0.029	0.238
High	2019:Fall-2018:Fall	0.761	0.014	0.880	0.011	0.119	0.067	0.172	0
High	2019:Spring-2018:Spring	0.652	0.020	0.878	0.010	0.225	0.173	0.278	0

TABLE S4: Results from generalized linear mixed effects models on *Pinus strobiformis* seedling garden specific overall survival (surv19) and rare allele counts for unfavorable, favorable or phenological SNPs. A significant result in all models indicated that individuals with a higher number of rare alleles had greater garden specific mortality. β represents the model estimate for the slope of the relationship between each set of variables, slope estimate from the model for each parameter, SE(β) is the standard error of the slope, p-value is based on a Z-score (α=0.05), and the 95% confidence intervals were calculated using program R base stats package.

Garden	Survival (Response)	Minor Allele Count Group (Predictor)	β	SE(β)	*p-value*		95% confidence interval	
$Low_{(Lammas)}$	surv19	Unfavorable Minor Allele Counts	-0.005	0.002	0.023	*	-0.009	-0.001
Low	surv19	Unfavorable Minor Allele Counts	-0.013	0.003	3.70E-05	***	-0.020	-0.007
Low	surv19	Favorable Minor Allele Counts	-0.011	0.007	0.126		-0.024	0.003
Low	surv19	Phenology Minor Allele Counts	-0.012	0.004	0.005	**	-0.020	-0.004
Inter	surv19	Unfavorable Minor Allele Counts	-0.006	0.002	0.002	**	-0.010	-0.002
Inter	surv19	Favorable Minor Allele Counts	-0.005	0.007	0.479		-0.018	0.008
Inter	surv19	Phenology Minor Allele Counts	-0.004	0.002	0.059	.	-0.008	1.40E-04
High	surv19	Unfavorable Minor Allele Counts	-0.007	0.003	0.007	**	-0.012	-0.002
High	surv19	Favorable Minor Allele Counts	-0.008	0.011	0.467		-0.029	0.013
High	surv19	Phenology Minor Allele Counts	-0.006	0.003	0.047	*	-0.013	-7.90E-05

*Indicates a significant relationship.

TABLE S5: Results from generalized linear mixed effects models for *Pinus strobiformis* minor allele counts and seed source climate variables. The table only includes models that were significant or approaching significance (α=0.05). β represents the model estimate for the slope of the relationship between each set of variables, slope estimate from the model for each parameter, SE(β) is the standard error of the slope, *p-value* is based on a Z-score (α=0.05), and the 95% confidence intervals were calculated using program R base *stats* package.

Climate Variable (Predictor)	Garden	Minor Allele Count Group (Response)	β	SE(β)	*p-value*		95 % confidence interval	
CMD	Low	Unfavorable Minor Allele Counts	8.98E-03	3.92E-03	2.19E-02	*	8.98E-03	3.92E-03
	Low	Favorable Minor Allele Counts	3.21E-03	1.93E-03	9.60E-02	.	-5.70E-04	6.99E-03
	Intermediate	Unfavorable Minor Allele Counts	7.52E-03	3.29E-03	2.22E-02	*	1.07E-03	1.40E-02
	High	Unfavorable Minor Allele Counts	1.04E-02	3.93E-03	7.90E-03	**	2.74E-03	1.82E-02
	All	Phenology Minor Allele Counts	8.23E-03	3.06E-03	7.13E-03	**	2.23E-03	1.42E-02
PPT_wt	Low	Unfavorable Minor Allele Counts	-1.07E-02	5.48E-03	5.03E-02	.	-2.14E-02	1.46E-05
	Low	Favorable Minor Allele Counts	-8.09E-03	2.64E-03	2.21E-03	**	-1.33E-02	-2.91E-03
	Intermediate	Unfavorable Minor Allele Counts	-9.87E-03	4.59E-03	3.14E-02	*	-1.89E-02	-8.79E-04
	Intermediate	Favorable Minor Allele Counts	-2.77E-03	1.33E-03	3.80E-02	*	-5.38E-03	-1.53E-04
	High	Unfavorable Minor Allele Counts	-1.45E-02	5.47E-03	7.93E-03	**	-2.53E-02	-3.80E-03
	All	Phenology Minor Allele Counts	-1.21E-02	4.25E-03	4.35E-03	**	-2.04E-02	-3.79E-03
TD	$Low_{(Lammas)}$	Unfavorable Minor Allele Counts	8.21E-01	4.20E-01	5.04E-02	.	-1.94E-01	8.07E-01

*Indicates a significant relationship.

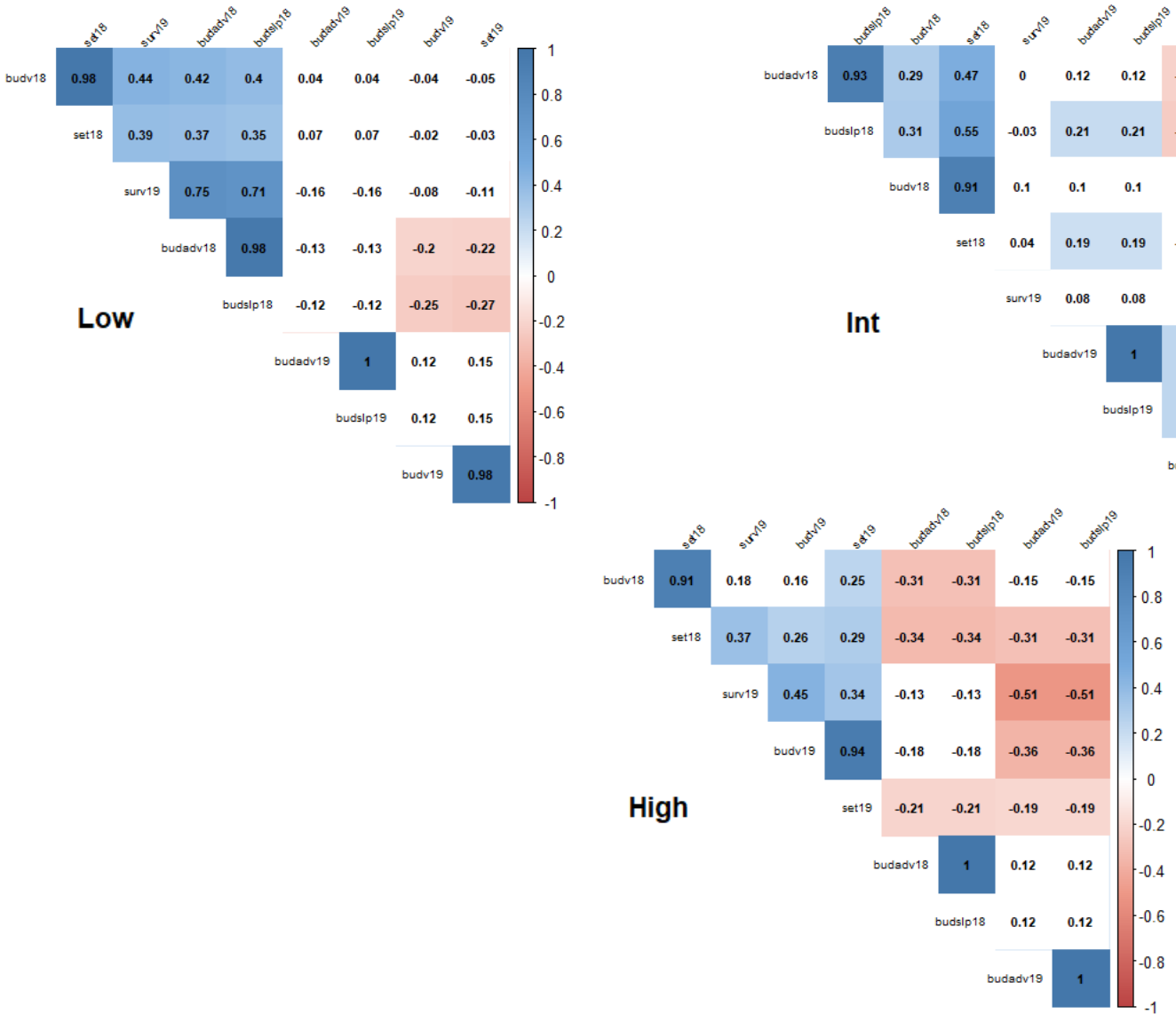

FIGURE S1: Correlation matrices for maternal tree trait averages at each garden. Red indicates a significant negative relationship whereas blue gradients represent significant positive relationships.

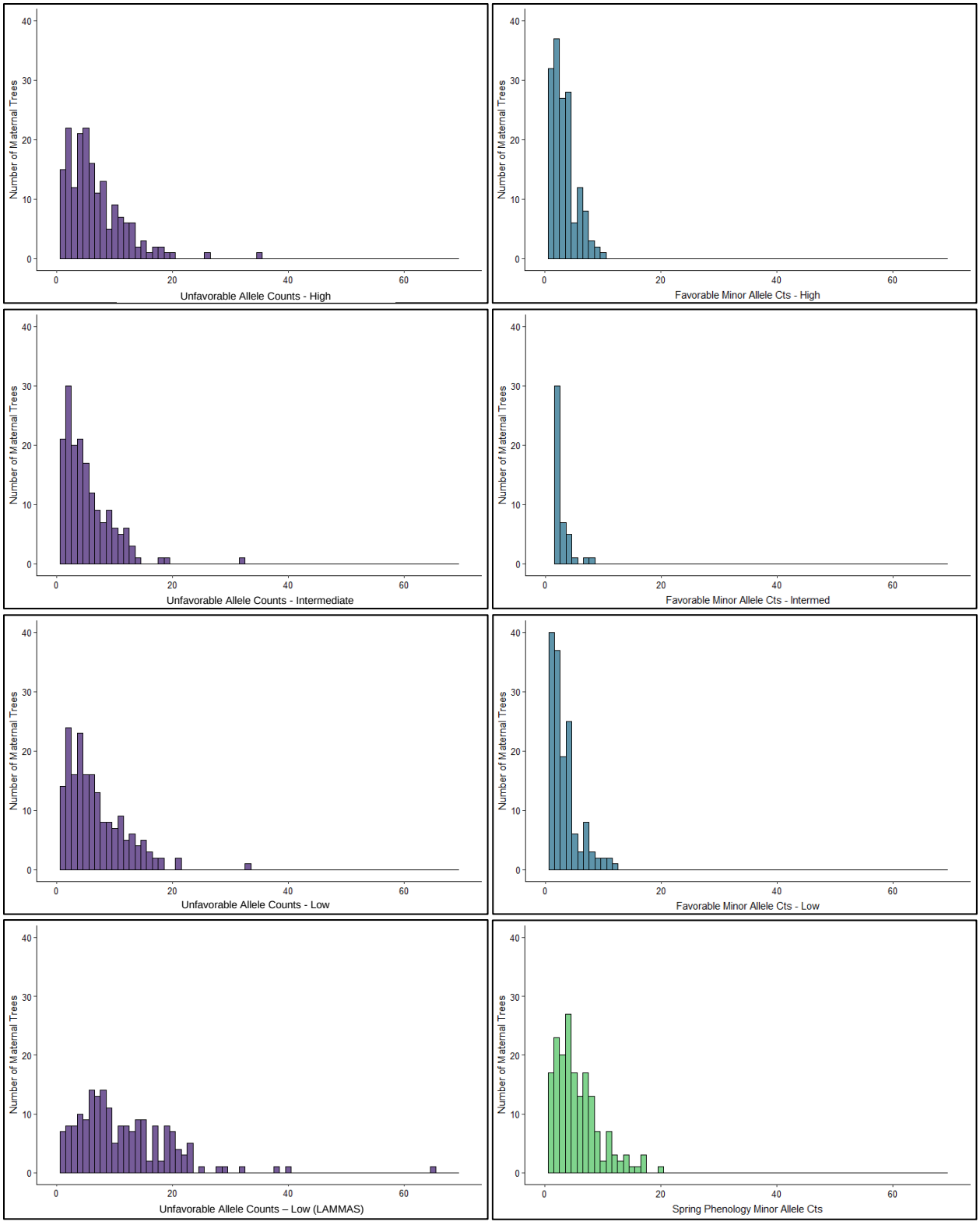

FIGURE S2: Distribution of minor allele counts. The graph displays the number of maternal trees (y-axis) that had a particular minor allele count (x-axis) for unfavorable (purple), favorable (blue), or spring phenological (green) minor alleles.

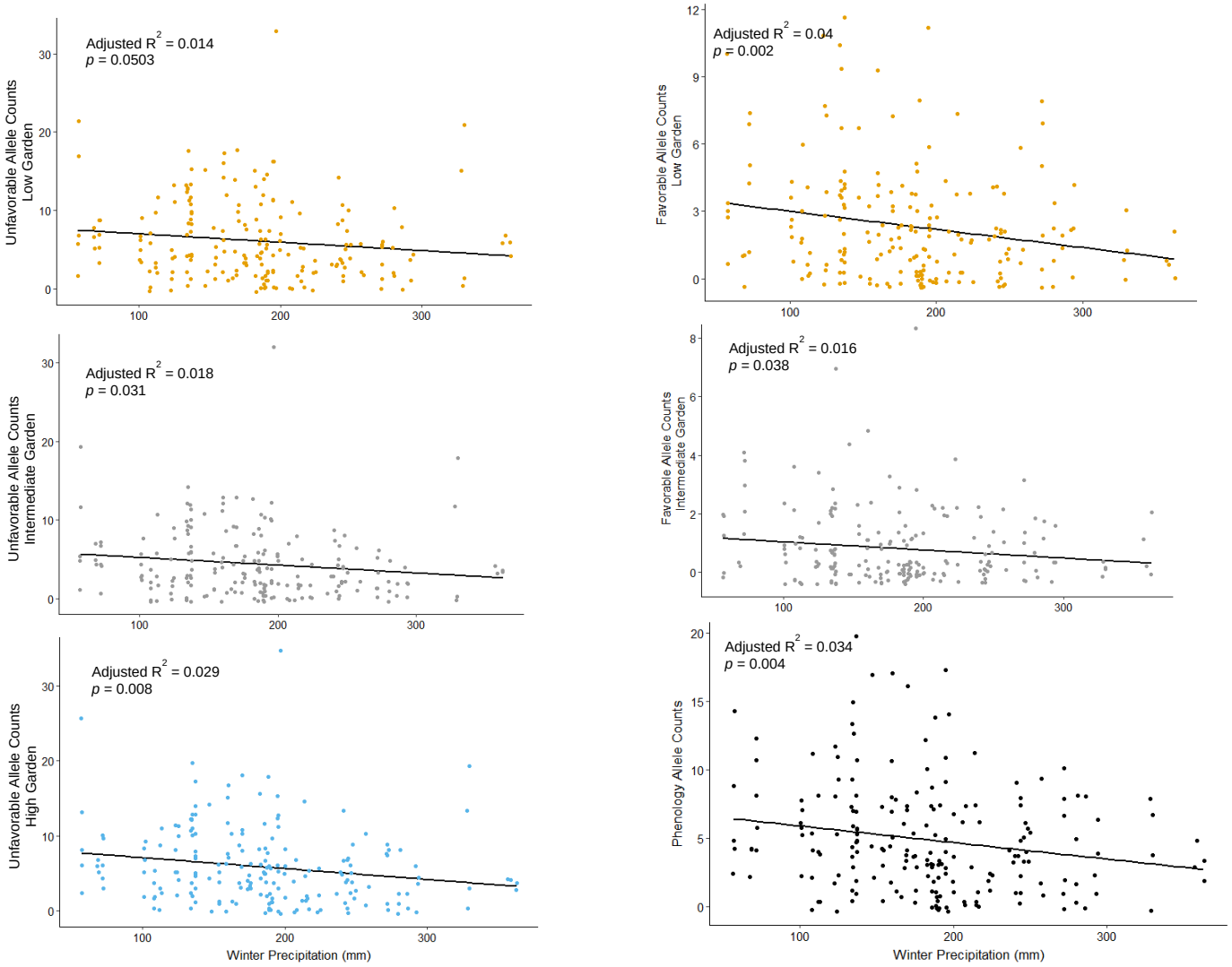

FIGURE S3: Relationships between *P. strobiformis* minor allele counts and seed source winter precipitation. Higher counts of minor alleles were significantly associated with less winter precipitation at all three gardens for unfavorable and favorable allele groupings (see Table S2 for model details). Higher counts of minor alleles for spring phenology was also significantly related to less winter precipitation (p=0.004, β= –0.01 ±0.004).

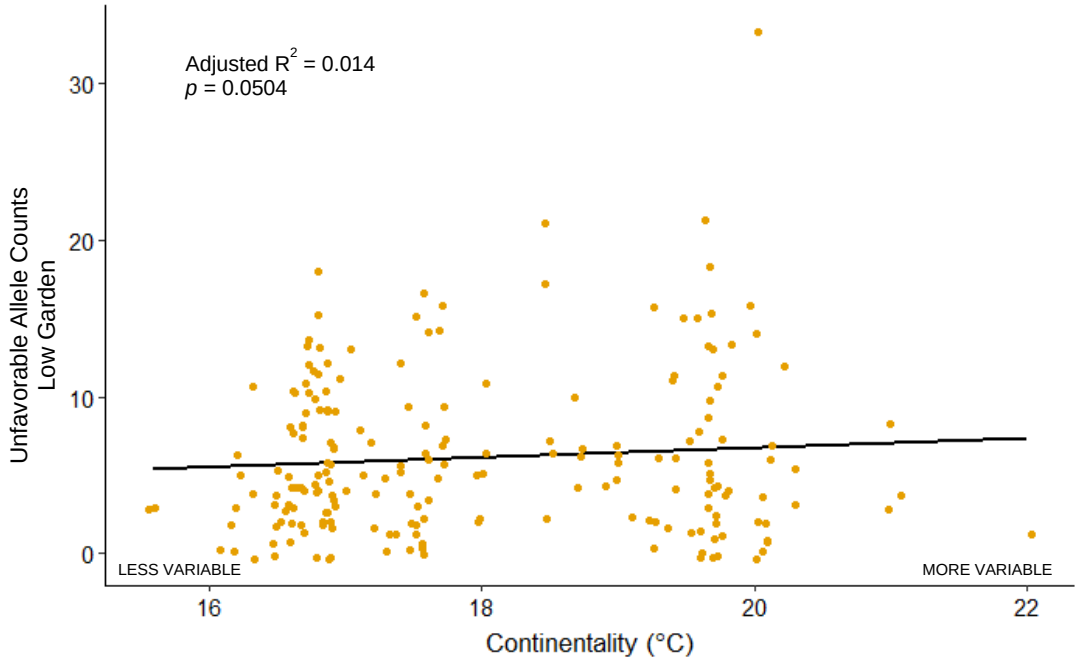

FIGURE S4: Relationships between *P. strobiformis* minor allele counts and seed source continentality. A higher number of minor alleles were present in maternal trees from areas with more continental, or variable, maternal seed source sites. This unfavorable minor allele group included minor alleles that were associated with lammas growth. This relationship was approaching significance (p= 0.0504, CI: -0.19, 0.81, β=0.82 ±0.42)

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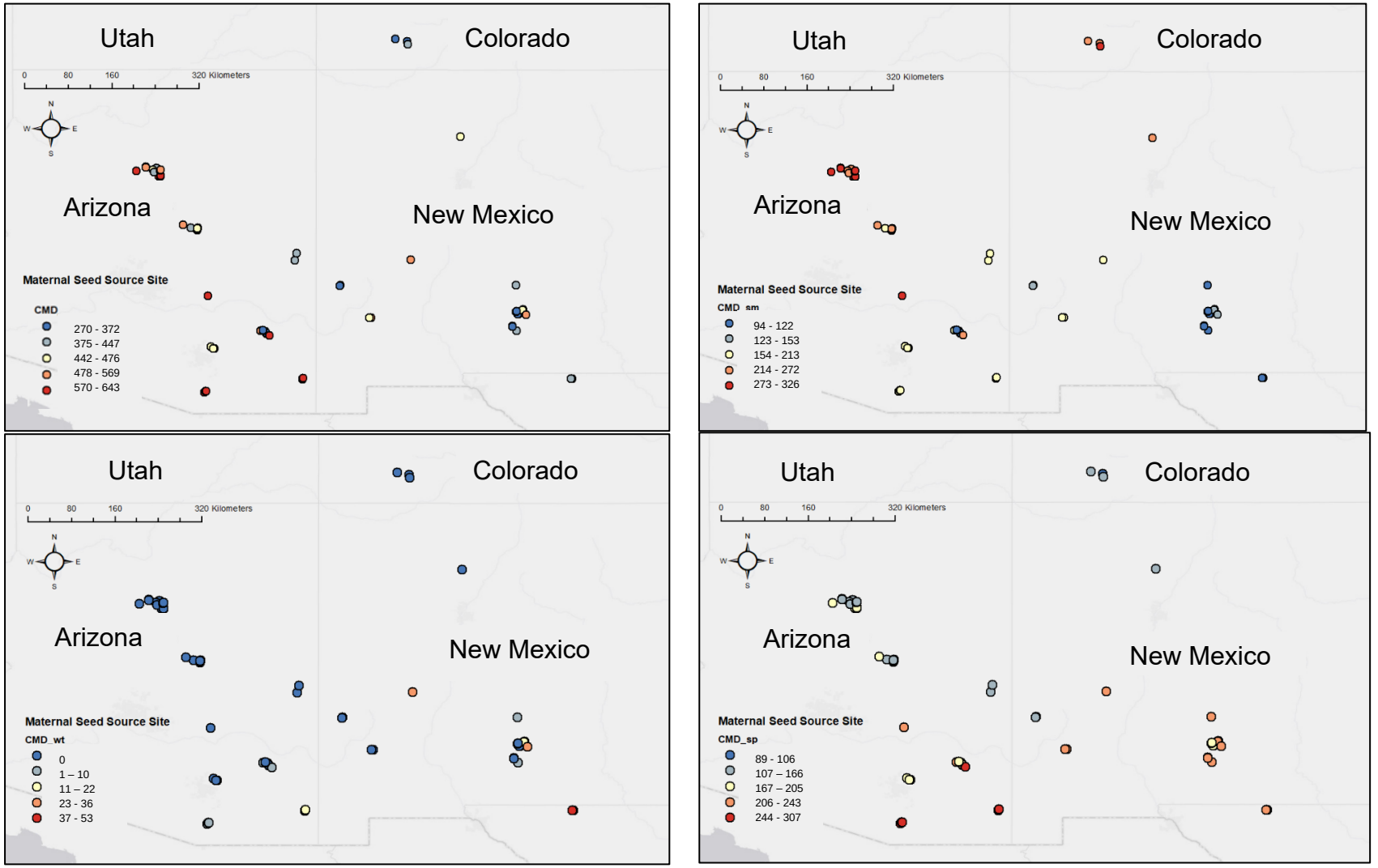

FIGURE S5: Map of annual and seasonal CMD across maternal seed source sites (n=202). These maps include the distribution of 30-year averages for annual, summer, (sm), winter (wt), and spring (sp) climate moisture deficit.

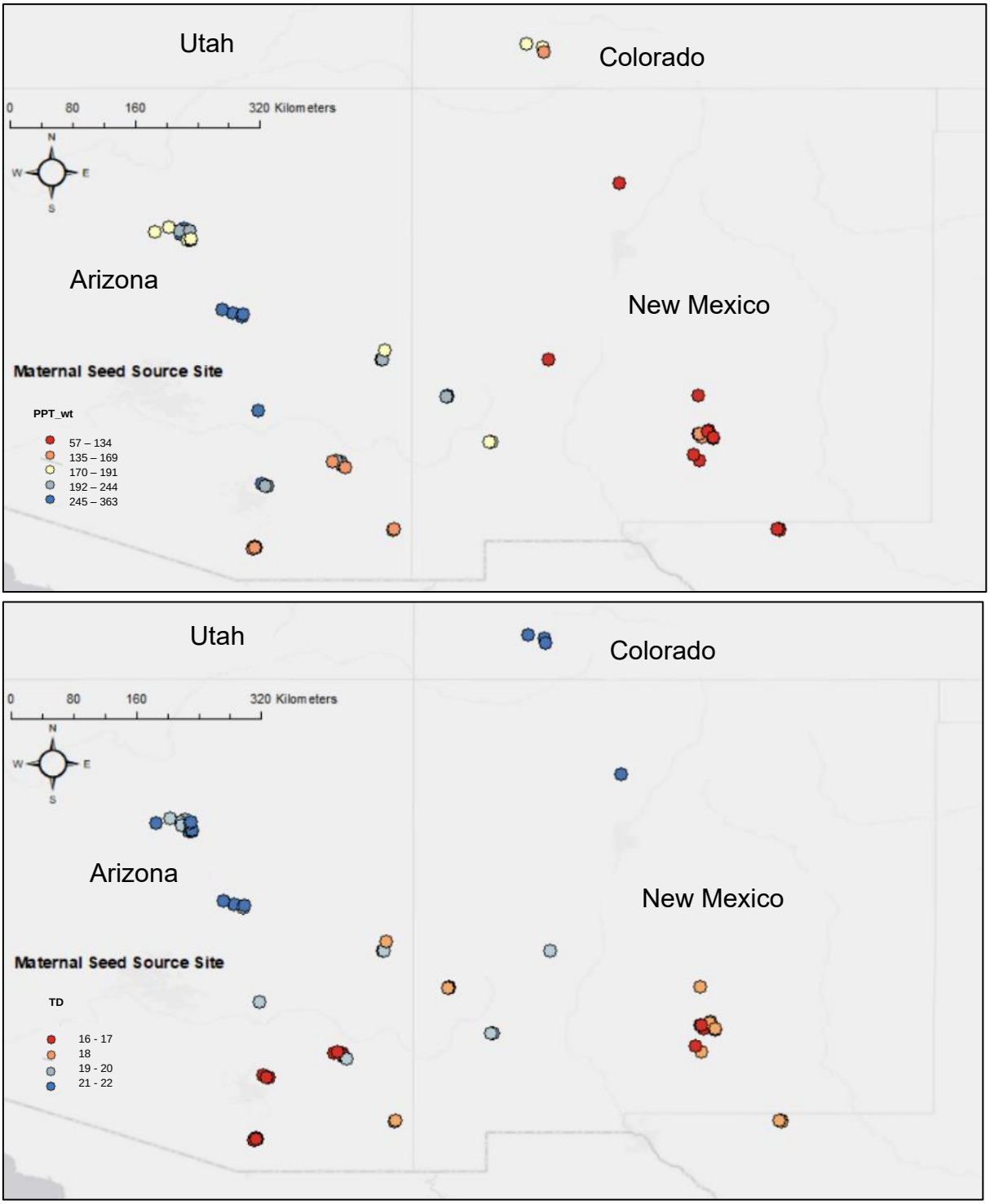

FIGURE S6: Map of winter precipitation (PPT_wt) and continentality (TD) across maternal seed source sites (n=202). These maps include the distribution of 30-year averages for winter precipitation (PPT_wt), and continentality (TD).