

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

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

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### 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

52 **Introduction**

53 Descriptions of the genetic components underlying fitness-related phenotypic variation  
54 have been a focus of quantitative genetics for over a century (Shull 1908; Fisher 1918; Mather  
55 1941; Ford 1975; Mackay *et al.* 1994; Ritland *et al.* 2011 and references therein). These  
56 descriptions have progressed from identifications of the genetic elements affecting trait variation  
57 (e.g. Jermstad *et al.* 2001) to analysis of interactions among these elements with one another  
58 and the environment (e.g. Jermstad *et al.* 2003). Uniting all these descriptions are foundational  
59 questions about the structure, function, and evolution of genotype-phenotype maps in natural  
60 populations. For forest trees, these descriptions historically addressed traits of economic  
61 importance such as specific gravity of wood (e.g. Groover *et al.* 1994), microfibril angle (e.g.  
62 Sewell *et al.* 2000), growth (e.g. Wu 1998), and phenology (e.g. Pelgas *et al.* 2011), with the  
63 ultimate goals of marker-assisted breeding (Neale and Savolainen 2004) and trait prediction  
64 from genotypic data (Grattapaglia and Resende 2011). These traits, while economically  
65 important, often also affect fitness (especially phenology, see Sorensen 1983), so that these  
66 efforts can also be leveraged to understand the genetic basis of ecologically relevant trait  
67 variation. The linkage between traits measured in common gardens and fitness in natural  
68 populations, however, is usually assumed *post hoc*, which can lead to storytelling (Barrett and  
69 Hoekstra 2011) and oversimplification of the ecological ramifications of quantitative genetic  
70 results. Here, we address this disconnect through simultaneous use of species distribution  
71 modeling and quantitative trait locus (QTL) mapping to dissect the genetic architecture of an  
72 ecologically important phenotypic trait for foxtail pine (*Pinus balfouriana* Grev. & Balf).

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

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

90 Species distribution models (SDMs) are commonly utilized as predictive tools with which  
91 to assess the importance of environmental variables to current geographical ranges of species  
92 (Elith *et al.* 2006). At a minimum, these models are built from known occurrences of a certain  
93 species and the environmental and ecological attributes of these locations derived from either  
94 field measurements or information stored in geographical information systems (GIS) layers.  
95 Numerous approaches are available with which to build models from these data (Segurado and  
96 Araujo 2004; Elith *et al.* 2006; Phillips *et al.* 2006). Once constructed, SDMs are often used  
97 subsequently to study the evolutionary development of ranges (e.g. McCormack *et al.* 2010), as  
98 well as the effects of continued climate change on current geographical ranges (e.g. Pearson  
99 and Dawson 2003). However, there are limitations to equating SDMs, even those with good  
100 predictive abilities of current geographical ranges, with realized ecological niches and hence  
101 measures of fitness limits (Hampe 2004; Soberon and Peterson 2005; Warren and Seifert  
102 2011). For example, individuals used to create SDMs are considered exchangeable, so that  
103 fitness variation among individuals is ignored (Hampe 2004). Some of these issues, especially

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

108 Water is crucial to the survival of many plant species (e.g. Sorenson 1983), although its  
109 importance relative to other environmental factors varies depending upon the environmental  
110 factors that are most limiting within local environments (Dudley 1996). The intrinsic efficiency by  
111 which plants use water (WUE) is defined as the ratio of net assimilation of carbon from CO<sub>2</sub>  
112 during photosynthesis to the loss of water during transpiration (Bacon 2004). Carbon isotopic  
113 composition ( $\delta^{13}\text{C}$ ) is an indirect measure of intrinsic WUE and is based upon the ratio of two  
114 isotopes of carbon (<sup>13</sup>C and <sup>12</sup>C) within plant tissue standardized to a reference. This ratio is  
115 related to WUE because it has been demonstrated that the discrimination by C<sub>3</sub> plants of <sup>13</sup>CO<sub>2</sub>  
116 relative to <sup>12</sup>CO<sub>2</sub> is correlated to the ratio of carbon assimilation during photosynthesis to  
117 stomatal conductance (Farquhar *et al.* 1982; Farquhar and Richards 1984; e.g. Zhang and  
118 Marshall 1994). The physiological and environmental mechanisms, however, driving the linkage  
119 between  $\delta^{13}\text{C}$  and intrinsic WUE at various levels of biological organization are numerous, so  
120 that the expected linear relationship between  $\delta^{13}\text{C}$  and WUE may not always hold (Seibt *et al.*  
121 2008). For example, differences in  $\delta^{13}\text{C}$  across individual plants at the leaf level can result from  
122 changes in carbon to nitrogen allocation during carboxylation, variation in leaf structure and  
123 morphology, and/or variation in available CO<sub>2</sub> (Seibt *et al.* 2008). Within a common  
124 environment, however, it is assumed that variation in available amounts of atmospheric CO<sub>2</sub> is  
125 negligible. Variation for  $\delta^{13}\text{C}$  across individual plants in these common environments should  
126 therefore reflect variation for intrinsic WUE. Indeed, previous research in conifers has  
127 established that variation in  $\delta^{13}\text{C}$  across individual plants is heritable (Seiler and Johnson 1988;  
128 Cregg 1993; Brendel *et al.* 2002; Baltunis *et al.* 2008; Cumbie *et al.* 2011), is polygenic, yet

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

133 Water availability is often an important driver of tree distributions (Stephenson 1990 and  
134 references therein), especially in Mediterranean climates (e.g. Baldocchi and Xu 2007; Lutz *et*  
135 *al.* 2010). This importance is evident through increased tree mortality as a function of both direct  
136 and indirect consequences associated with changing water availability (van Mantgem *et al.*  
137 2009; Allen *et al.* 2010). Regional and local water availability will likely be altered, either through  
138 changes to annual precipitation totals or the seasonality of precipitation, under most climate  
139 change scenarios, especially in ecosystems dependent on residual summer snow-packs  
140 (Barnett *et al.* 2005). The ability of natural populations of forest trees to respond to changing  
141 water availability is linked to segregating genetic variation for traits responsive to water  
142 availability (Aitken *et al.* 2008). Knowledge of the genetic architecture of such traits, therefore,  
143 provides an important resource for assessing forest health, as well as the genetics of adaptation  
144 (Neale and Kremer 2011). Here, we test two hypotheses about the genetic architecture of WUE  
145 for foxtail pine – (i) water availability is an important determinant of the geographical range of  
146 foxtail pine and hence fitness and (ii) variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  is genetically based,  
147 differentiated between regional populations, and has genetic architectures that include loci of  
148 large effect. We subsequently discuss how the integration of results from disparate fields of  
149 research (i.e. genomics, ecology, and quantitative genetics) provides information useful to  
150 foundational tests about the genetic architecture of local adaptation and its evolution (*cf.*  
151 Friedline *et al.* 2015).

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155 **Materials and Methods**

156 **Focal Species**

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

165 **Common Garden**

166 A common garden representing 141 maternal foxtail pine trees was established at the  
167 Institute of Forest Genetics (Placerville, CA) during 2011 and 2012 using a randomized block  
168 design. Cones were collected from 141 maternal trees sampled range-wide, with 72 sampled  
169 from the Klamath Mountains and 69 from the southern Sierra Nevada region. For each maternal  
170 tree, 35 – 100 seeds were germinated and grown in standard conditions as outlined in Eckert *et*  
171 *al.* (2015). More information about the common garden can be obtained from Friedline *et al.*  
172 (2015). Of these 141 maternal trees, offspring, assumed to be half-siblings, from five were  
173 selected for analysis of water-use efficiency (see **Phenotype determination**, Table 1). The  
174 megagametophyte associated with each germinated seed from these five maternal trees was  
175 rescued and used to construct a high-density linkage map based on four of the five maternal  
176 trees (Friedline *et al.* 2015). The seedlings from each maternal tree were allowed to grow for a  
177 full year after which needles were sampled ( $n = 32$  to 40/maternal tree) for determination of  
178 phenotypes and genotypes. As done by Friedline *et al.* (2015), families were named using  
179 colors (i.e. these were the colors of family identifier tags in the common garden), with families

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

## 182 **Phenotype Determination**

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

## 200 **Sequence Analysis and Genotype Determination**

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

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

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

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

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

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

### 248 **Species Distribution Modeling**

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

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

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

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

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283 statistics given by Warren *et al.* (2008). The former null hypothesis was tested using the  
284 *niche.equivalency.test* function in the *phyloclim* library in R, while the latter null hypothesis was  
285 tested using the *bg.similarity.test* function in the same R library. Both tests were based on  $n =$   
286 100 permutations to derive null distributions of test statistics.

### 287 **Quantitative Genetic Analysis**

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

294 The genetic basis for each measured trait was assessed using linear models. We fit  
295 three different linear models to the observed data for each trait: (1) a fixed effect model  
296 containing only a grand mean (i.e. intercept), (2) a linear mixed model with a grand mean as a  
297 fixed effect plus a random effect of family, and (3) a linear mixed model of a grand mean as a  
298 fixed effect plus a random effect of region plus a random effect of family nested within region.  
299 Uncertainty in parameter estimates from each model was assessed using parametric  
300 bootstrapping ( $n = 1,000$  replicated simulations) as carried out with the *simulate* function in R.  
301 Models were compared using the Akaike Information Criterion (AIC), with Akaike weights used  
302 to assess the conditional probabilities for each model (Burnham and Anderson 2002). If models  
303 containing random effects for families or models containing random effects for regions and  
304 families nested within regions fit the data better than a model with only a grand mean, then we  
305 concluded that there were non-zero heritabilities for these traits. If we assume that all offspring  
306 within each family were half-siblings, we could estimate narrow-sense heritability as  $h^2 =$   
307  $4\sigma_{\text{fam}}^2 / (\sigma_{\text{fam}}^2 + \sigma_{\text{res}}^2)$ , where  $\sigma_{\text{fam}}^2$  is the variance due to family nested within region and  $\sigma_{\text{res}}^2$  is the  
308 residual variance. Given the small number of families, however, we avoided this estimation, as

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

314 The genetic basis of each trait was dissected using the least squares regression  
315 approach of Knott *et al.* (1996) for outbred, half-sibling families, where probabilities of allelic  
316 inheritance due to the common parent were used as predictors for each trait. Significance of the  
317 regression model was determined using a *F*-test calculated at 1-cM intervals, with the  
318 distribution of this statistic under a null model of no QTLs generated via a permutation scheme  
319 (Churchill and Doerge 1994). The common parent in our analyses was the maternal tree, we  
320 assumed that all offspring per maternal tree were half-siblings, and we used 1,000 permutations  
321 to generate null distributions of *F*-statistics. Permutations were used to create null distributions  
322 for *F*-statistics at the level of the entire genome (i.e. all linkage groups) and for each  
323 chromosome (i.e. linkage group) separately. We initially fit models of one QTL per linkage group  
324 using three significance thresholds: (1)  $\alpha = 0.05$  at the level of the entire genome (major QTL),  
325 (2)  $\alpha = 0.01$  at the level of a particular chromosome (minor QTL), and (3)  $\alpha = 0.05$  at the level of  
326 a particular chromosome (suggestive QTL). For each QTL, we estimated the percent variance  
327 explained (PVE) as  $PVE = 4[1 - (MSE_{full}/MSE_{reduced})]$ , where  $MSE_{full}$  and  $MSE_{reduced}$  are the  
328 mean square errors of the full and reduced models, respectively (*cf.* Everett and Seeb 2014).  
329 Following Knott *et al.* (1996), estimates of PVE were scaled by  $(1 - 2r)^2$ , where  $r$  is the  
330 recombination frequency between the marker and QTL (i.e.  $r = 0.01$  for a 1-cM scan of each  
331 linkage group). Uncertainty in the position of the QTL was assessed using bootstrapping ( $n =$   
332 1,000 replicates). For each linkage group with a statistically significant QTL, we subsequently fit  
333 a model of two QTLs using the same approach, with the only differences being the use of  
334 asymptotic null distributions to test the statistical significance of the observed *F*-statistics and

335 the lack of adjustments to estimates of the PVE for multiple QTL models. All analyses were  
336 conducted with the HSportlets module on GridQTL ver. 3.3.0 (Seaton *et al.* 2006; Allen *et al.*  
337 2012) using the linkage map for foxtail pine reported by Friedline *et al.* (2015).

## 338 **Results**

### 339 **Sequence Analysis and Genotype Determination**

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

### 353 **Species Distribution Modeling**

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

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

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

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

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

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

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

### 419 Quantitative Genetic Analysis

420 Variation across siblings measured within the common garden was genetically based for  
421 each trait (Table 3). Family identifiers nested within regional populations accounted for sizeable  
422 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  $\delta^{15}\text{N}$  ( $\sigma^2_{\text{fam}}/[\sigma^2_{\text{reg}} +$   
423  $\sigma^2_{\text{fam}} + \sigma^2_{\text{res}}] = 24.45\%$ ). This was consistent with the differences among predicted family means  
424 for both traits (Figure 2), which were positively correlated (Figure 3), but not significantly so  
425 (Pearson's  $r = 0.415$ ;  $P = 0.487$ ). Regional identifiers, however, were differentially important  
426 across traits, with these identifiers accounting for marginally more variance than family  
427 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  
428 effect of family and regional identifiers (i.e. the total genetic effect =  $[\sigma^2_{\text{reg}} + \sigma^2_{\text{fam}}]/[\sigma^2_{\text{reg}} + \sigma^2_{\text{fam}} +$   
429  $\sigma^2_{\text{res}}]$ ), however, was large for each trait ( $\delta^{13}\text{C}$ : 50.78%;  $\delta^{15}\text{N}$ : 29.75%). Comparisons of linear  
430 models progressing from intercept only to an intercept plus families nested within regions using  
431 AIC, revealed that a linear mixed model with an intercept and families was the best fit (AIC =  
432 310.29 for  $\delta^{13}\text{C}$ ; AIC = 1031.26 for  $\delta^{15}\text{N}$ ; Table 4). Comparison to other models using AIC  
433 weights, however, revealed that the most complex model of an intercept plus region plus  
434 families nested within regions had a reasonably high conditional probability (AIC weight = 0.36  
435  $\delta^{13}\text{C}$ ; AIC weight = 0.28 for  $\delta^{15}\text{N}$ ; Table 4) relative to those for the best model ( $\delta^{13}\text{C} = 0.64$ ;  $\delta^{15}\text{N}$   
436 = 0.72) for each phenotypic trait.

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437 We dissected the genetic basis of the heritable variation evident for each trait from the  
438 linear mixed model analysis using the regression-based approach to QTL mapping of Knott *et*  
439 *al.* (1996). Application of one-locus models (i.e. a maximum of one-locus per linkage group)  
440 resulted in a set of 11 QTLs across all linkage groups and both traits (Table 5; Figure 4). For  
441  $\delta^{13}\text{C}$ , six QTLs were discovered, with two discovered at the most stringent significance level  
442 (genome-wide permutation-based  $\alpha = 0.05$ ) and four at the least stringent significance level  
443 (linkage group specific permutation-based  $\alpha = 0.05$ ). Effect sizes for these QTLs were large to  
444 moderate, with the percent variation explained (PVE) ranging from 47.807% to 24.066%. For  
445  $\delta^{15}\text{N}$ , five QTLs were discovered, with one QTL at the most stringent significance level, two at  
446 the intermediate significance level (linkage group specific permutation-based  $\alpha = 0.01$ ), and two  
447 at the least stringent significance level. Effect sizes for these QTLs were also large to moderate,  
448 with PVE varying from 39.773% to 25.058%. There was moderate autocorrelation for the *F*-  
449 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  
450 no correlation between *F*-statistics for each trait (Pearson's *r*: -0.014, *P* = 0.734; Figure S17). In  
451 general, 95% confidence levels of positions for each QTL were large (Table 5).

452 For the 11 QTLs detected using one-locus models, 10 were consistent with multiple  
453 QTLs using two-locus models (Table 6). In general, the QTLs from the one-locus models were  
454 one of the pair of QTLs detected in the two-locus models. There were four exceptions to this  
455 pattern, with two of these exceptions being a minor modification in position of the original QTL  
456 equal to 1.0 cM. The other two exceptions included significant changes to the position of the  
457 original QTL, with the QTL on linkage group 3 for  $\delta^{15}\text{N}$  changing from 93.0 cM to 52.0 cM and  
458 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  
459 (Tables 5 and 6). The average spacing between QTLs on the same linkage group was 29.4 cM,  
460 with a minimum of 3 cM to a maximum of 85 cM. The multi-QTL PVE for each trait ranged from  
461 a minimum of 42.685% to a maximum of 71.315%, with only one instance of positional overlap

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462 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,  
463 there was a negative relationship between distance (cM) and the correlation of family effects  
464 (Pearson's  $r$ ) between QTLs on the same linkage group (Figure S18), so that strong positive  
465 correlations of family effects were observed when QTLs were close together (<15 cM) and  
466 strong negative correlations when QTLs were farther apart (>20 cM).

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

## 474 Discussion

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

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

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

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

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

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511 example, small differences in soil texture observed across the Southern Sierra Nevada Critical  
512 Zone Observatory, a site not far removed from the regional population of foxtail pine in the  
513 southern Sierra Nevada, result in large differences in the available soil moisture (Bales *et al.*  
514 2011). Soil texture also varied by elevation, with soils at the highest elevations being coarser  
515 and less developed. As such, water availability in these soils was more limited even though  
516 snowfall was typically higher. Soils between regional populations of foxtail pine are  
517 fundamentally different, and so is the local distribution of foxtail pine. In the Klamath Mountains,  
518 soils are primarily ultramafic, while in the southern Sierra Nevada they are largely granitic.  
519 Foxtail pine grows near tops of local peaks in the Klamath Mountains, whereas in the southern  
520 Sierra Nevada it is distributed broadly across large swathes of high elevation sites. Thus, one  
521 explanation for the apparent contradiction is that soil properties are different, so as to create  
522 patterns of soil moisture not reflective of regional mean precipitation patterns. Foxtail pine in the  
523 Klamath Mountains often inhabits areas with high levels of boulder cover (Eckert and Sawyer  
524 2002; Eckert 2006), which are expected to house soils with less capacity to hold water over long  
525 periods of time. When coupled with the higher average temperatures in the Klamath Mountains,  
526 this suggests that water may be more limited throughout the year (e.g. summer drought) than  
527 expected based on annual precipitation totals. Additional work, however, would be needed to  
528 quantify trait variation within each regional population and correlate it to both climate and soil  
529 characteristics.

### 530 **Genetic architecture of water-use efficiency**

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

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536 consistent with previous estimates of quantitative genetic parameters for these phenotypic traits  
537 in other conifers. For example,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  are both heritable in a variety of pine species  
538 (Brendel *et al.* 2002; Baltunis *et al.* 2008; Gonzalez-Martinez *et al.* 2008; Cumbie *et al.* 2011;  
539 Joao Gaspar *et al.* 2013; Marguerit *et al.* 2014; Eckert *et al.* 2015). Populations within many  
540 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*  
541 *al.* unpublished). Further work, however, would be needed to precisely estimate the level of  
542 differentiation for these traits, as well as to test whether this level of differentiation is larger than  
543 that expected for neutral loci (i.e. this pattern is consistent with local adaptation).

544         Estimates of narrow-sense heritabilities ( $h^2$ ) resulted in values greater than 1.0 for each  
545 phenotypic trait no matter which model with a family effect was used (i.e. families or regions  
546 plus families nested within regions). This could be due to tissue sampling occurring prior to  
547 formation of randomized blocks in the common garden, as family groups would be confounded  
548 with micro-environmental variation. Use of data from Eckert *et al.* (2015) and Maloney *et al.*  
549 (unpublished data) for sugar pine (*P. lambertiana* Dougl.), western white pine (*P. monticola*  
550 Dougl.), and whitebark pine (*P. albicaulis* Engelm.) grown at the same facility in the same  
551 experimental conditions, however, reveals that block effects for  $\delta^{13}\text{C}$  were present only for the  
552 relatively fast growing western white pine (Type III Wald  $F$ -tests with Kenward-Rogers degrees  
553 of freedom; sugar pine:  $F_{1,416.49} = 3.5166$ ,  $P = 0.06146$ ; western white pine:  $F_{1,630.24}$ ,  $P =$   
554  $0.00068$ ; whitebark pine:  $F_{1,452.75} = 0.0147$ ;  $P = 0.9037$ ). In contrast, block had a statistically  
555 significant effect on  $\delta^{15}\text{N}$  for sugar pine and western white pine ( $P < 0.001$ ), but not whitebark  
556 pine ( $F_{1,429.22} = 1.6252$ ,  $P = 0.20305$ ). Thus, our results should be taken with caution, but family  
557 effects estimated here were similar in magnitude to those from Eckert *et al.* (2015) and  
558 randomized blocks tended to have no effect on the same phenotypic traits measured in  
559 whitebark pine at the same facility, a species with a similar pattern of early slow growth  
560 (McCune 1988).

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

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

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

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

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

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

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

637 We leveraged the annotations of contigs at or near ( $\pm 3$  cM) the estimated QTL positions  
638 to search for putatively functional genes as the drivers of the genotype-phenotype correlations  
639 for each QTL (Table S3). Annotations for foxtail pine contigs were derived through similarity  
640 searches against the loblolly pine genome. Annotations were obtained from any locus on a  
641 loblolly pine scaffold containing a significant hit to a RADtag from foxtail pine, with significance  
642 justified by the estimated substitution rate and divergence time between these species (Friedline  
643 *et al.* 2015). Several statistically significant QTLs had no annotation information available. For  
644 example, the QTL on linkage group 1 for  $\delta^{13}\text{C}$  had no annotations available within a 6-cM  
645 window encapsulating the QTL, despite 24 of 76 RADtags having significant similarity to  
646 scaffolds in loblolly pine. This is consistent with reports of gene densities reported for conifers  
647 (Nystedt *et al.* 2013; Neale *et al.* 2014). For the QTL related to  $\delta^{13}\text{C}$  on linkage group 2 (Table  
648 5), however, two of the 18 RADtags for foxtail pine had sequence similarity to loblolly pine  
649 scaffolds, with annotated InterPro domains suggestive of loci encoding stress responsive  
650 proteins (Table S3; Toka *et al.* 2010; Karijolic *et al.* 2015). Another example of potentially  
651 biologically informative results included the QTL on linkage group 9 for  $\delta^{15}\text{N}$  where putative  
652 homologs for proteins with domains such as ribosomal protein L38e, cytochrome P450, and  
653 thiolase were present. Proteins containing these domains have been implicated in lipid turnover  
654 during leaf senescence (Troncoso-Ponce *et al.* 2013), as well as plant growth and drought  
655 stress response (Tamiru *et al.* 2015). Care should be taken in interpreting these results,  
656 however, as QTL intervals were wide, annotations were based on statements of homology with  
657 gene predictions in an early release of the loblolly pine genome sequence (Wegrzyn *et al.*  
658 2014), and *post hoc* explanations linking gene products to phenotypic traits is prone to  
659 storytelling (Barrett and Hoekstra 2011; Pavlidis *et al.* 2012). It is important to note, however,

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

### 663 **Conclusions**

664 We have used a mixture of species distribution modeling and quantitative genetics to  
665 test two hypotheses about WUE, as measured by  $\delta^{13}\text{C}$ , for foxtail pine. We showed that  
666 precipitation-related variables structured the geographical range of foxtail pine, that climate-  
667 based niches differed between regional populations, and that similar patterns were apparent for  
668  $\delta^{13}\text{C}$ , which was also demonstrated to be heritable. We subsequently dissected this heritability  
669 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  
670 in light of population genetic theory about local adaptation. While we cannot definitely say that  
671 WUE, as measured by  $\delta^{13}\text{C}$ , contributes to local adaptation, we have described to a first  
672 approximation its genetic architecture, while noting several patterns consistent with  $\delta^{13}\text{C}$  being a  
673 fitness-related trait affected by natural selection. These are useful results with which to generate  
674 further hypotheses about the evolution of genetic architecture contributing to local adaptation in  
675 natural populations (e.g. Holliday *et al.* 2015). Our results also shed light on ecologically  
676 relevant phenotypic trait variation useful for management decisions and predictions for range  
677 shifts under changing climates.

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690 was the basis for this work.

691

#### 692 **Data Archiving Statement**

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

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### 949 **Compliance with Ethical Standards**

- 950 • **Disclosure of potential conflicts of interest:** Funding for this work was provided from  
951 Virginia Commonwealth University (start-up funds awarded to A. J. Eckert) and from the  
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- 953 • **Research involving Human Participants and/or Animals:** There were no human  
954 participants or animals used in this research.
- 955 • **Informed consent:** There were no human participants used in this research, so  
956 informed consent is not applicable.

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962 **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

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

964 Additional siblings for each family are still growing within the common garden (see **Materials**

965 **and Methods**).

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980 **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)

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999 **Table 3.** Attributes of linear mixed models used to estimate familial and regional effects for each  
 1000 phenotypic trait. Values in parentheses are 95% parametric bootstrap confidence intervals (see  
 1001 **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)

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Genetic architecture of water-use efficiency

1013 **Table 4.** Comparisons of linear mixed models using the Akaike Information Criterion (AIC) by  
1014 trait were used to select the best model (bolded text). In these models, the intercept was a fixed  
1015 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

1016 <sup>a</sup>The AIC weight is calculated using the standardized relative likelihoods, where the relative  
1017 likelihood is given as  $e^{(-0.5 \times \Delta\text{AIC})}$ . For this calculation,  $\Delta\text{AIC}$  is the difference between the AIC for  
1018 each model and the AIC for the best model (bolded text), where the best model is the one with  
1019 the lowest AIC. The weights are then calculated as each of relative likelihoods over the sum of  
1020 the relative likelihoods, thus making the sum of the weights equal to 1. Akaike weights can also  
1021 be considered as the conditional probabilities for each model.

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1034 **Table 5.** Summary of QTLs for each trait that survive multiple test corrected significance  
 1035 thresholds at either the level of the whole genome ( $\alpha = 0.05$  for  $G_{0.05}$ ) or a chromosome ( $\alpha =$   
 1036  $0.01$  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

1037 <sup>a</sup>LG, Linkage group

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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)

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Genetic architecture of water-use efficiency

1066 **Table 6.** Summary of two QTL models fit to each significant QTL from Table 4. Bolded *P*-values  
 1067 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>
$\delta^{15}\text{N}$	1	0.0	79.0	3.89	<b>0.0023</b>	54.518
$\delta^{13}\text{C}$	1	98.0	13.0	2.92	<b>0.0149</b>	64.725
$\delta^{13}\text{C}$	2	77.0	66.0	3.76	<b>0.0030</b>	61.685
$\delta^{13}\text{C}$	3	34.0	14.0	3.18	<b>0.0091</b>	44.459
$\delta^{15}\text{N}$	3	52.0	35.0	4.24	<b>0.0012</b>	57.594
$\delta^{13}\text{C}$	5	64.0	88.0	1.81	0.1135	37.745
$\delta^{13}\text{C}$	6	46.0	56.0	3.84	<b>0.0026</b>	48.892
$\delta^{15}\text{N}$	7	62.0	80.0	4.69	<b>0.0005</b>	71.315
$\delta^{15}\text{N}$	8	71.0	68.0	2.57	<b>0.0287</b>	49.661
$\delta^{15}\text{N}$	9	95.0	64.0	2.90	<b>0.0155</b>	53.602
$\delta^{13}\text{C}$	12	23.0	43.0	3.20	<b>0.0088</b>	42.685

1068 <sup>a</sup>LG, Linkage group

1069 <sup>b</sup>PVE, percent variance explained by both QTLs

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## Figure Legends

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1079 **Figure 1.** Species distribution models (SDMs) created using MaxEnt are good predictors of the  
1080 current geographical range of foxtail pine (inlaid maps; AUC = area under the receiver operating  
1081 characteristic curve). Precipitation and temperature-related variables are differentially important,  
1082 as measured by variable contributions to each model, to the SDM of each regional population of  
1083 foxtail pine, with precipitation-related variables more important for the Klamath Region and  
1084 temperature-related variables more important for the southern Sierra Nevada. Variable  
1085 contribution scores ( $\pm 1$  standard deviation derived from 10 replicated runs of MaxEnt per  
1086 SDM) are uncorrelated (Spearman's  $\rho = -0.065$ ). For symbols without apparent error bars, the  
1087 diameter of the circle was greater than the standard deviation.

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1089 **Figure 2.** Ranks of variable importance (low rank = more important) based on variable  
1090 contribution (VC) scores and permutation importance (PI) scores to the SDM for each regional  
1091 population are moderately correlated ( $r = \text{Spearman's } \rho$ ). Variable types are denoted using filled  
1092 circles, with black used for temperature-related variables, white for precipitation-related  
1093 variables, and gray for variables related to both temperature and precipitation.

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1095 **Figure 3.** Familial and regional level means ( $\pm 1$  standard error) by trait (left:  $\delta^{13}\text{C}$ , right:  $\delta^{15}\text{N}$ )  
1096 are differentiated across families and regions relative to the global mean. Dashed gray lines  
1097 give global means across all families for each trait. Estimates for the Klamath Mountains (KM)  
1098 are given as filled circles, while estimates for the southern Sierra Nevada (SN) are given as  
1099 filled triangles. Familial names are given as colors (see **Materials and Methods**).

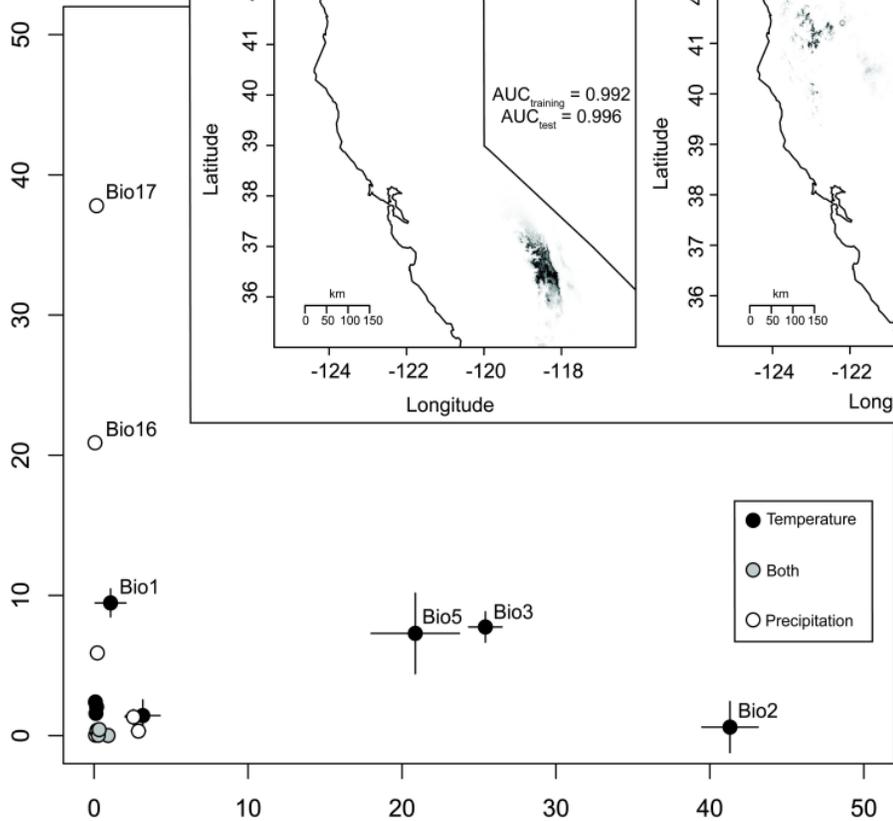
1100  
1101 **Figure 4.** The relationship between traits based on family means ( $\pm 1$  standard error) is  
1102 positive (Pearson's  $r = 0.415$ ), although statistically non-significant at  $\alpha = 0.05$  ( $P = 0.487$ ).  
1103 Dashed gray lines give global means across all families for each trait.

## Genetic architecture of water-use efficiency

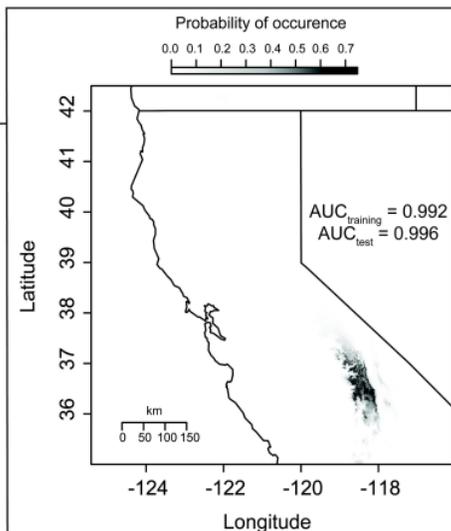
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1105 **Figure 5.** The distributions of the  $F$ -statistic derived from single QTL models across each  
1106 linkage group for carbon isotope discrimination and nitrogen content of needles reveals the  
1107 isolated nature of QTLs. The dashed horizontal 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).

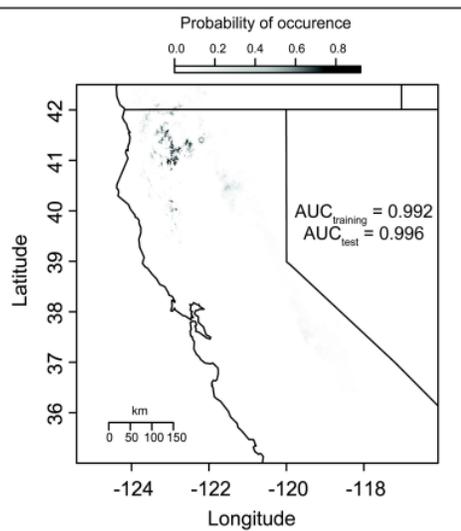
Variable contribution to *P. balfouriana* subsp. *balfouriana* SDM



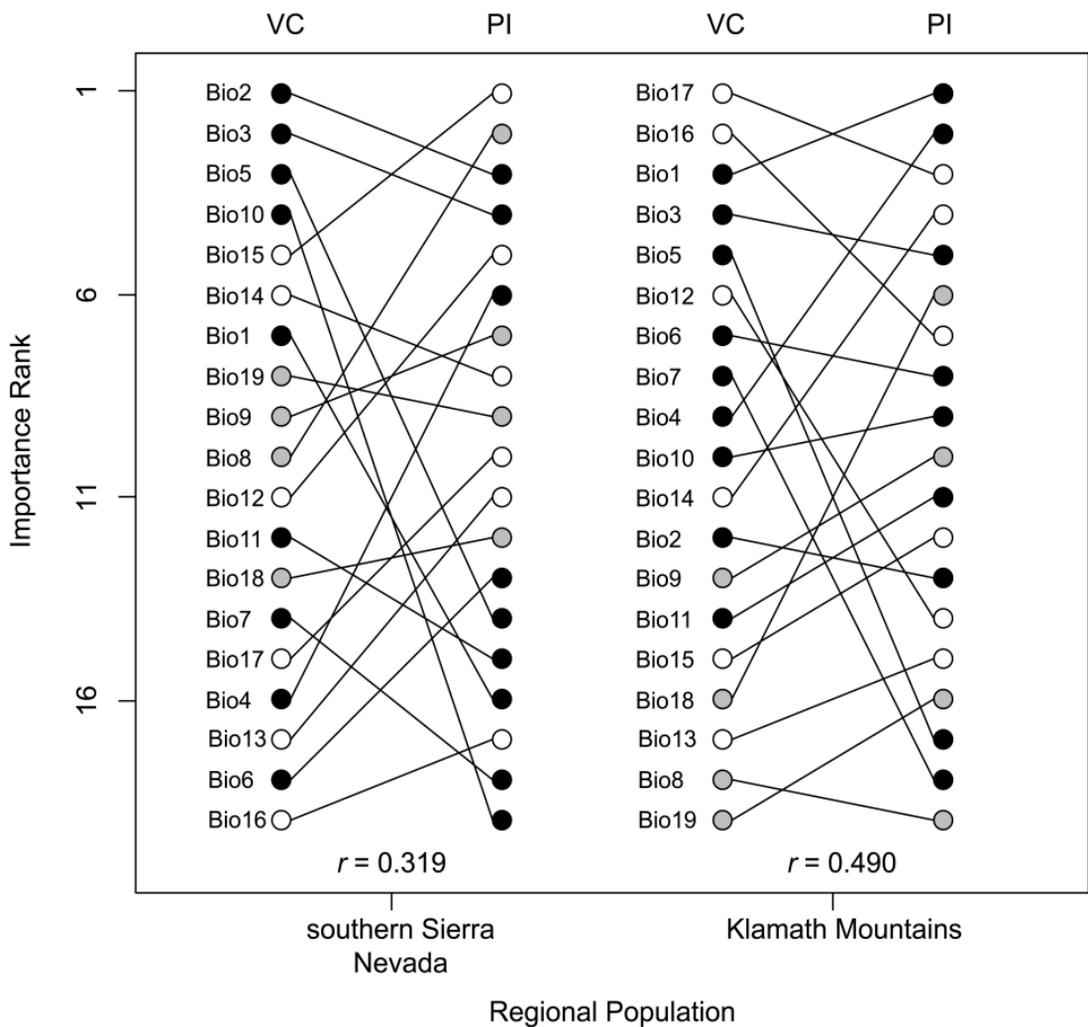
southern Sierra Nevada SDM



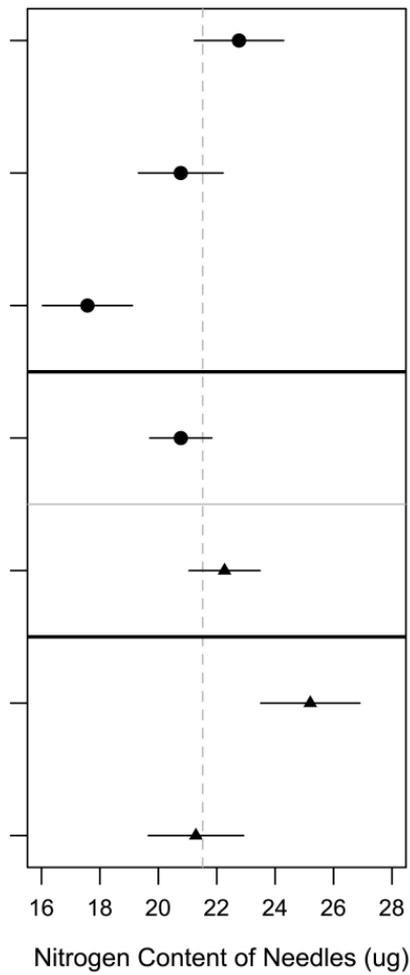
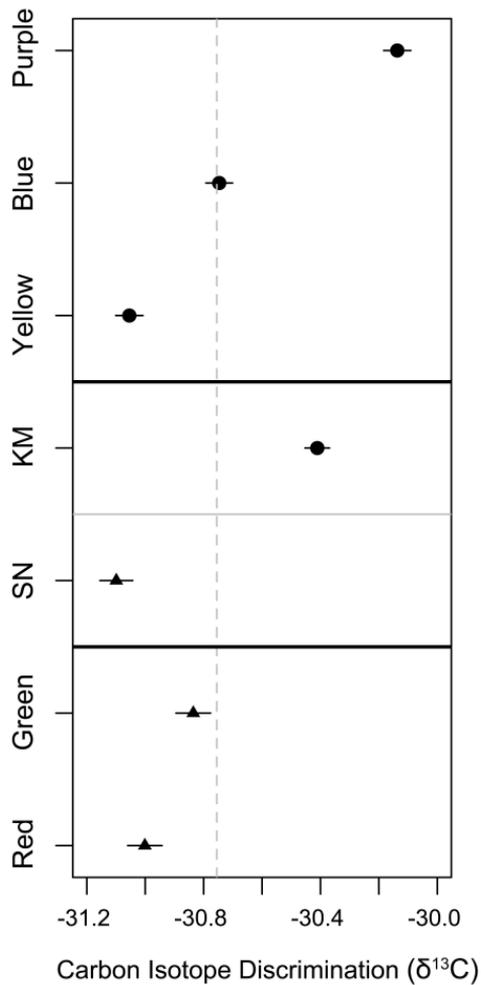
Klamath Mountains SDM

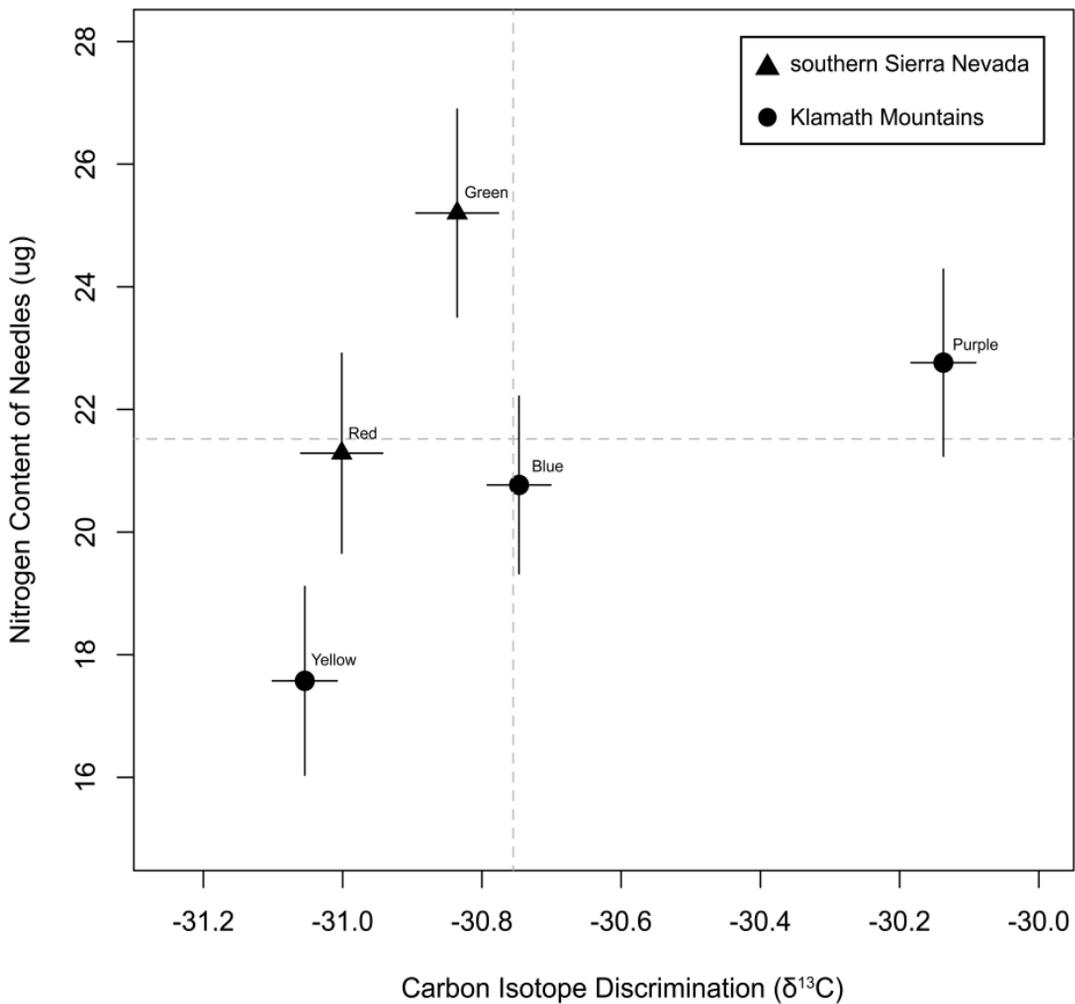


Variable contribution to *P. balfouriana* subsp. *austrina* SDM

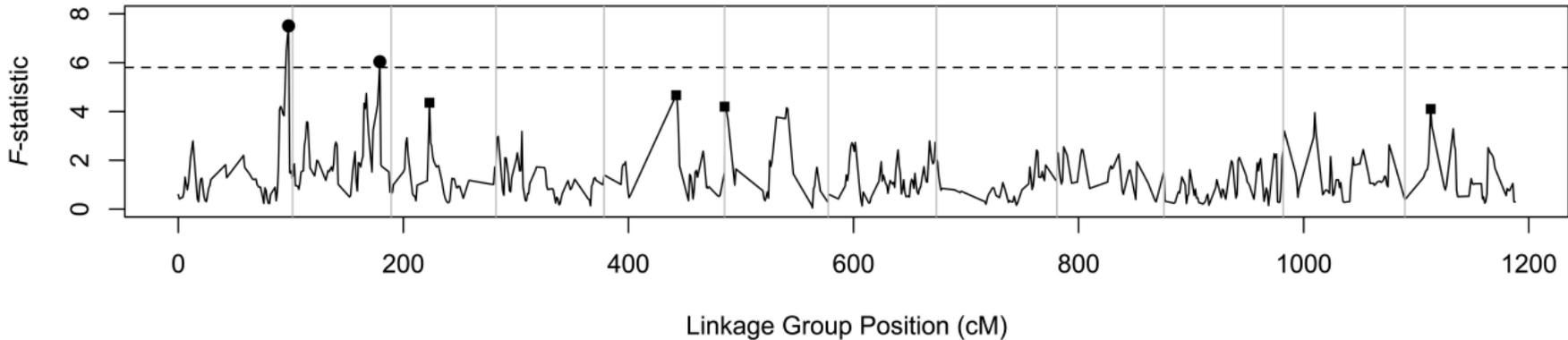


Family or Region





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



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

