

# Genetic architecture of water-use efficiency

**Running Header:** Genetic architecture of water-use efficiency

## The genetic architecture of local adaptation II: The QTL landscape of water-use efficiency for foxtail pine (*Pinus balfouriana* Grev. & Balf.)

Andrew J. Eckert<sup>1\*</sup>, Douglas E. Harwood<sup>2</sup>, Brandon M. Lind<sup>2</sup>, Erin M. Hobson<sup>1</sup>, Annette Delfino Mix<sup>3</sup>, Patricia E. Maloney<sup>4</sup>, and Christopher J. Friedline<sup>1</sup>

<sup>1</sup>Department of Biology, Virginia Commonwealth University, Richmond, VA 23284

<sup>2</sup>Integrative Life Sciences Program, Virginia Commonwealth University, Richmond, VA 23284

<sup>3</sup>Institute of Forest Genetics, USDA Pacific Southwest Research Station, Placerville, CA 95667

<sup>4</sup>Department of Plant Pathology, University of California, Davis, CA 95616

### \*Author for correspondence:

Andrew J. Eckert

Department of Biology

Virginia Commonwealth University

Richmond, VA 23284

e-mail: [aeckert2@vcu.edu](mailto:aeckert2@vcu.edu)

phone: +1-804-828-0800

fax: +1-804-828-0503

## Abstract

Water availability is an important driver of the geographic distribution of many plant species, although its importance relative to other climatic variables varies across climate regimes and species. A common indirect measure of water-use efficiency (WUE) is the ratio of carbon isotopes ( $\delta^{13}\text{C}$ ) fixed during photosynthesis, especially when analyzed in conjunction with a measure of leaf-level resource utilization ( $\delta^{15}\text{N}$ ). Here, we test two hypotheses about the genetic architecture of WUE for foxtail pine (*Pinus balfouriana* Grev. & Balf.) using a novel mixture of double digest restriction site associated DNA sequencing, species distribution modeling, and quantitative genetics. First, we test the hypothesis that water availability is an important determinant of the geographical range of foxtail pine. Second, we test the hypothesis that variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  is genetically based, differentiated between regional populations, and has genetic architectures that include loci of large effect. We show that precipitation-related variables structured the geographical range of foxtail pine, climate-based niches differed between regional populations, and  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were heritable with moderate signals of differentiation between regional populations. A set of large-effect QTLs ( $n = 11$  for  $\delta^{13}\text{C}$ ;  $n = 10$  for  $\delta^{15}\text{N}$ ) underlying  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  variation, with little to no evidence of pleiotropy, was discovered using multiple-marker, half-sibling regression models. Our results represent a first approximation to the genetic architecture of these phenotypic traits, including documentation of several patterns consistent with  $\delta^{13}\text{C}$  being a fitness-related trait affected by natural selection.

**Key words:** Adaptation; double digest restriction site associated DNA sequencing; ddRADSeq; foxtail pine; genetic architecture; *Pinus balfouriana*; quantitative trait locus

## Introduction

Descriptions of the genetic components underlying fitness-related phenotypic variation have been a focus of quantitative genetics for over a century (Shull 1908; Fisher 1918; Mather 1941; Ford 1975; Mackay *et al.* 1994; Ritland *et al.* 2011 and references therein). These descriptions have progressed from identifications of the genetic elements affecting trait variation (e.g. Jermstad *et al.* 2001) to analysis of interactions among these elements with one another and the environment (e.g. Jermstad *et al.* 2003). Uniting all these descriptions are foundational questions about the structure, function, and evolution of genotype-phenotype maps in natural populations. For forest trees, these descriptions historically addressed traits of economic importance such as specific gravity of wood (e.g. Groover *et al.* 1994), microfibril angle (e.g. Sewell *et al.* 2000), growth (e.g. Wu 1998), and phenology (e.g. Pelgas *et al.* 2011), with the ultimate goals of marker-assisted breeding (Neale and Savolainen 2004) and trait prediction from genotypic data (Grattapaglia and Resende 2011). These traits, while economically important, often also affect fitness (especially phenology, see Sorensen 1983), so that these efforts can also be leveraged to understand the genetic basis of ecologically relevant trait variation. The linkage between traits measured in common gardens and fitness in natural populations, however, is usually assumed *post hoc*, which can lead to storytelling (Barrett and Hoekstra 2011) and oversimplification of the ecological ramifications of quantitative genetic results. Here, we address this disconnect through simultaneous use of species distribution modeling and quantitative trait locus (QTL) mapping to dissect the genetic architecture of an ecologically important phenotypic trait for foxtail pine (*Pinus balfouriana* Grev. & Balf).

The spatial and temporal distribution of all viable individuals across the Earth's landscape for a given species is defined as its geographical range (Brown *et al.* 1996). Evolution of range sizes and structural attributes of these ranges have been studied for a variety of taxa for many decades (e.g. Mayr 1963; Antonovics 1976; Brown *et al.* 1996; Gaston 2003; Eckert *et al.* 2008; Sheth and Angert 2014). The common thread underlying these interests is

## Genetic architecture of water-use efficiency

the assumption that fitness of individuals within species is related to the known geographical range for each species based on the environments defined by this range, other selective pressures (i.e. competition) across this range, and the phylogeographic history that resulted in the current geographical range (Hutchinson 1957; Pulliam 2000; Chuine 2010). For example, relative fitness values within plant populations tend to be highest in their home environments and lower in novel environments at the margin or outside of known geographical ranges (reviewed in Leimu and Fischer 2008). Regardless of the relationship between this pattern and evolutionary concepts such as local adaptation, it is clear that current geographical ranges are to some degree projections of ecological niches (i.e. realized versus fundamental niches), or at least some aspect of these niches, onto geographical space (Pulliam 2000; Ettinger *et al.* 2011). Knowledge of the environmental and climatic drivers of geographical ranges can therefore be informative about links between traits responsive to these drivers and fitness.

Species distribution models (SDMs) are commonly utilized as predictive tools with which to assess the importance of environmental variables to current geographical ranges of species (Elith *et al.* 2006). At a minimum, these models are built from known occurrences of a certain species and the environmental and ecological attributes of these locations derived from either field measurements or information stored in geographical information systems (GIS) layers. Numerous approaches are available with which to build models from these data (Segurado and Araujo 2004; Elith *et al.* 2006; Phillips *et al.* 2006). Once constructed, SDMs are often used subsequently to study the evolutionary development of ranges (e.g. McCormack *et al.* 2010), as well as the effects of continued climate change on current geographical ranges (e.g. Pearson and Dawson 2003). However, there are limitations to equating SDMs, even those with good predictive abilities of current geographical ranges, with realized ecological niches and hence measures of fitness limits (Hampe 2004; Soberon and Peterson 2005; Warren and Seifert 2011). For example, individuals used to create SDMs are considered exchangeable, so that fitness variation among individuals is ignored (Hampe 2004). Some of these issues, especially

## Genetic architecture of water-use efficiency

those related to exchangeability of individuals within species, can be addressed through a careful matching of modeling units (e.g. genetically differentiated populations within species; *sensu* Davis *et al.* 2005), geographical scale (e.g. the geographical scale relevant to the genetically differentiated populations), and the research questions of interest.

Water is crucial to the survival of many plant species (e.g. Sorenson 1983), although its importance relative to other environmental factors varies depending upon the environmental factors that are most limiting within local environments (Dudley 1996). The intrinsic efficiency by which plants use water (WUE) is defined as the ratio of net assimilation of carbon from CO<sub>2</sub> during photosynthesis to the loss of water during transpiration (Bacon 2004). Carbon isotopic composition ( $\delta^{13}\text{C}$ ) is an indirect measure of intrinsic WUE and is based upon the ratio of two isotopes of carbon ( $^{13}\text{C}$  and  $^{12}\text{C}$ ) within plant tissue standardized to a reference. This ratio is related to WUE because it has been demonstrated that the discrimination by C<sub>3</sub> plants of  $^{13}\text{CO}_2$  relative to  $^{12}\text{CO}_2$  is correlated to the ratio of carbon assimilation during photosynthesis to stomatal conductance (Farquhar *et al.* 1982; Farquhar and Richards 1984; e.g. Zhang and Marshall 1994). The physiological and environmental mechanisms, however, driving the linkage between  $\delta^{13}\text{C}$  and intrinsic WUE at various levels of biological organization are numerous, so that the expected linear relationship between  $\delta^{13}\text{C}$  and WUE may not always hold (Seibt *et al.* 2008). For example, differences in  $\delta^{13}\text{C}$  across individual plants at the leaf level can result from changes in carbon to nitrogen allocation during carboxylation, variation in leaf structure and morphology, and/or variation in available CO<sub>2</sub> (Seibt *et al.* 2008). Within a common environment, however, it is assumed that variation in available amounts of atmospheric CO<sub>2</sub> is negligible. Variation for  $\delta^{13}\text{C}$  across individual plants in these common environments should therefore reflect variation for intrinsic WUE. Indeed, previous research in conifers has established that variation in  $\delta^{13}\text{C}$  across individual plants is heritable (Seiler and Johnson 1988; Cregg 1993; Brendel *et al.* 2002; Baltunis *et al.* 2008; Cumbie *et al.* 2011), is polygenic, yet

## Genetic architecture of water-use efficiency

comprised of a mixture of large and small effect loci (Brendel *et al.* 2002; Gonzalez-Martinez *et al.* 2008; Cumbie *et al.* 2011; Marguerit *et al.* 2014), and that it often reflects variation for intrinsic WUE through leaf level assimilation (Zhang and Marshall 1994; Brendel *et al.* 2002; Cumbie *et al.* 2011; Marguerit *et al.* 2014).

Water availability is often an important driver of tree distributions (Stephenson 1990 and references therein), especially in Mediterranean climates (e.g. Baldocchi and Xu 2007; Lutz *et al.* 2010). This importance is evident through increased tree mortality as a function of both direct and indirect consequences associated with changing water availability (van Mantgem *et al.* 2009; Allen *et al.* 2010). Regional and local water availability will likely be altered, either through changes to annual precipitation totals or the seasonality of precipitation, under most climate change scenarios, especially in ecosystems dependent on residual summer snow-packs (Barnett *et al.* 2005). The ability of natural populations of forest trees to respond to changing water availability is linked to segregating genetic variation for traits responsive to water availability (Aitken *et al.* 2008). Knowledge of the genetic architecture of such traits, therefore, provides an important resource for assessing forest health, as well as the genetics of adaptation (Neale and Kremer 2011). Here, we test two hypotheses about the genetic architecture of WUE for foxtail pine – (i) water availability is an important determinant of the geographical range of foxtail pine and hence fitness and (ii) variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  is genetically based, differentiated between regional populations, and has genetic architectures that include loci of large effect. We subsequently discuss how the integration of results from disparate fields of research (i.e. genomics, ecology, and quantitative genetics) provides information useful to foundational tests about the genetic architecture of local adaptation and its evolution (*cf.* Friedline *et al.* 2015).

## Materials and Methods

### Focal Species

Foxtail pine is one of three species classified within subsection *Balfourianae* of section *Parrya* within subgenus *Strobus*. It is generally regarded as the sister taxon to Great Basin bristlecone pine (*P. longaeva* D. K. Bailey; see Eckert and Hall 2006). The distribution of this species is relegated to the high elevation mountains of California, with all known occurrences being in either the Klamath Mountains of northern California or in the high elevations of the southern Sierra Nevada (Figure S1). These two regions are separated by approximately 500 km and differ in climate, soils, and forest composition (Ornduff 1974; Eckert and Sawyer 2002; Barbour *et al.* 2007).

### Common Garden

A common garden representing 141 maternal foxtail pine trees was established at the Institute of Forest Genetics (Placerville, CA) during 2011 and 2012 using a randomized block design. Cones were collected from 141 maternal trees sampled range-wide, with 72 sampled from the Klamath Mountains and 69 from the southern Sierra Nevada region. For each maternal tree, 35 – 100 seeds were germinated and grown in standard conditions as outlined in Eckert *et al.* (2015). More information about the common garden can be obtained from Friedline *et al.* (2015). Of these 141 maternal trees, offspring, assumed to be half-siblings, from five were selected for analysis of water-use efficiency (see **Phenotype determination**, Table 1). The megagametophyte associated with each germinated seed from these five maternal trees was rescued and used to construct a high-density linkage map based on four of the five maternal trees (Friedline *et al.* 2015). The seedlings from each maternal tree were allowed to grow for a full year after which needles were sampled ( $n = 32$  to 40/maternal tree) for determination of phenotypes and genotypes. As done by Friedline *et al.* (2015), families were named using colors (i.e. these were the colors of family identifier tags in the common garden), with families

## Genetic architecture of water-use efficiency

sampled from the Klamath Mountains being labeled as blue, yellow, and purple and families sampled from the southern Sierra Nevada being labeled as red and green.

### Phenotype Determination

Two phenotypic traits were measured from needle tissue sampled from each growing seedling – carbon isotope discrimination ( $\delta^{13}\text{C}$ ) and foliar nitrogen content ( $\delta^{15}\text{N}$ ). These were chosen because ( $\delta^{13}\text{C}$ ) is a proxy for intrinsic WUE (Farquhar *et al.* 1982; Farquhar and Richards 1984), while  $\delta^{15}\text{N}$  is a proxy for plant growth and resource utilization during photosynthesis (Prasolova *et al.* 2000). Tissue was sampled in year 1 of growth, which was also prior to formation of randomized blocks in the common garden. Given the age of the seedlings, sampling of enough needle tissue for determination of phenotypes and genotypes was destructive. Thus, only a subset of the seedlings per maternal tree was used. For these seedlings, all available needles were sampled, cleaned and separated into those used for genotype determination and those used for phenotype determination. For phenotype determination, needles were placed into a mortar with liquid nitrogen and coarsely ground by hand using a pestle. The resulting needle tissue was then transferred into 20 ml glass vials and oven-dried at 60°C for 96 hrs. Approximately, 2 to 3 mg of ground and dried needle tissue from each seedling was subsequently placed into individual wells comprising a 96 well microtiter plate. Samples were analyzed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  at the Stable Isotope Facility at UC Davis (<http://stableisotopefacility.ucdavis.edu/>). Data are presented as carbon isotope ratios for  $\delta^{13}\text{C}$  (‰) and weight for  $\delta^{15}\text{N}$  (μg).

### Sequence Analysis and Genotype Determination

Total genomic DNA was extracted from the remaining needles from each sampled seedling using Qiagen DNeasy 96 Plant Kits following the manufacturer's protocol. The resulting total genomic DNA for each seedling was quantified using spectrophotometry as implemented with a Thermo Scientific NanoDrop 8000. Following quantification, samples were prepared for



## Genetic architecture of water-use efficiency

double digest restriction site associated DNA sequencing (ddRADseq) following the protocols of Parchman *et al.* (2012) as implemented for foxtail pine by Friedline *et al.* (2015). All samples had concentrations of total genomic DNA in the range of 15 to 60 ng/ul. In brief, this protocol proceeds via restriction digests of total genomic DNA for each sample using EcoR1 and Mse1, ligation of adapters that include the Illumina primer, universal M13 primers, and 8 – 10 bp barcodes, PCR amplification, and size selection of the PCR amplified and ligated restriction digests. In our protocol, multiplexing (i.e. pooling) occurred post PCR and size selection was carried out using 1.0% agarose gels run for 1 hour at 110 volts in 1X TAE buffer. All data are based on sequencing fragments in the size range of 300 to 500 bp on the Illumina HiSeq 2500. DNA sequencing was performed at the VCU Nucleic Acid Research Facility (<http://www.narf.vcu.edu/index.html>).

Raw FASTQ sequences were quality-checked and filtered as in Friedline *et al.* (2015). Briefly, reads must pass a three-stage filtering procedure to be retained for downstream analysis. First, if the average quality for all bases in the read was below 30, the read was discarded. Second, a five-base pair sliding window was evaluated along each raw sequence. Consecutive windows were retained if their mean quality was greater-than or equal-to 30. If the mean score of a window fell below this threshold, the read was trimmed at this point. If the length after trimming was at least 50% of the original read length, the read was kept, otherwise it was discarded. Finally, if 20% of the bases in the original read had quality scores below 30, the entire read was discarded, even if its average quality met the inclusion threshold. The reads that passed quality filtering were demultiplexed and assigned to individual trees in one of five families: Blue, Green, Purple, Red, or Yellow.

Sequences were aligned to the linkage map assembly (Friedline *et al.* 2015) and read groups were added using Bowtie2 version 2.2.4 (Langmead and Salzberg 2012) using the `very-sensitive-local` set of options. Each alignment was checked and marked for PCR artifacts using Picard (<http://picard.sourceforge.net>, svn 03a1d72). Variants were called using

## Genetic architecture of water-use efficiency

the multiallelic caller from samtools version 1.1 (Li *et al.* 2009), specifying diploidy for all individuals. The resulting VCF file was processed using VCFtools version 0.1.12.b (Danecek *et al.* 2011), retaining only biallelic SNPs that mapped to positions on the linkage map defined in Friedline *et al.* (2015) with quality (`--minQ`) of at least 20. All read processing and variant calling pipeline code, Python 3.4.3 and R version 3.2.0 (R Core Team 2015), can be found as IPython (Pérez and Granger 2007) notebooks and associated files at [http://www.github.com/cfriedline/foxtail\\_wue](http://www.github.com/cfriedline/foxtail_wue).

Once genotypes were called for all loci on the linkage map of Friedline *et al.* (2015), we selected one SNP per position on the linkage map based on minimizing the amount of missing data and being polymorphic in the most families. Missing genotype data were subsequently imputed for each linkage group using the default settings of the program fastPHASE ver. 1.2 (Scheet and Stephens 2006), with families used as populations. To account for uncertainty in genotype imputation, we estimated posterior probabilities of each possible genotype (i.e. 0, 1, or 2) at each locus using 1,000 haplotype reconstructions provided by fastPHASE, which were used subsequently as weights in a weighted average of the minor allele count. These weighted averages were then rounded to the closest value (0, 1, or 2) following normal rounding rules (i.e. round downward if the tenths position is less than five, otherwise round up).

### Species Distribution Modeling

We used species distribution models (SDMs) to justify water-use efficiency as a fitness-related trait and to quantify niches of each regional population relative to one another. The former provides an *a priori* justification for the measured traits as ecologically relevant, while the latter provides an estimate of niche differentiation between regional populations comparable to the effect of region on trait differentiation (see **Quantitative Genetic Analysis**).

Species distribution models were used to assess the relative importance of precipitation-related and temperature-related variables to the distribution of foxtail pine. We utilized the approach of maximum entropy (MaxEnt; Phillips *et al.* 2006) to construct SDMs. Known

## Genetic architecture of water-use efficiency

locations of foxtail pine within each regional population ( $n = 93$  Klamath Mountains,  $n = 207$  southern Sierra Nevada) were gathered from digitized herbarium records available through the Jepson Herbarium located at the University of California, Berkeley (<http://ucjeps.berkeley.edu/>). When the latitude and longitude of locations associated with these herbarium records were missing, visual inspections of maps from Google Earth were used to find the best approximation to the locality described on the herbarium sample. Climate data for each regional population were obtained from WorldClim (<http://www.worldclim.org/>) and are represented as 19 bioclimatic variables, which are functions of temperature and precipitation variables (Table S1), given at a resolution of 30 arc-seconds (~1 km). The generic grid files available from the WorldClim website were trimmed for each climate variable using the *raster* library in R and the following geographical extent: minimum longitude: -124.0°, maximum longitude: -117.5°, minimum latitude: 35.0°, maximum latitude: 42.5°. Using these trimmed grid files and the location information pruned of duplicate observations ( $n_{\text{pruned}} = 65$  Klamath Mountains,  $n_{\text{pruned}} = 144$  southern Sierra Nevada), the MaxEnt software version 3.3.3k (<https://www.cs.princeton.edu/~schapire/maxent/>) was used to build a SDM for each regional population. MaxEnt was run using the cross-validation option for model assessment, 10 replicates, a maximum number of background points of 10,000, and jackknife analysis to evaluate variable importance. Measures of variable importance (i.e. variable contribution and permutation importance scores) and the results of the jackknife analyses were used to assess the relative roles of temperature-related and precipitation-related variables to each SDM.

We used also used SDMs to quantify niche differentiation between regional populations of foxtail pine (Warren *et al.* 2008). We tested two null hypotheses. First, we tested the null hypothesis that the two SDMs were based on a single, underlying SDM common to each regional population. Second, we tested the null hypothesis that the two SDMs are no more differentiated than those randomly drawn from a common SDM with non-overlapping geographical distributions for each regional population. Both tests are based on the  $D$  and  $I$

statistics given by Warren *et al.* (2008). The former null hypothesis was tested using the *niche.equivalency.test* function in the *phyloclim* library in R, while the latter null hypothesis was tested using the *bg.similarity.test* function in the same R library. Both tests were based on  $n = 100$  permutations to derive null distributions of test statistics.

## Quantitative Genetic Analysis

We performed two sets of analyses to dissect the genetic basis of water-use efficiency for foxtail pine. First, we demonstrated that variation for the measured traits was genetically based using standard methods to decompose trait variance into effects of families, regions, and environment (Lynch and Walsh 1998). Second, we fit single and multiple QTL models to dissect the genetic basis of each trait into their genetic components using the regression methods of Knott *et al.* (1996).

The genetic basis for each measured trait was assessed using linear models. We fit three different linear models to the observed data for each trait: (1) a fixed effect model containing only a grand mean (i.e. intercept), (2) a linear mixed model with a grand mean as a fixed effect plus a random effect of family, and (3) a linear mixed model of a grand mean as a fixed effect plus a random effect of region plus a random effect of family nested within region. Uncertainty in parameter estimates from each model was assessed using parametric bootstrapping ( $n = 1,000$  replicated simulations) as carried out with the *simulate* function in R. Models were compared using the Akaike Information Criterion (AIC), with Akaike weights used to assess the conditional probabilities for each model (Burnham and Anderson 2002). If models containing random effects for families or models containing random effects for regions and families nested within regions fit the data better than a model with only a grand mean, then we concluded that there were non-zero heritabilities for these traits. If we assume that all offspring within each family were half-siblings, we could estimate narrow-sense heritability as  $h^2 = 4\sigma_{\text{fam}}^2 / (\sigma_{\text{fam}}^2 + \sigma_{\text{res}}^2)$ , where  $\sigma_{\text{fam}}^2$  is the variance due to family nested within region and  $\sigma_{\text{res}}^2$  is the residual variance. Given the small number of families, however, we avoided this estimation, as

## Genetic architecture of water-use efficiency

we were interested only in detecting non-zero heritability and not precise estimation of its magnitude. Linear models with fixed effects were fit using the *lm* function, while linear mixed models were fit using maximum likelihood as employed in the *lmer* function of the *lme4* library of R. Log-likelihood and AIC values were extracted for each fitted model using the *logLik* and *AIC* functions in R, respectively.

The genetic basis of each trait was dissected using the least squares regression approach of Knott *et al.* (1996) for outbred, half-sibling families, where probabilities of allelic inheritance due to the common parent were used as predictors for each trait. Significance of the regression model was determined using a *F*-test calculated at 1-cM intervals, with the distribution of this statistic under a null model of no QTLs generated via a permutation scheme (Churchill and Doerge 1994). The common parent in our analyses was the maternal tree, we assumed that all offspring per maternal tree were half-siblings, and we used 1,000 permutations to generate null distributions of *F*-statistics. Permutations were used to create null distributions for *F*-statistics at the level of the entire genome (i.e. all linkage groups) and for each chromosome (i.e. linkage group) separately. We initially fit models of one QTL per linkage group using three significance thresholds: (1)  $\alpha = 0.05$  at the level of the entire genome (major QTL), (2)  $\alpha = 0.01$  at the level of a particular chromosome (minor QTL), and (3)  $\alpha = 0.05$  at the level of a particular chromosome (suggestive QTL). For each QTL, we estimated the percent variance explained (PVE) as  $PVE = 4[1 - (MSE_{full}/MSE_{reduced})]$ , where  $MSE_{full}$  and  $MSE_{reduced}$  are the mean square errors of the full and reduced models, respectively (*cf.* Everett and Seeb 2014). Following Knott *et al.* (1996), estimates of PVE were scaled by  $(1 - 2r)^2$ , where  $r$  is the recombination frequency between the marker and QTL (i.e.  $r = 0.01$  for a 1-cM scan of each linkage group). Uncertainty in the position of the QTL was assessed using bootstrapping ( $n = 1,000$  replicates). For each linkage group with a statistically significant QTL, we subsequently fit a model of two QTLs using the same approach, with the only differences being the use of asymptotic null distributions to test the statistical significance of the observed *F*-statistics and

the lack of adjustments to estimates of the PVE for multiple QTL models. All analyses were conducted with the HSporelets module on GridQTL ver. 3.3.0 (Seaton *et al.* 2006; Allen *et al.* 2012) using the linkage map for foxtail pine reported by Friedline *et al.* (2015).

## Results

### Sequence Analysis and Genotype Determination

From two lanes of HiSeq sequencing, we obtained 148,685,598 and 160,770,417 reads from lane 1 (length = 101 bp, %GC = 40) and lane 2 (length = 101 bp, %GC = 41), respectively. Following read filtering, we retained 77,568,370 (length = 49 - 101 bp, %GC = 40) reads from lane 1 and 107,372,313 (length = 49 - 101, %GC = 40) reads from lane 2. A summary of the sequencing output and quality can be found in Table 2. The highest quality and most reads came from the Blue and Red families, while the Green family produced the smallest number of reads. Similarly, the Blue and Red families had the highest percentages of reads mapping to the assembly. The quality of reads across all families was sufficiently high, with average quality of any base of approximately 38. Graphical summaries of missing data and quality metrics are available in Figures S2 and S3. We filtered SNPs at the same position on the linkage map down to a set of 843 loci with the least amount of missing data and polymorphism in the most families. At these 843 SNPs, missing data averaged 58.0% (0% - 95.6%). Missing data were subsequently imputed using the marker ordering from Friedline *et al.* (2015) and fastPHASE.

### Species Distribution Modeling

Species distribution models were good predictors of the current geographical ranges for each regional population of foxtail pine (Figures 1, S1). Estimates of the area under the receiver operating characteristic curves (ROC curves) were near 1.0 for each model for both the training and test set of samples (Figure S4). Exceptions to this pattern included low to moderate probabilities of occurrence outside the current geographical distribution for the Klamath Mountains, which were centered on the northern Sierra Nevada, and a slightly expanded range north and south of the known range limits in the southern Sierra Nevada. Foxtail pine is known

## Genetic architecture of water-use efficiency

to be absent from these regions. In both cases, the probabilities of occurrence were less, often much less, than 0.40. The SDM based on the Klamath Mountains predicted a near zero probability for cells within the range of the southern Sierra Nevada and vice versa.

Foxtail pine inhabits the cooler portions of each region in which it is currently located (Figures S5 – S6). For precipitation-related variables, however, foxtail pine in the Klamath Mountains inhabits slightly wetter localities relative to background localities, while in the southern Sierra Nevada foxtail pine inhabits drier localities relative to background localities. The climates inhabited by foxtail pine in each region also differ. In general, differences between the climates inhabited by each regional population were consistent with the Klamath Mountains being warmer, yet less variable in temperature throughout the year, and wetter, yet slightly more variable in precipitation throughout the year, relative to the southern Sierra Nevada. For example, mean annual precipitation was almost twice as high in the Klamath Mountains as in the southern Sierra Nevada (1179.66 mm versus 650.03 mm, respectively), yet the distribution of precipitation was slightly more variable throughout the year (e.g. precipitation of the driest month: 11.78 mm versus 12.41 mm, respectively; coefficient of variation across months: 65.86 versus 65.02, respectively).

Bioclimatic variables used to predict occurrences of foxtail pine within each regional population were highly correlated with one another (Figure S7). Sets of correlated variables are difficult to evaluate as contributing to SDMs (Warren and Seifert 2011). We, therefore, used several different measures of variable importance. Inspection of variable contribution scores revealed that temperature-related and precipitation-related variables were differentially important across SDMs for each region (Figure 1; Table S2). Temperature-related variables, specifically mean diurnal range (Bio2), isothermality (Bio3), and maximum temperature of the warmest month (Bio5), were most important for the southern Sierra Nevada population, whereas precipitation-related variables, specifically precipitation of the driest quarter (Bio17) and precipitation of the wettest quarter (Bio16), were most important for the Klamath Mountains



## Genetic architecture of water-use efficiency

population. This pattern, however, was reversed when using permutation importance scores, despite a moderate correlation between rankings of importance based on variable contribution and permutation importance scores (Figures 2, S8; Table S4). Temperature-related variables became more important for the Klamath Mountains, specifically annual temperature (Bio1), while precipitation-related variables became more important for the southern Sierra Nevada population, specifically precipitation seasonality (Bio15) and mean temperature of the wettest quarter (Bio8). Jackknife analysis of variable importance based on AUC, test gain, and regularized test gain, however, were consistent with both temperature-related and precipitation-related variables as being important for the Klamath Mountains population (Figures S9 – S11). For example, mean annual temperature (Bio1), maximum temperature of the warmest quarter (Bio5), mean temperature of the driest quarter (Bio9), mean temperature of the warmest quarter (Bio10), precipitation of the driest quarter (Bio17), and precipitation of the warmest quarter (Bio18) all contributed significantly to the SDM for the Klamath Mountains population (Figure S11), although no one variable contained much information that was not present in at least one of the others. In contrast, jackknife analysis of variable importance based on AUC, test gain, and regularized test gain were consistent with primarily temperature-related variables, specifically mean annual temperature (Bio1), mean diurnal range (Bio2), maximum temperature of the warmest month (Bio5), and the mean temperature of the warmest quarter (Bio10), driving the SDM for the southern Sierra Nevada population (Figures S12 – S14). As with the SDM for the Klamath Mountains population, however, no one variable contained information that was not present in at least one of the others (Figure S14).

Predicted niches based on SDMs for each regional population were dissimilar, with estimates of  $D$  (0.072) and  $I$  (0.258) being much closer to zero (dissimilar) than to 1 (similar) (Figure S15). These differences were significant enough to reject a null model of a single shared SDM common to both regional populations ( $P < 0.01$  for  $D$  and  $I$ ). Even if differences were accounted for in the background environments of each regional population (Figure S5), the



## Genetic architecture of water-use efficiency

predicted niches were statistically different ( $P < 0.05$  for both  $D$  and  $I$ ). Replicating the analyses for climate variables related only to temperature or only to precipitation revealed that niche divergence was stronger for precipitation-related variables ( $D_{\text{precip}} = 0.074$ ;  $I_{\text{precip}} = 0.271$ ) relative to temperature-related variables ( $D_{\text{temp}} = 0.124$ ,  $I_{\text{temp}} = 0.376$ ). Therefore, regional populations of foxtail pine have divergent climatic niches, with precipitation-related variables more differentiated than temperature-related variables.

### Quantitative Genetic Analysis

Variation across siblings measured within the common garden was genetically based for each trait (Table 3). Family identifiers nested within regional populations accounted for sizeable portions of the total variance for  $\delta^{13}\text{C}$  ( $\sigma^2_{\text{fam}}/[\sigma^2_{\text{reg}} + \sigma^2_{\text{fam}} + \sigma^2_{\text{res}}] = 24.76\%$ ) and  $\delta^{15}\text{N}$  ( $\sigma^2_{\text{fam}}/[\sigma^2_{\text{reg}} + \sigma^2_{\text{fam}} + \sigma^2_{\text{res}}] = 24.45\%$ ). This was consistent with the differences among predicted family means for both traits (Figure 2), which were positively correlated (Figure 3), but not significantly so (Pearson's  $r = 0.415$ ;  $P = 0.487$ ). Regional identifiers, however, were differentially important across traits, with these identifiers accounting for marginally more variance than family identifiers for  $\delta^{13}\text{C}$  (26.01%) but less than 10% of the total variance for  $\delta^{15}\text{N}$  (Figure 2). The joint effect of family and regional identifiers (i.e. the total genetic effect =  $[\sigma^2_{\text{reg}} + \sigma^2_{\text{fam}}]/[\sigma^2_{\text{reg}} + \sigma^2_{\text{fam}} + \sigma^2_{\text{res}}]$ ), however, was large for each trait ( $\delta^{13}\text{C}$ : 50.78%;  $\delta^{15}\text{N}$ : 29.75%). Comparisons of linear models progressing from intercept only to an intercept plus families nested within regions using AIC, revealed that a linear mixed model with an intercept and families was the best fit (AIC = 310.29 for  $\delta^{13}\text{C}$ ; AIC = 1031.26 for  $\delta^{15}\text{N}$ ; Table 4). Comparison to other models using AIC weights, however, revealed that the most complex model of an intercept plus region plus families nested within regions had a reasonably high conditional probability (AIC weight = 0.36  $\delta^{13}\text{C}$ ; AIC weight = 0.28 for  $\delta^{15}\text{N}$ ; Table 4) relative to those for the best model ( $\delta^{13}\text{C} = 0.64$ ;  $\delta^{15}\text{N} = 0.72$ ) for each phenotypic trait.

## Genetic architecture of water-use efficiency

We dissected the genetic basis of the heritable variation evident for each trait from the linear mixed model analysis using the regression-based approach to QTL mapping of Knott *et al.* (1996). Application of one-locus models (i.e. a maximum of one-locus per linkage group) resulted in a set of 11 QTLs across all linkage groups and both traits (Table 5; Figure 4). For  $\delta^{13}\text{C}$ , six QTLs were discovered, with two discovered at the most stringent significance level (genome-wide permutation-based  $\alpha = 0.05$ ) and four at the least stringent significance level (linkage group specific permutation-based  $\alpha = 0.05$ ). Effect sizes for these QTLs were large to moderate, with the percent variation explained (PVE) ranging from 47.807% to 24.066%. For  $\delta^{15}\text{N}$ , five QTLs were discovered, with one QTL at the most stringent significance level, two at the intermediate significance level (linkage group specific permutation-based  $\alpha = 0.01$ ), and two at the least stringent significance level. Effect sizes for these QTLs were also large to moderate, with PVE varying from 39.773% to 25.058%. There was moderate autocorrelation for the  $F$ -statistic at a resolution of 6 cM or less for  $\delta^{13}\text{C}$  and 3 cM or less  $\delta^{15}\text{N}$  (Figure S16), but there was no correlation between  $F$ -statistics for each trait (Pearson's  $r$ : -0.014,  $P = 0.734$ ; Figure S17). In general, 95% confidence levels of positions for each QTL were large (Table 5).

For the 11 QTLs detected using one-locus models, 10 were consistent with multiple QTLs using two-locus models (Table 6). In general, the QTLs from the one-locus models were one of the pair of QTLs detected in the two-locus models. There were four exceptions to this pattern, with two of these exceptions being a minor modification in position of the original QTL equal to 1.0 cM. The other two exceptions included significant changes to the position of the original QTL, with the QTL on linkage group 3 for  $\delta^{15}\text{N}$  changing from 93.0 cM to 52.0 cM and 35.0 cM and the QTL on linkage group 6 for  $\delta^{13}\text{C}$  changing from 0.0 cM to 46.0 cM and 56.0 cM (Tables 5 and 6). The average spacing between QTLs on the same linkage group was 29.4 cM, with a minimum of 3 cM to a maximum of 85 cM. The multi-QTL PVE for each trait ranged from a minimum of 42.685% to a maximum of 71.315%, with only one instance of positional overlap

## Genetic architecture of water-use efficiency

in QTLs for each trait (linkage group 3 at 34.0 cM for  $\delta^{13}\text{C}$  and 35.0 cM for  $\delta^{15}\text{N}$ ). On average, there was a negative relationship between distance (cM) and the correlation of family effects (Pearson's  $r$ ) between QTLs on the same linkage group (Figure S18), so that strong positive correlations of family effects were observed when QTLs were close together (<15 cM) and strong negative correlations when QTLs were farther apart (>20 cM).

QTL effects from the one-locus QTL models were consistent with differentiation between regional populations, with family effects opposite in sign more often than expected by chance for  $\delta^{13}\text{C}$  (Fisher's exact test: odds ratio = 0.113,  $P$  = 0.009), but not for  $\delta^{15}\text{N}$  (Fisher's exact test: odds ratio = 1.319,  $P$  = 1.0). Trait differentiation was similarly structured (Tables 3 and 4), with the clearest signal of differentiation for  $\delta^{13}\text{C}$ . The same patterns were observed for family effects in the two-locus models for the original QTL from Table 5, but not for the second QTL ( $P$  > 0.05 for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ).

## Discussion

Climate is one of the main drivers for the distribution and diversification of forest tree species (MacArthur 1972; Royce and Barbour 2000; Ettinger *et al.* 2011; Alberto *et al.* 2013). The relative importance of specific climate variables as drivers of natural selection, however, is often assumed. For example, if a phenotypic trait is correlated to water availability in one species, the same trait is often studied in a different focal species without documenting water availability as having a large impact on fitness variation in the latter. The problem lies in the assumption that this correlation is also indicative of similar fitness consequences across species. Here, we address this issue for foxtail pine using a novel combination of species distribution modeling and quantitative genetics. We illustrate the importance of water availability to the distribution of foxtail pine and hence fitness, as well as describe the genetic architecture of WUE, a phenotypic trait responsive to water availability, so that this trait and the markers

correlated to it can be used to test hypotheses about local adaptation and its genetic architecture.

# **Climate drivers of the current geographical distribution and WUE**

In many situations, drivers of geographical distributions for tree species are obvious. For example, links between light availability, temperature, precipitation, and phenological traits are commonly noted for forest trees (Howe *et al.* 2003; Chuine 2010). In other situations, however, climate drivers are less clear, so that quantification of the relative importance for a suite of climate variables is needed. For foxtail pine, the drivers of its current geographical distribution appear to be a mixture of temperature-related and precipitation-related variables, with a clear pattern that precipitation-related variables are necessary to explain the current geographical range. This implies that phenotypic traits correlated to precipitation-related variables likely have fitness consequences for foxtail pine, as precipitation-related variables appear to structure its current range. Additionally, the importance of these drivers is differentiated between regional populations, with precipitation-related variables more differentiated than temperature-related variables, which mimicked differentiation of phenotypic trait values. Thus, if we leverage the correlations between  $\delta^{13}\text{C}$  and water availability, a crucial component of survival and hence fitness, observed in other plant species (Ehleringer *et al.* 1993) and the conclusion that precipitation-related variables are important for the distribution of foxtail pine, it is likely that  $\delta^{13}\text{C}$  variation in foxtail pine is linked with fitness.

In general, increases in  $\delta^{13}\text{C}$  reflect higher WUE (Farquhar *et al.* 1982). Inspection of mean values for  $\delta^{13}\text{C}$  for each region (see Figure 3), in light of the documented precipitation patterns, however, appears contradictory. On average, maternal trees in the Klamath Mountains had higher  $\delta^{13}\text{C}$  values, which suggests higher WUE, yet precipitation is much higher in the Klamath Mountains than in the southern Sierra Nevada. It is well known, however, that soil properties, such as coarseness and depth to bedrock, affect available soil moisture. For

## Genetic architecture of water-use efficiency

example, small differences in soil texture observed across the Southern Sierra Nevada Critical Zone Observatory, a site not far removed from the regional population of foxtail pine in the southern Sierra Nevada, result in large differences in the available soil moisture (Bales *et al.* 2011). Soil texture also varied by elevation, with soils at the highest elevations being coarser and less developed. As such, water availability in these soils was more limited even though snowfall was typically higher. Soils between regional populations of foxtail pine are fundamentally different, and so is the local distribution of foxtail pine. In the Klamath Mountains, soils are primarily ultramafic, while in the southern Sierra Nevada they are largely granitic. Foxtail pine grows near tops of local peaks in the Klamath Mountains, whereas in the southern Sierra Nevada it is distributed broadly across large swathes of high elevation sites. Thus, one explanation for the apparent contradiction is that soil properties are different, so as to create patterns of soil moisture not reflective of regional mean precipitation patterns. Foxtail pine in the Klamath Mountains often inhabits areas with high levels of boulder cover (Eckert and Sawyer 2002; Eckert 2006), which are expected to house soils with less capacity to hold water over long periods of time. When coupled with the higher average temperatures in the Klamath Mountains, this suggests that water may be more limited throughout the year (e.g. summer drought) than expected based on annual precipitation totals. Additional work, however, would be needed to quantify trait variation within each regional population and correlate it to both climate and soil characteristics.

### Genetic architecture of water-use efficiency

Both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were consistent with non-zero heritabilities. Families and regions accounted for approximately 50% of the total phenotypic variance for  $\delta^{13}\text{C}$  and 30% for  $\delta^{15}\text{N}$ . Models with effects due to families or families nested within regions were also strongly preferred over models without these effects (Table 4). The effect of region, however, was highest in magnitude for  $\delta^{13}\text{C}$ , with the variance component for region larger than that for family. This is

## Genetic architecture of water-use efficiency

consistent with previous estimates of quantitative genetic parameters for these phenotypic traits in other conifers. For example,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  are both heritable in a variety of pine species (Brendel *et al.* 2002; Baltunis *et al.* 2008; Gonzalez-Martinez *et al.* 2008; Cumbie *et al.* 2011; Joao Gaspar *et al.* 2013; Marguerit *et al.* 2014; Eckert *et al.* 2015). Populations within many species are also often differentiated for  $\delta^{13}\text{C}$ , but not for  $\delta^{15}\text{N}$  (e.g. Eckert *et al.* 2015; Maloney *et al.* unpublished). Further work, however, would be needed to precisely estimate the level of differentiation for these traits, as well as to test whether this level of differentiation is larger than that expected for neutral loci (i.e. this pattern is consistent with local adaptation).

Estimates of narrow-sense heritabilities ( $h^2$ ) resulted in values greater than 1.0 for each phenotypic trait no matter which model with a family effect was used (i.e. families or regions plus families nested within regions). This could be due to tissue sampling occurring prior to formation of randomized blocks in the common garden, as family groups would be confounded with micro-environmental variation. Use of data from Eckert *et al.* (2015) and Maloney *et al.* (unpublished data) for sugar pine (*P. lambertiana* Dougl.), western white pine (*P. monticola* Dougl.), and whitebark pine (*P. albicaulis* Engelm.) grown at the same facility in the same experimental conditions, however, reveals that block effects for  $\delta^{13}\text{C}$  were present only for the relatively fast growing western white pine (Type III Wald  $F$ -tests with Kenward-Rogers degrees of freedom; sugar pine:  $F_{1,416.49} = 3.5166$ ,  $P = 0.06146$ ; western white pine:  $F_{1,630.24}$ ,  $P = 0.00068$ ; whitebark pine:  $F_{1,452.75} = 0.0147$ ;  $P = 0.9037$ ). In contrast, block had a statistically significant effect on  $\delta^{15}\text{N}$  for sugar pine and western white pine ( $P < 0.001$ ), but not whitebark pine ( $F_{1,429.22} = 1.6252$ ,  $P = 0.20305$ ). Thus, our results should be taken with caution, but family effects estimated here were similar in magnitude to those from Eckert *et al.* (2015) and randomized blocks tended to have no effect on the same phenotypic traits measured in whitebark pine at the same facility, a species with a similar pattern of early slow growth (McCune 1988).

## Genetic architecture of water-use efficiency

If our results are indicative of true signal, effect sizes could be over-estimated on average due to the small number of sampled families (Beavis 1994). To illustrate this effect, we re-analyzed the data from Eckert *et al.* (2015) for sugar pine, which was grown in a common garden at the same facility and measured for  $\delta^{13}\text{C}$  using the same methodology, by resampling smaller numbers of families ( $n = 108$  families resampled in decreasing numbers from 108 to three families) and estimating  $h^2$ . As the number of sampled families decreased, estimates of mean  $h^2$  became larger (Figure S19), with a 1.5-fold increase in the mean  $h^2$  as the number of sampled families dropped from 108 to three. This is likely also the case for foxtail pine and for  $\delta^{15}\text{N}$ . Regardless of the precise value of  $h^2$ , it is clear that at least a moderate amount of segregating genetic variation exists for this trait in natural populations of foxtail pine.

There was also a moderate, but statistically insignificant, positive correlation between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Figure 4). This has been noted in other species, such as loblolly pine (Cumbie *et al.* 2011), although general patterns in the sign of the correlation are lacking. In this context, positive correlations could indicate that WUE is determined primarily through leaf-level assimilation (e.g. Johnson *et al.* 1999; Prasolova *et al.* 2005), while a negative correlation could indicate that WUE is determined primarily through stomatal conductance. Despite the observed positive correlation, little evidence of pleiotropy was detected, with only a single QTL on linkage group 3 shared between traits. The lack of pleiotropy for these traits has been noted in several other conifer species (e.g. Marguerit *et al.* 2014). Correlations between  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , or growth traits more generally, can also be driven environmentally and can change depending on water availability. For example, Joao Gaspar *et al.* (2013) have shown that in water limiting environments  $\delta^{13}\text{C}$  correlates with survival, but in less water limited environments  $\delta^{13}\text{C}$  correlates with height growth for maritime pine (*P. pinaster* Ait.). A similar case might be occurring for foxtail pine, where in the wetter Klamath Mountains  $\delta^{13}\text{C}$  variation is correlated with overall growth and in the more xeric southern Sierra Nevada it is correlated with survival. In this



## Genetic architecture of water-use efficiency

context, WUE would be realized through leaf-level assimilation in the Klamath region (as in Weih *et al.* 2011 for *Salix*), and through stomatal conductance in the southern Sierra Nevada. Sampling more families, measurement of other traits (e.g. growth), and experimentation in multiple environments, however, would be needed to test these ideas. Importantly,  $\delta^{13}\text{C}$  should be measured within natural populations to assess correspondence between inferences from common gardens and natural populations.

Using one-locus QTL models, the observed segregating genetic variance for  $\delta^{13}\text{C}$  was dissected into two major QTLs and four suggestive QTLs (Table 5). Each QTL explained a large fraction of total phenotypic variance (23.113% to 47.807%), which suggests that the genetic architecture of this fitness-related trait includes loci of large effect. Under many models of adaptation, however, is difficult to separate QTLs composed of a single, large-effect locus from those composed of several small-effect loci (Yeaman and Whitlock 2011). The observed large values of PVE may also be over-estimated (Beavis 1994), although there is precedence for large effect QTLs for  $\delta^{13}\text{C}$  in other species of *Pinus*, especially those distributed in water-limited regions displaying moderate levels of genetic differentiation among populations. For example, Marguerit *et al.* (2014) identified a QTL explaining 67% of phenotypic variance for  $\delta^{13}\text{C}$  in maritime pine, which is distributed across the Mediterranean regions of Europe and has moderate levels of genetic structure across this range (Eveno *et al.* 2008). For foxtail pine, water availability is an important driver of its current geographical distribution and genetic structure is moderate to high between regional populations and among stands within regional populations (Eckert *et al.* 2008, but see Oline *et al.* 2000). Furthermore, family effects for these QTLs were consistent with differentiation among regions, so it is plausible that the architecture discovered here for  $\delta^{13}\text{C}$  largely represents genomic regions underlying trait divergence between the regional populations. If this is the case, this architecture has evolved since the



## Genetic architecture of water-use efficiency

divergence of the regional populations from their common ancestor on the order of one million years ago (Eckert *et al.* 2008).

Summaries of the results from two-locus QTL models were largely consistent with those from the one-locus models. For the 11 QTLs reported in Table 5, 10 were consistent with at least two segregating QTLs. This brings the total number of QTLs to four major and seven suggestive QTLs for  $\delta^{13}\text{C}$  and two major, four minor, and four suggestive QTLs for  $\delta^{15}\text{N}$ . Interestingly, the correlation of family-level effects for the two QTLs on the same linkage group was negatively related to the distance between these QTLs, so that QTLs close together tended to have similar patterns of family-level effects, whereas QTLs at larger distances tended to have opposite family-level effects (Figure S14). This trend was uncorrelated with the difference in effect sizes between QTLs. When added to the observation that family effects were often consistent within regions and differentiated between regions, a likely explanation for this pattern is some form of natural selection driving clustering of loci dependent on consistency of their effects on a fitness-related trait. The fitness benefit of clustering, however, is related to the level of gene flow (Yeaman and Whitlock 2011), so that clustering of adaptive alleles is expected under high levels of gene flow, reduced recombination, and strong magnitudes of selection. This is especially pronounced when genomic rearrangements are common. Inspection of the family-level linkage maps from Friedline *et al.* (2015), however, revealed little evidence for clustered QTLs displaying differing marker orders across families more so than random positions on the linkage map. This explanation, however, is complicated given that gene flow is approximately zero between these regions (Eckert *et al.* 2008) and populations of foxtail pine are unlikely to be at selection – migration equilibrium due to large effective population sizes and long generation times. For example, patterns of segregating ancestral variation after divergence are similar to those predicted by gene flow (Pamilo and Nei 1988), so that it becomes difficult to separate pattern from process with regard to the effects of gene flow on adaptive genetic architectures.

## Genetic architecture of water-use efficiency

Additional work within natural populations, including fine mapping of trait values in the linkage bins defined by Friedline *et al.* (2015), would be needed to test these ideas further.

We leveraged the annotations of contigs at or near ( $\pm 3$  cM) the estimated QTL positions to search for putatively functional genes as the drivers of the genotype-phenotype correlations for each QTL (Table S3). Annotations for foxtail pine contigs were derived through similarity searches against the loblolly pine genome. Annotations were obtained from any locus on a loblolly pine scaffold containing a significant hit to a RADtag from foxtail pine, with significance justified by the estimated substitution rate and divergence time between these species (Friedline *et al.* 2015). Several statistically significant QTLs had no annotation information available. For example, the QTL on linkage group 1 for  $\delta^{13}\text{C}$  had no annotations available within a 6-cM window encapsulating the QTL, despite 24 of 76 RADtags having significant similarity to scaffolds in loblolly pine. This is consistent with reports of gene densities reported for conifers (Nystedt *et al.* 2013; Neale *et al.* 2014). For the QTL related to  $\delta^{13}\text{C}$  on linkage group 2 (Table 5), however, two of the 18 RADtags for foxtail pine had sequence similarity to loblolly pine scaffolds, with annotated InterPro domains suggestive of loci encoding stress responsive proteins (Table S3; Toka *et al.* 2010; Karijovich *et al.* 2015). Another example of potentially biologically informative results included the QTL on linkage group 9 for  $\delta^{15}\text{N}$  where putative homologs for proteins with domains such as ribosomal protein L38e, cytochrome P450, and thiolase were present. Proteins containing these domains have been implicated in lipid turnover during leaf senescence (Troncoso-Ponce *et al.* 2013), as well as plant growth and drought stress response (Tamiru *et al.* 2015). Care should be taken in interpreting these results, however, as QTL intervals were wide, annotations were based on statements of homology with gene predictions in an early release of the loblolly pine genome sequence (Wegrzyn *et al.* 2014), and *post hoc* explanations linking gene products to phenotypic traits is prone to storytelling (Barrett and Hoekstra 2011; Pavlidis *et al.* 2012). It is important to note, however,

## Genetic architecture of water-use efficiency

that these concerns are with interpretations of putative functions of genes located within the QTL as sensible in their effect on the measured phenotypic trait, and not with the biological signal of linkage driving the discovery of the QTL.

### Conclusions

We have used a mixture of species distribution modeling and quantitative genetics to test two hypotheses about WUE, as measured by  $\delta^{13}\text{C}$ , for foxtail pine. We showed that precipitation-related variables structured the geographical range of foxtail pine, that climate-based niches differed between regional populations, and that similar patterns were apparent for  $\delta^{13}\text{C}$ , which was also demonstrated to be heritable. We subsequently dissected this heritability into a set of large-effect QTLs ( $n = 21$  total, with 11 for  $\delta^{13}\text{C}$  and 10 for  $\delta^{15}\text{N}$ ), which we interpret in light of population genetic theory about local adaptation. While we cannot definitely say that WUE, as measured by  $\delta^{13}\text{C}$ , contributes to local adaptation, we have described to a first approximation its genetic architecture, while noting several patterns consistent with  $\delta^{13}\text{C}$  being a fitness-related trait affected by natural selection. These are useful results with which to generate further hypotheses about the evolution of genetic architecture contributing to local adaptation in natural populations (e.g. Holliday *et al.* 2015). Our results also shed light on ecologically relevant phenotypic trait variation useful for management decisions and predictions for range shifts under changing climates.

**Acknowledgements** – We are grateful to the staff at the USDA Institute of Forest Genetics for space and logistical support in the establishment of the common garden, as well as the staff at the VCU Nucleic Acids Research Facility and Center for High Performance Computing for help in generating and analyzing the Illumina short read data. Seeds for foxtail pine were obtained from Tom Blush and Tom Burt. Rodney Dyer, Christopher Gough, William Eggleston and Salvatore Agosta all made helpful comments on D. E. Harwood's M.S. thesis at VCU, which was the basis for this work.

# **Data Archiving Statement**

- **Genotype data** are available as raw short read data as part of NCBI BioProject PRJNA310118, processed short read data in VCF format (File S1), and imputed data in a tab-delimited text file (File S2).
- **Phenotypic trait data** are available for all half-siblings within each of the five families used for QTL mapping in a tab-delimited text file (File S3).
- **Location data** used for species distribution modeling are available in a tab-delimited text file (File S4).

# **References**

- Aitken SN, Yeaman S, Holliday JA, Wang T, Curtis-McLane S (2008) Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evol Appl* 1:95-111.
- Alberto FJ, Aitken SN, Alia R, Gonzalez-Martinez SC, Hanninen H, et al. (2013) Potential for evolutionary responses to climate change – evidence from tree populations. *Glob Chang Biol* 19:1645-1661.
- Allen CD, Macalady AK, Chenchouni H, Bachelet D, McDowell N, et al. (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecol Manag* 259:660-684.

# Genetic architecture of water-use efficiency

- 710 Allen J, Scott D, Illingworth M, Dobrzelecki B, Virdee D, Thorn S, Knott S (2012) CloudQTL:  
711 Evolving a Bioinformatics Application to the Cloud. Digital Research 2012, September  
712 10-12, 2012. Oxford, UK.
- 713 Antonovics J (1976) The nature of limits to natural selection. *Ann Mo Bot Gard* 63:224-247.
- 714 Bacon MA (2004) Water use efficiency in plant biology. In: Bacon MA (ed) *Water Use Efficiency*  
715 in Plant Biology, Blackwell Publishing Ltd, Oxford, UK, pp 1-26.
- 716 Baldocchi DD, Xu L (2007) What limits evaporation from Mediterranean oak woodlands – the  
717 supply of moisture in the soil, physiological control by plants or the demand by the  
718 atmosphere? *Adv Water Res* 30:2113-2122.
- 719 Bales RC, Hopmans J, O’Green AT, Meadows M, Hartsough PC, Kirchner P, Hunsaker CT,  
720 Beaudette D (2011) Soil moisture response to snowmelt and rainfall in a Sierra Nevada  
721 mixed-conifer forest. *Vadose Zone J* 10:786-799.
- 722 Baltunis BS, Martin TA, Huber DA, Davis JM (2008) Inheritance of foliar stable carbon isotope  
723 discrimination and third-year height in *Pinus taeda* clones on contrasting sites in Florida  
724 and Georgia. *Tree Genet Genomes* 4:797-807.
- 725 Barbour M, Keeler-Wolf T, Schoenherr AA (2007) *Terrestrial Vegetation of California*. Third  
726 edition. University of California Press, Berkeley, California, USA.
- 727 Barnett TP, Adam JC, Lettenmaier (2005) Potential impacts of a warming climate on water  
728 availability in snow-dominated regions. *Nature* 438:303-309.
- 729 Barrett RD, Hoekstra HE (2011) Molecular spandrels: tests of adaptation at the genetic level.  
730 *Nat Rev Genet* 12:767-780.
- 731 Beavis WD (1994) The power and deceit of QTL experiments: Lessons from comparative QTL  
732 studies. In: *Proceedings of the 49th Annual Corn and Sorghum Industry Research*  
733 *Conference*, 250–266. American Seed Trade Association, Washington, DC, USA.
- 734 Brendel O, Pot D, Plomion C, Rozenberg P, Guehl J-M (2002) Genetic parameters and QTL  
735 analysis of  $\delta^{13}\text{C}$  and ring width in maritime pine. *Plant Cell Environ* 25:945-953.

# Genetic architecture of water-use efficiency

- 736 Brown JH, Stevens GC, Kaufman DM (1996) The geographic range: size, shape, boundaries,  
737 and internal structure. *Annu Rev Ecol Syst* 27:597-623.
- 738 Burnham KP, Anderson DR (2002) Model Selection and Multimodal Inference – A Practical  
739 Information-Theoretic Approach. Springer, New York, New York, USA.
- 740 Chuine I (2010) Why does phenology drive species distribution? *Philos Trans R Soc Lond B Biol*  
741 *Sci* 365:3149-3160.
- 742 Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait  
743 mapping. *Genetics* 138:963-971.
- 744 Cregg, BM 1993. Seed source variation in water relations, gas exchange, and needle  
745 morphology of mature ponderosa pine trees. *Can J For Res* 23:749-755.
- 746 Cumbie WP, Eckert A, Wegrzyn J, Whetten R, Neale D, Goldfarb B (2011) Association genetics  
747 of carbon isotope discrimination, height and foliar nitrogen in a natural population of  
748 *Pinus taeda* L. *Heredity* 107:105-114.
- 749 Danecek P, Auton A, Abecasis G, *et al.* (2011) The variant call format and VCFtools.  
750 *Bioinformatics* 27:2156-2158.
- 751 Davis MB, Shaw RG, Etterson JR (2005) Evolutionary responses to changing climate. *Ecology*  
752 86:1704-1714.
- 753 Dudley SA (1996) Differing selection on plant physiological traits in response to environmental  
754 water availability: a test of adaptive hypotheses. *Evolution* 50:92-102.
- 755 Eckert AJ (2006) Influence of substrate type and microsite availability on the persistence of  
756 foxtail pine (*Pinus balfouriana*, Pinaceae) in the Klamath Mountains, California. *Am J*  
757 *Bot* 93:1615-1624.
- 758 Eckert AJ, Sawyer JO (2002) Foxtail pine importance and conifer diversity in the Klamath  
759 Mountains and southern Sierra Nevada, California. *Madroño* 49:33-45.

# Genetic architecture of water-use efficiency

- 760 Eckert AJ, Hall BD (2006) Phylogeny, historical biogeography, and patterns of diversification  
761 for *Pinus* (Pinaceae) – Phylogenetic tests of fossil-based hypotheses. Mol Phylogenet  
762 Evol 40:166-182.
- 763 Eckert AJ, Tarse BR, Hall BD (2008) A phylogeographical analysis of the range disjunction for  
764 foxtail pine (*Pinus balfouriana*, Pinaceae): the role of Pleistocene glaciation. Mol  
765 Ecol 17:1983-1997.
- 766 Eckert AJ, Maloney PE, Vogler DR, Jensen CE, Delfino Mix A, Neale DB (2015) Local  
767 adaptation at fine spatial scales: an example from sugar pine (*Pinus lambertiana*,  
768 Pinaceae). Tree Genet Genomes 11:42.
- 769 Eckert CG, Samis KE, Loughheed SC (2008) Genetic variation across species' geographical  
770 ranges: the central-marginal hypothesis and beyond. Mol Ecol 17:1170-1188.
- 771 Ehleringer JR, Hall AE, Farquhar GD (1993) Stable isotopes and plant carbon/water relations.  
772 Academic Press, San Diego, California, USA.
- 773 Elith J, Graham CH, Anderson RP, Dudik M, Ferrier S, et al. (1996) Novel methods improve  
774 prediction of species' distributions from occurrence data. Ecography 29:129-151.
- 775 Ettinger AK, Ford KR, HilleRisLambers J (2011) Climate determines upper, but not lower,  
776 altitudinal range limits of Pacific Northwest conifers. Ecology 92:1323-1331.
- 777 Eveno E, Collada, Angeles Guevara M, Leger V, Soto A, et al. (2008) Contrasting patterns of  
778 selection at *Pinus pinaster* Ait. drought stress candidate genes as revealed by genetic  
779 differentiation analyses. Mol Biol Evol 25:417-437.
- 780 Everett MV, Seeb JE (2014) Detection and mapping of QTL for temperature tolerance and body  
781 size in Chinook salmon (*Oncorhynchus tshawytscha*) using genotyping by sequencing.  
782 Evol Appl 7:480-492.
- 783 Farquhar GD, Richards RA (1984) Isotopic composition of plant carbon correlates with water-  
784 use efficiency of wheat genotypes. Aust J Plant Physiol 11:539-552.

# Genetic architecture of water-use efficiency

785 Farquhar GD, O'Leary MH, Berry JA (1982) On the relationship between carbon isotope  
786 discrimination and the intercellular carbon dioxide concentrations in leaves. Aust J Plant  
787 Physiol 9:121-137.

788 Fisher RA (1918) The correlation between relative on the supposition of Mendelian inheritance.  
789 Philos T R Soc Edinburgh 52:399-433.

790 Ford EB (1975) Ecological Genetics, 4<sup>th</sup> edition. Chapman and Hall, London, UK.

791 Friedline CJ, Lind BM, Hobson EM, Harwood DE, Delfino Mix A, Maloney PE, Eckert AJ (2015)  
792 The genetic architecture of local adaptation I: the genomic landscape of foxtail pine  
793 (*Pinus balfouriana* Grev. & Balf.) as revealed from a high-density linkage map. Tree  
794 Genet Genomes 11:49.

795 Gaston KJ (2003) The Structure and Dynamics of Geographic Ranges. Oxford University Press,  
796 Oxford, UK.

797 Gonzalez-Martinez SC, Huber D, Ersoz E, Davis JM, Neale DB (2008) Association genetics in  
798 *Pinus taeda* L. II. Carbon isotope discrimination. Heredity 101:19-26.

799 Grattapaglia D, Resende MDV (2011) Genomic selection in forest tree breeding. Tree Genet  
800 Genomes 7:241-255.

801 Groover A, Devey M, Fiddler T, Lee J, Megraw R, Mitchell-Olds T, Sherman B, Vujcic S,  
802 Williams C, Neale DB (1994) Identification of quantitative trait loci influencing wood  
803 specific gravity in an outbred pedigree of loblolly pine. Genetics 138:1293-1300.

804 Hampe A (2004) Bioclimate envelope models: what they detect and what they hide. Global Ecol  
805 Biogeogr 13:469-471.

806 Holliday JA, Zhou L, Bawa R, Zhang M, Oubida RW (2015) Evidence for extensive parallelism  
807 but divergent genomic architecture of adaptation along altitudinal and latitudinal  
808 gradients in *Populus trichocarpa*. New Phyt 209: 1240-1251.



# Genetic architecture of water-use efficiency

809 Howe GT, Aitken SN, Neale DB, Jermstad KD, Wheeler NC, Chen THH (2003) From genotype  
810 to phenotype: unraveling the complexities of cold adaptation in forest trees. *Can J Bot*  
811 81:1247-1266.

812 Hutchinson GE (1957) Concluding remarks. *Cold Spring Harb Symp Quant Biol* 22:415-427.

813 Jermstad KD, Bassoni DL, Wheeler NC, Anekonda TS, Aitken SN, Adams WT, Neale DB  
814 (2001) Mapping of quantitative trait loci controlling adaptive traits in coastal Douglas-fir.  
815 II. Spring and fall cold-hardiness. *Theor Appl Genet* 102:1152-1158.

816 Jermstad KD, Bassoni DL, Jech KS, Ritchie GA, Wheeler NC, Neale DB (2003) Mapping of  
817 quantitative trait loci controlling adaptive traits in coastal Douglas-fir. III. Quantitative trait  
818 loci-by-environment interactions. *Genetics* 165:1489-1506.

819 Joao Gaspar M, Velasco T, Feito I, Alia R, Majada J (2013) Genetic variation of drought  
820 tolerance in *Pinus pinaster* at three hierarchical levels: a comparison of induced osmotic  
821 stress and field testing. *PLoS ONE* 8:e79094.

822 Johnson KH, Flanagan LB, Huber DA, Major JE (1999) Genetic variation in growth, carbon  
823 isotope discrimination, and foliar N concentration in *Picea mariana*: analyses from a half-  
824 diallel mating design using field-grown trees. *Can J For Res* 29:1727-1735.

825 Karijolic J, Yi C, Yu Y-T (2015) Transcriptome-wide dynamics of RNA pseudouridylation. *Nat*  
826 *Rev Mol Cell Biol* 16:581-585.

827 Knott SA, Elsen, JM, Haley CS (1996) Methods for multiple-marker mapping of quantitative trait  
828 loci in half-sib populations. *Theor Appl Genet* 93:71–80.

829 Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. *Nat Meth* 9:357-  
830 359.

831 Lemiu R, Fischer M (2008) A meta-analysis of local adaptation in plants. *PLoS ONE* 3:e4010.

832 Li H, Handsaker B, Wysoker A, *et al.* (2009) The Sequence Alignment/Map format and  
833 SAMtools. *Bioinformatics* 25:2078-2079.

# Genetic architecture of water-use efficiency

- 834 Lutz JA, van Wagtendonk JW, Franklin JF (2010) Climatic water deficit, tree species ranges,  
835 and climate change in Yosemite National Park. J Biogeogr 37:936-950.
- 836 Lynch M, Walsh B (1998) Genetics and Analysis of Quantitative Traits. Sinauer Associates, Inc.,  
837 Sunderland, Massachusetts, USA.
- 838 MacArthur RH (1984) Geographical Ecology: Patterns in the Distribution of Species. Princeton  
839 University Press, Princeton, New Jersey, USA.
- 840 Mackay TF, Fry JD, Lyman RF, Nuzhdin SV (1994) Polygenic mutation in *Drosophila*  
841 *melanogaster*: estimates from response to selection of inbred strains. Genetics 136:937-  
842 951.
- 843 Marguerit E, Bouffier L, Chancerel E, Costa P, Lagane F, Guehl J-M, Plomion C, Brendel O.  
844 The genetics of water-use efficiency and its relation to growth in maritime pine. J Exp  
845 Bot 65:4757-4768.
- 846 Mather K (1941) Variation and selection of polygenic characters. J Genetics 41:159-193.
- 847 Mayr E (1963) Animal Species and Evolution. Belknap Press, Cambridge, Massachusetts, USA.
- 848 McCormack JE, Zellmer AJ, Knowles LL (2010) Does niche divergence accompany allopatric  
849 divergence in *Aphelocoma* jays as predicted under ecological speciation?: Insights from  
850 tests with niche models. Evolution 64:1231-1244.
- 851 McCune B (1988) Ecological diversity in North American pines. Am J Bot 75:353-368
- 852
- 853 Neale DB, Savolainen O (2004) Association genetics of complex traits in conifers. Trends Plant  
854 Sci 9:325-330.
- 855 Neale DB, Kremer A (2011) Forest tree genomics: growing resources and applications. Nat Rev  
856 Genet 12:111-122.
- 857 Neale DB, Wegrzyn JL, Stevens KA, Zimin AV, Puiu D, et al. (2014) Decoding the massive  
858 genome of loblolly pine using haploid DNA and novel assembly strategies. Genom Biol  
859 15:R59.

# Genetic architecture of water-use efficiency

- 860 Nystedt B, Street NR, Wetterbom A, Zuccolo A, Lin Y-C, et al. (2013) The Norway spruce  
861 genome sequence and conifer genome evolution. *Nature* 497:579-584.
- 862 Oline DK, Mitton JB, Grant MC (2000) Population and subspecific genetic differentiation in the  
863 foxtail pine (*Pinus balfouriana*). *Evolution* 54:1813-1819.
- 864 Ornduff R (1974) *Introduction to California Plant Life*. University of California Press, Berkeley,  
865 California, USA.
- 866 Pamilo P, Nei M (1988) Relationships between gene trees and species trees. *Mol Biol Evol*  
867 5:568-583.
- 868 Parchman TL, Gompert Z, Mudge J, Schilkey FD, Benkman CW, Buerkle CA (2012) Genome-  
869 wide association genetics of an adaptive trait in lodgepole pine. *Mol Ecol* 21:2991-3005.
- 870 Pavlidis P, Jensen JD, Stephan W, Stamatakis A (2012) A critical assessment of storytelling:  
871 Gene Ontology categories and the importance of validating genomic scans. *Mol Biol*  
872 *Evol* 29:3237-3248.
- 873 Pearson RG, Dawson TP (2003) Predicting the impacts of climate change on the distribution of  
874 species: are bioclimate envelope models useful? *Global Ecol Biogeogr* 12:361-371.
- 875 Pelgas B, Bousquet J, Merimans PG, Ritland K, Isabel N (2011) QTL mapping in white spruce:  
876 gene maps and genomic regions underlying adaptive traits across pedigrees, years and  
877 environments. *BMC Genomics* 12:145.
- 878 Pérez F, Granger BE (2007) IPython: A System for Interactive Scientific Computing. *Comput Sci*  
879 *Eng* 9:21-29.
- 880 Phillips SJ, Anderson RP, Schapire RE (2006) Maximum entropy modeling of species  
881 geographic distributions. *Ecol Model* 190:231-259.
- 882 Prasolova NV, Xu ZH, Farquhar GD, Saffigna PG, Dieters MJ (2000) Variation in canopy  $\delta^{13}\text{C}$  of  
883 8-year-old hoop pine families (*Araucaria cunninghamii*) in relation to canopy nitrogen  
884 concentration and tree growth in subtropical Australia. *Tree Physiol* 20:1049-1055.

## Genetic architecture of water-use efficiency

- 885 Prasolova NV, Lundkvist K, Xu ZH (2005) Genetic variation in foliar nutrient concentration in  
886 relation to foliar carbon isotope composition and tree growth with clones of the F1 hybrid  
887 between slash pine and Caribbean pine. For Ecol Manag 210:173–191.
- 888 Pulliam HR (2000) On the relationship between niche and distribution. Ecol Lett 3:349-361.
- 889 R Core Team (2015) R: A language and environment for statistical computing. R Foundation for  
890 Statistical Computing, Vienna, Austria. URL: <http://www.R-project.org/>
- 891 Ritland K, Krutovsky KV, Tsumura Y, Pelgas B, Isabel N, Bousquet J (2011) Genetic mapping in  
892 conifers. In: Plomion C, Bousquet J, Koe C (eds) Genetics, Genomics and Breeding of  
893 Conifers, CRC Press, New York, USA, pp 196-238.
- 894 Royce EB, Barbour MG (2000) Mediterranean climate effects. II. Conifer growth phenology  
895 across a Sierra Nevada ecotone. Am J Bot 88:919-932.
- 896 Scheet P, Stephens M (2006) A fast and flexible statistical model for large-scale population  
897 genotype data: applications to inferring missing genotypes and haplotypic phase. Am J  
898 Hum Genet 78:629-644.
- 899 Seaton G, Hernandez J, Grunchev JA, White I., Allen J, De Koning DJ, Wei W, Berry D, Haley  
900 C, Knott S (2006) GridQTL: A Grid Portal for QTL Mapping of Compute Intensive  
901 Datasets. Proceedings of the 8th World Congress on Genetics Applied to Livestock  
902 Production, August 13-18, 2006. Belo Horizonte, Brazil.
- 903 Segurado P, Araujo MB (2004) An evaluation of methods for modelling species distributions. J  
904 Biogeogr 31:1555-1568.
- 905 Seibt U, Rajabi A, Griffiths H, Berry JA (2008) Carbon isotopes and water use efficiency: sense  
906 and sensibility. Oecologia 155:441-454.
- 907 Seiler JR, Johnson JD (1988) Physiological and morphological response of three half-sib  
908 families of loblolly pine to water-stress conditioning. For Sci 34:487-495.
- 909 Sewell MM, Bassoni DL, Megraw RA, Wheeler NC, Neale DB (2000) Identification of QTLs  
910 influencing wood property traits in loblolly pine (*Pinus taeda* L.). I. Physical wood

## Genetic architecture of water-use efficiency

- 911 properties. Theor Appl Genet 101:1273-1281.
- 912 Sheth SN, Angert AL (2014) The evolution of environmental tolerance and range size: a
- 913 comparison of geographically restricted and widespread *Mimulus*. Evolution 68:2917-
- 914 2931.
- 915 Shull GH (1908) The composition of a field of maize. Am Breeders Assoc Rep 5:51-59.
- 916 Soberon J, Peterson AT (2005) Interpretation of models of fundamental ecological niches and
- 917 species' distributional areas. Biodiversity Informatics 2:1-10.
- 918 Sorenson FC (1983) Geographic variation in seedling Douglas-fir (*Pseudotsuga menziesii*) from
- 919 the western Siskiyou Mountains of Oregon. Ecology 64:696-702.
- 920 Stephenson NL (1990) Climatic control of vegetation distribution - the role of the water balance.
- 921 Am Nat 135:649-670.
- 922 Tamiru M, Undan JR, Takagi H, Abe A, Yoshida K, et al. (2015) A cytochrome P450, OsDSS1,
- 923 is involved in growth and drought stress responses in rice (*Oryza sativa* L.). Plant Mol
- 924 Biol 88:85-99.
- 925 Toka I, Planchais S, Cabassa C, Justin AM, De Vos D, Richard L, et al. (2010) Mutations in the
- 926 hyperosmotic stress-responsive mitochondrial BASIC AMINO ACID CARRIER2 enhance
- 927 proline accumulation in *Arabidopsis*. Plant Physiol. 152:1851-1862.
- 928 Troncoso-Ponce MA, Cao X, Yang Z, Ohlrogge JB (2013) Lipid turnover during senescence.
- 929 Plant Sci 205-206:13-19.
- 930 van Mantgem PJ, Stephenson NL, Byrne JC, Daniels LD, Franklin JF, et al. (2009) Widespread
- 931 increase of tree mortality rates in the western United States. Science 323:521-524.
- 932 Warren DL, Glor RE, Turelli M (2008) Environmental niche equivalency versus conservatism:
- 933 quantitative approaches to niche evolution. Evolution 62:2868-2883.
- 934 Warren DL, Seifert SN (2011) Ecological niche modeling in Maxent: the importance of model
- 935 complexity and the performance of model selection criteria. Ecol Appl 21:335-342.

## Genetic architecture of water-use efficiency

- Wegrzyn JL, Liechty JD, Stevens KA, Wu L-S, Loopstra CA, et al. (2014) Unique features of the loblolly pine (*Pinus taeda* L.) megagenome revealed through sequence annotation. *Genetics* 196:891-909.
- Weih M, Bonosi L, Ghelardini L, Ronnberg-Wastljung AC (2011) Optimizing nitrogen economy under drought: increased leaf nitrogen is an acclimation to water stress in willow (*Salix* spp.). *Ann Bot* 108:1347-1353.
- Wu RL (1998) Genetic mapping of QTLs affecting tree growth and architecture in *Populus*: implication for ideotype breeding. *Theor Appl Genet* 96:447-457.
- Yeaman S, Whitlock MC (2011) The genetic architecture of adaptation under migration-selection balance. *Evolution* 65:1897-1911.
- Zhang J, Marshall JD (1994) Population differences in water-use efficiency of well-watered and water-stressed western larch seedlings. *Can J Forest Res* 24:92-99.

## Compliance with Ethical Standards

- **Disclosure of potential conflicts of interest:** Funding for this work was provided from Virginia Commonwealth University (start-up funds awarded to A. J. Eckert) and from the National Science Foundation (NSF-NPGI-PRFB-1306622 awarded to C. J. Friedline).
- **Research involving Human Participants and/or Animals:** There were no human participants or animals used in this research.
- **Informed consent:** There were no human participants used in this research, so informed consent is not applicable.

# Genetic architecture of water-use efficiency

**Table 1.** Summary of the families ( $n = 5$ ) used for QTL mapping

	Red	Green	Purple	Blue	Yellow
Latitude	36.448075	36.448075	41.319871	41.195910	41.748267
Longitude	-118.170644	-118.170644	-122.479184	-122.792240	-123.133233
Elevation (m)	3352.80	3352.80	2397.56	2103.12	2103.12
Siblings <sup>a</sup>	35	40	34	40	32
Locality	Cottonwood	Cottonwood	Mt. Eddy	East Boulder	Lake
	Pass	Pass		Lake	Mountain
Region	SN	SN	KM	KM	KM

<sup>a</sup>These counts represent the numbers of siblings genotyped and phenotyped for each family.

Additional siblings for each family are still growing within the common garden (see **Materials and Methods**).

Genetic architecture of water-use efficiency

**Table 2.** Mean and standard deviation (in parentheses) of read metrics by family

Family	Number of reads	Length (bp)	Quality	% Aligned
Blue	1,092,446 (319,903)	89.0 (8.28)	38.0 (1.05)	31.00 (4.881)
Green	691,141 (119,272)	87.6 (10.32)	37.5 (1.16)	26.08 (1.614)
Purple	724,998 (126,585)	88.1 (9.98)	37.6 (1.15)	24.81 (1.398)
Red	1,289,156 (304,551)	89.0 (8.10)	38.1 (1.05)	33.14 (3.577)
Yellow	952,597 (377,357)	88.6 (9.17)	37.8 (1.12)	28.89 (4.185)



Genetic architecture of water-use efficiency

**Table 3.** Attributes of linear mixed models used to estimate familial and regional effects for each phenotypic trait. Values in parentheses are 95% parametric bootstrap confidence intervals (see **Materials and Methods**).

Model Attribute	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
$\log L$	-151.705 (-167.029 – -130.520)	-512.587 (-528.588 – -491.444)
Intercept	-30.755 (-31.439 – -30.075)	21.519 (18.615 – 24.596)
Family variance component ( $\sigma^2_{\text{fam}}$ )	0.159 (0.002 – 0.432)	7.826 (0.000 – 17.521)
Region variance component ( $\sigma^2_{\text{reg}}$ )	0.167 (0.000 – 0.538)	1.696 (0.000 – 9.933)
Residual variance component ( $\sigma^2_{\text{res}}$ )	0.316 (0.249 – 0.384)	22.486 (17.927 – 27.912)

# Genetic architecture of water-use efficiency

**Table 4.** Comparisons of linear mixed models using the Akaike Information Criterion (AIC) by trait were used to select the best model (bolded text). In these models, the intercept was a fixed effect, while families nested within regions and regions were random effects.

Model	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
	AIC	AIC weight <sup>a</sup>	AIC	AIC weight <sup>a</sup>
Intercept	408.10	$3.66 \times 10^{-22}$	1071.76	$1.16 \times 10^{-9}$
<b>Intercept + family</b>	<b>310.29</b>	<b>0.64</b>	<b>1031.26</b>	<b>0.72</b>
Intercept + family + region	311.41	0.36	1033.17	0.28

<sup>a</sup>The AIC weight is calculated using the standardized relative likelihoods, where the relative likelihood is given as  $e^{(-0.5 \times \Delta\text{AIC})}$ . For this calculation,  $\Delta\text{AIC}$  is the difference between the AIC for each model and the AIC for the best model (bolded text), where the best model is the one with the lowest AIC. The weights are then calculated as each of relative likelihoods over the sum of the relative likelihoods, thus making the sum of the weights equal to 1. Akaike weights can also be considered as the conditional probabilities for each model.

# Genetic architecture of water-use efficiency

**Table 5.** Summary of QTLs for each trait that survive multiple test corrected significance thresholds at either the level of the whole genome ( $\alpha = 0.05$  for  $G_{0.05}$ ) or a chromosome ( $\alpha = 0.01$  for  $C_{0.01}$ ,  $\alpha = 0.05$  for  $C_{0.05}$ )

Trait	LG <sup>a</sup>	Position (cM)	<i>F</i>	PVE <sup>b</sup> (PVE <sub>c</sub> )	Threshold <i>F</i> <sup>c</sup>	95% CI <sup>d</sup> (cM)
$\delta^{15}\text{N}$	1	0.0	4.422	26.540 (25.489)	3.818 ( $C_{0.05}$ )	0.0 – 97.0
$\delta^{13}\text{C}$	1	98.0	7.506	49.778 (47.807)	5.803 ( $G_{0.05}$ )	13.0 – 99.0
$\delta^{13}\text{C}$	2	78.0	6.040	39.139 (37.589)	5.803 ( $G_{0.05}$ )	3.0 – 78.0
$\delta^{13}\text{C}$	3	34.0	4.356	26.092 (25.058)	3.456 ( $C_{0.05}$ )	13.0 – 93.0
$\delta^{15}\text{N}$	3	93.0	4.475	27.065 (25.993)	3.725 ( $C_{0.05}$ )	14.0 – 93.0
$\delta^{13}\text{C}$	5	64.0	4.659	28.625 (27.491)	4.008 ( $C_{0.05}$ )	17.0 – 103.0
$\delta^{13}\text{C}$	6	0.0	4.198	24.825 (23.842)	3.835 ( $C_{0.05}$ )	0.0 – 85.0
$\delta^{15}\text{N}$	7	62.0	6.351	41.413 (39.773)	6.091 ( $G_{0.05}$ )	16.0 – 89.0
$\delta^{15}\text{N}$	8	72.0	5.784	37.182 (35.710)	5.559 ( $C_{0.01}$ )	1.0 – 100.0
$\delta^{15}\text{N}$	9	95.0	5.924	38.237 (36.809)	4.958 ( $C_{0.01}$ )	9.0 – 95.0
$\delta^{13}\text{C}$	12	23.0	4.105	24.066 (23.113)	4.072 ( $C_{0.05}$ )	15.0 – 91.0

<sup>a</sup>LG, Linkage group

# Genetic architecture of water-use efficiency

1038 <sup>b</sup>PVE, percent variance explained; PVE<sub>c</sub>, corrected percent variance explained

1039 <sup>c</sup>The threshold value for the *F*-statistic under the null model as determined using the listed value of  $\alpha$   
 1040 (0.05 or 0.01) and permutations following Churchill and Doerge (1994) for either individual linkage groups  
 1041 (C) or the entire genome (G).

1042 <sup>d</sup>95% CI, 95% confidence interval determined through bootstrap analysis ( $n = 1,000$  replicates)

1043

1044

1045

1046

1047

1048

1049

1050

1051

1052

1053

1054

1055

1056

1057

1058

1059

1060

1061

1062

1063

1064

1065

# Genetic architecture of water-use efficiency

**Table 6.** Summary of two QTL models fit to each significant QTL from Table 4. Bolded *P*-values are less than 0.05.

Trait	LG <sup>a</sup>	Position 1 (cM)	Position 2 (cM)	<i>F</i>	<i>P</i>	PVE <sup>b</sup>
δ <sup>15</sup> N	1	0.0	79.0	3.89	<b>0.0023</b>	54.518
δ <sup>13</sup> C	1	98.0	13.0	2.92	<b>0.0149</b>	64.725
δ <sup>13</sup> C	2	77.0	66.0	3.76	<b>0.0030</b>	61.685
δ <sup>13</sup> C	3	34.0	14.0	3.18	<b>0.0091</b>	44.459
δ <sup>15</sup> N	3	52.0	35.0	4.24	<b>0.0012</b>	57.594
δ <sup>13</sup> C	5	64.0	88.0	1.81	0.1135	37.745
δ <sup>13</sup> C	6	46.0	56.0	3.84	<b>0.0026</b>	48.892
δ <sup>15</sup> N	7	62.0	80.0	4.69	<b>0.0005</b>	71.315
δ <sup>15</sup> N	8	71.0	68.0	2.57	<b>0.0287</b>	49.661
δ <sup>15</sup> N	9	95.0	64.0	2.90	<b>0.0155</b>	53.602
δ <sup>13</sup> C	12	23.0	43.0	3.20	<b>0.0088</b>	42.685

<sup>a</sup>LG, Linkage group

<sup>b</sup>PVE, percent variance explained by both QTLs

# Figure Legends

**Figure 1.** Species distribution models (SDMs) created using MaxEnt are good predictors of the current geographical range of foxtail pine (inlaid maps; AUC = area under the receiver operating characteristic curve). Precipitation and temperature-related variables are differentially important, as measured by variable contributions to each model, to the SDM of each regional population of foxtail pine, with precipitation-related variables more important for the Klamath Region and temperature-related variables more important for the southern Sierra Nevada. Variable contribution scores ( $\pm 1$  standard deviation derived from 10 replicated runs of MaxEnt per SDM) are uncorrelated (Spearman's  $\rho = -0.065$ ). For symbols without apparent error bars, the diameter of the circle was greater than the standard deviation.

**Figure 2.** Ranks of variable importance (low rank = more important) based on variable contribution (VC) scores and permutation importance (PI) scores to the SDM for each regional population are moderately correlated ( $r = \text{Spearman's } \rho$ ). Variable types are denoted using filled circles, with black used for temperature-related variables, white for precipitation-related variables, and gray for variables related to both temperature and precipitation.

**Figure 3.** Familial and regional level means ( $\pm 1$  standard error) by trait (left:  $\delta^{13}\text{C}$ , right:  $\delta^{15}\text{N}$ ) are differentiated across families and regions relative to the global mean. Dashed gray lines give global means across all families for each trait. Estimates for the Klamath Mountains (KM) are given as filled circles, while estimates for the southern Sierra Nevada (SN) are given as filled triangles. Familial names are given as colors (see **Materials and Methods**).

**Figure 4.** The relationship between traits based on family means ( $\pm 1$  standard error) is positive (Pearson's  $r = 0.415$ ), although statistically non-significant at  $\alpha = 0.05$  ( $P = 0.487$ ). Dashed gray lines give global means across all families for each trait.

## Genetic architecture of water-use efficiency

1104

1105 **Figure 5.** The distributions of the  $F$ -statistic derived from single QTL models across each

1106 linkage group for carbon isotope discrimination and nitrogen content of needles reveals the

1107 isolated nature of QTLs. The dashed horizontal line in each panel is the genome-wide

1108 significance threshold ( $\alpha = 0.05$ ) for the  $F$ -statistic based on the permutation scheme ( $n = 1,000$

1109 permutations) suggested by Churchill and Doerge (1994). Significant QTLs are denoted with

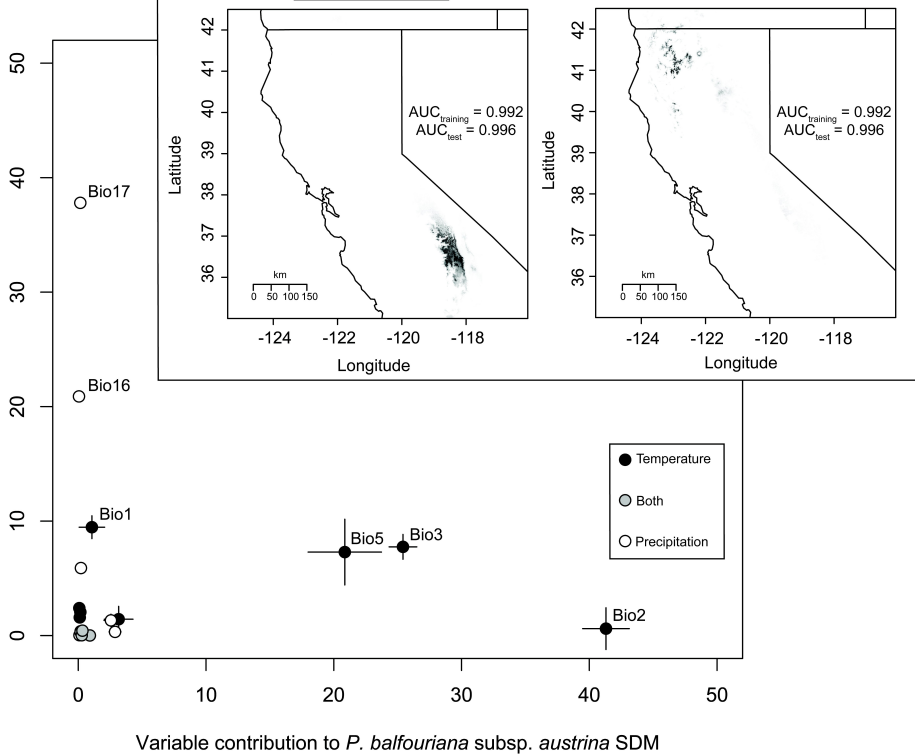
1110 filled circles ( $\alpha = 0.05$ , genome-wide), filled triangles ( $\alpha = 0.01$ , chromosome-wide) or filled

1111 squares ( $\alpha = 0.05$ , chromosome-wide).

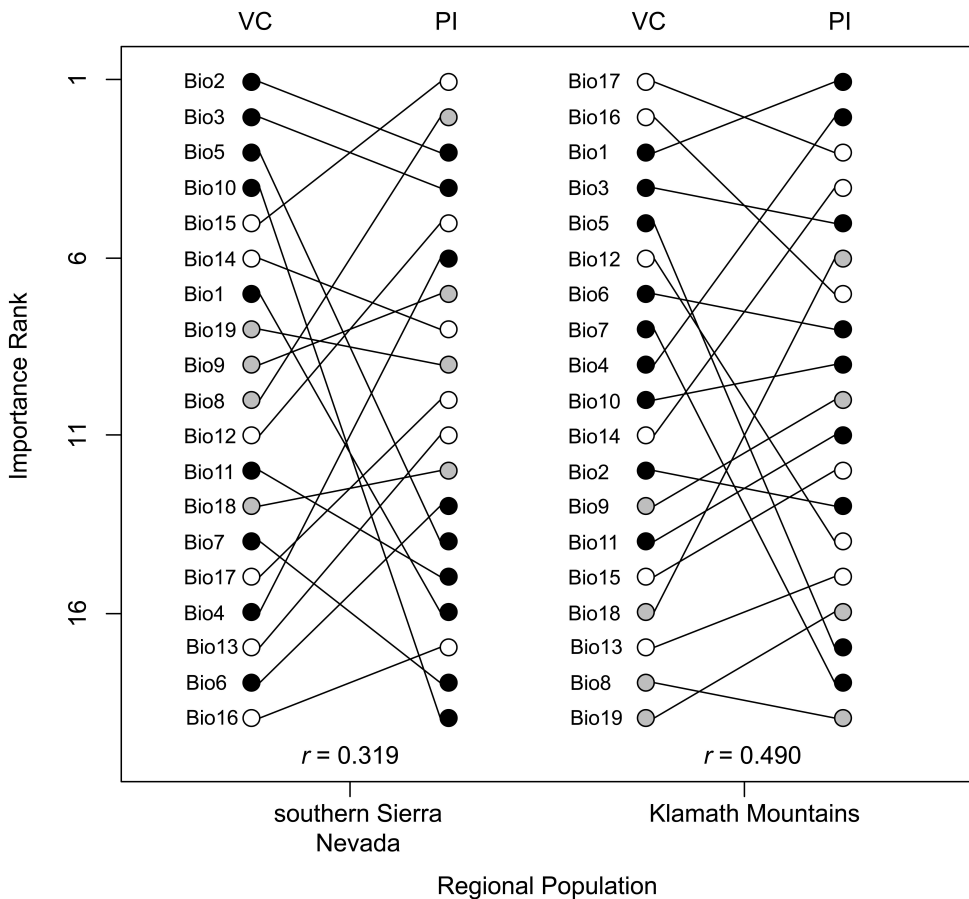
Variable contribution to *P. balfouriana* subsp. *balfouriana* SDM

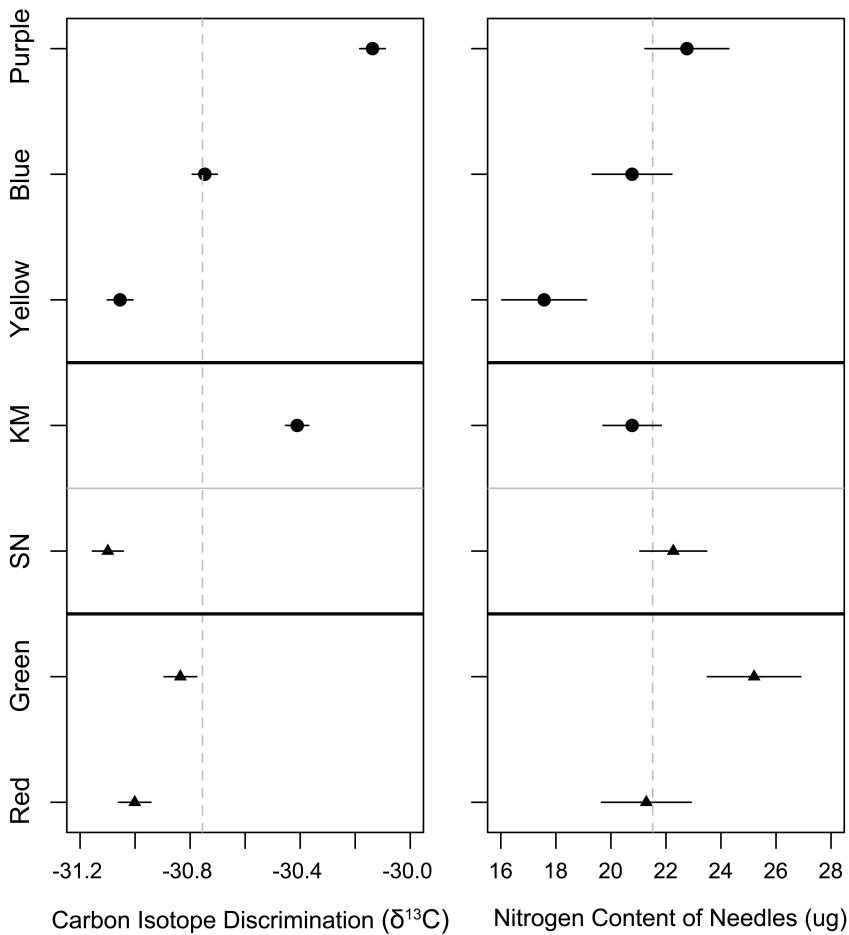
southern Sierra Nevada SDM

Klamath Mountains SDM

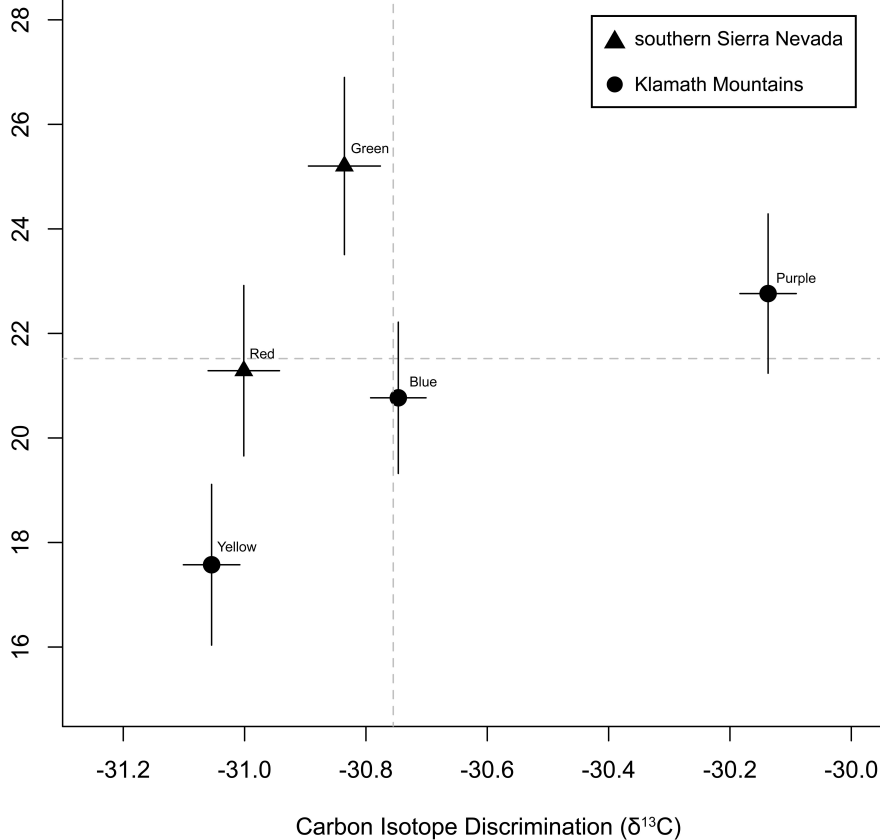




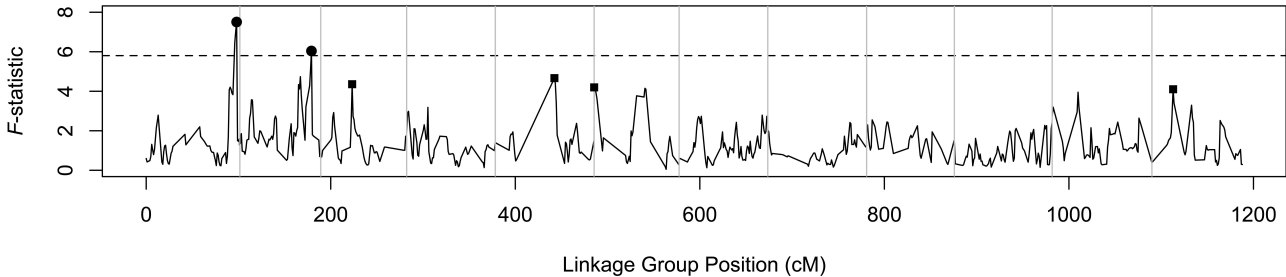




Nitrogen Content of Needles (ug)



## Carbon Isotope Discrimination ( $\delta^{13}\text{C}$ )



## Nitrogen Content of Needles ( $\mu\text{g}$ )

