

1 **Title**

2 Evolutionary quantitative genomics of *Populus trichocarpa*

3 **Authors**

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28 Supplementary material: All supplementary tables will be made available upon request.

29 **Abstract**

30 Forest trees generally show high levels of local adaptation and efforts focusing on understanding  
31 adaptation to climate will be crucial for species survival and management.

32 Merging quantitative genetics and population genomics, we studied the molecular basis of  
33 climate adaptation in 433 *Populus trichocarpa* (black cottonwood) genotypes originating across  
34 western North America. Variation in 74 field-assessed traits (growth, ecophysiology, phenology,  
35 leaf stomata, wood, and disease resistance) was investigated for signatures of selection  
36 (comparing  $Q_{ST}$  -  $F_{ST}$ ) using clustering of individuals by climate of origin. 29,354 SNPs were  
37 investigated employing three different outlier detection methods.

38 *Narrow-sense*  $Q_{ST}$  for 53% of distinct field traits was significantly divergent from expectations  
39 of neutrality (indicating *adaptive* trait variation); 2,855 SNPs showed signals of diversifying  
40 selection and of these, 118 SNPs (within 81 genes) were associated with adaptive traits (based on  
41 significant  $Q_{ST}$ ). Many SNPs were putatively pleiotropic for functionally uncorrelated adaptive  
42 traits, such as autumn phenology, height, and disease resistance.

43 Evolutionary quantitative genomics in *P. trichocarpa* provides an enhanced understanding  
44 regarding the molecular basis of climate-driven selection in forest trees. We highlight that  
45 important loci underlying *adaptive* trait variation also show relationship to climate of origin.

46 **Author summary**

47 Comparisons between population differentiation on the basis of quantitative traits and neutral  
48 genetic markers inform about the importance of natural selection, genetic drift and gene flow for  
49 local adaptation of populations. Here, we address fundamental questions regarding the molecular  
50 basis of adaptation in undomesticated forest tree populations to past climatic environments by  
51 employing an integrative quantitative genetics and landscape genomics approach. Marker-  
52 inferred relatedness was estimated to obtain the *narrow-sense* estimate of population  
53 differentiation in wild populations. We analyzed an unstructured population of common garden  
54 grown *Populus trichocarpa* individuals to uncover different extents of variation for a suite of  
55 field traits, wood quality and pathogen resistance with temperature and precipitation. We  
56 consider our approach the most comprehensive, as it uncovers the molecular mechanisms of  
57 adaptation using multiple methods and tests. We provide a detailed outline of the required  
58 analyses for studying adaptation to the environment in a population genomics context to better  
59 understand the species' potential adaptive capacity to future climatic scenarios.

## 60 **Introduction**

61 Knowledge about the genetic basis of *adaptive* quantitative traits in forest trees and genetic  
62 differentiation in response to selection facilitates the prediction of long-term responses to  
63 climate, but the genetic basis of adaptation is not comprehensively understood [1]. High levels of  
64 local adaptation due to consistent natural selection in a given environment resulted in local  
65 populations that have their highest fitness at their original provenance, and consequently, are  
66 differentiated from non-local populations. Within population diversity is fundamental to species  
67 survival in unpredictable environments, and therefore also relevant for conservation and forest  
68 management ([2]; [3]). Recent studies within forest trees have investigated the association of  
69 local climate and geography with either randomly identified loci (*Pinus taeda*: [4]; *Cryptomeria*  
70 *japonica*: [5], or candidate functional genes (*Picea abies*: bud set candidate genes, [6]; *Populus*  
71 *balsamifera*: flowering time candidate genes, [7]) to uncover genes underlying local adaptation.  
72 The genetic architecture underlying adaptive phenotypes of forest trees is generally highly  
73 complex (*e.g.* [8]). Therefore, untangling the relationships between adaptive loci and the role of  
74 climate in selection vs. neutral evolutionary processes is inherently difficult.

75 Evidence for potential adaptive significance of a genetic marker is often interpreted from  
76 ‘ $F_{ST}$  outlier’ analyses where genetic loci significantly differ in their allelic frequencies among  
77 populations. These ‘outliers’ can be efficiently detected using multilocus scans comparing  
78 patterns of nucleotide diversity and genetic differentiation to the simulated genome-wide neutral  
79 genetic background ([9]; [10]). For instance, this methodology has led to the detection of SNPs  
80 implicated in local climate adaptation in *Picea* ([11]; [12]; [13]). In order to obtain a detailed  
81 understanding of how populations have diverged in response to climate variation, such  $F_{ST}$   
82 outliers can be tested for associations with an adaptive trait and an environmental variable to

83 substantiate the evidence for their involvement in local adaptation ([14]; [15]). Integrating  
84 quantitative and population genomics is therefore essential to determine the degree to which  
85 genetic and phenotypic variation are driven by selection as opposed to neutral processes (*e.g.*  
86 genetic drift). Specifically, this allows for comprehensive information from genome-wide  
87 association studies (GWAS),  $Q_{ST}$  quantitative genetics analysis (*i.e.* ‘top-down’ approaches,  
88 [16]) and landscape population  $F_{ST}$  outlier analysis (*i.e.* ‘bottom-up’ approaches, [17]) be  
89 merged.

90         The existence of interaction effects among different loci within co-adapted gene  
91 complexes has long been recognized [18]. Yeaman (2013) suggested that ecological selection  
92 might even promote the physical clustering of locally adaptive loci through genomic  
93 rearrangements [19]. Landscape population genomics can identify genome regions significantly  
94 associated with spatial and temporal environmental gradients [3]. For instance, the study using  
95 natural *Arabidopsis* genotypes spanning the species’ range revealed that local adaptation might  
96 be maintained by independent target loci enriched for molecular processes that exhibit their  
97 major genetic effects within distinct local environments but are neutral in others [20]. The  
98 geographic variation in the degree to which a genetic region under selection responds is termed  
99 “conditional neutrality” [21] and suggests a given species has not uniformly responded to an  
100 environmental pressure or that the pressure is not equally active across a species range.  
101 Importantly, the assessment of local adaptation in this work on *Arabidopsis* involves the study of  
102 fitness traits such as fecundity and survival (viability) ([20]; [22]). In addition, there also exist  
103 traits that increase fitness in one environment, but reduce it in another. Ecological genetics can  
104 more easily explore the genetic changes over time in annuals (due to their short generation times)  
105 involving multiple generations studied under a changing environment ([23]; [15]). This is less

106 feasible for long-lived forest trees. However, the estimation of quantitative genetic parameters  
107 using SNP marker-inferred relatedness estimation to obtain *narrow-sense* estimates of  
108 heritability and  $Q_{ST}$  in wild populations [24] can allow monitoring adaptive genetic responses  
109 along an ecological time-scale [15].

110         In this study, we integrated an extensive body of results on the genetics of wild *Populus*  
111 *trichocarpa* Torr. & A. Gray (black cottonwood) to understand adaptation to climate. All  
112 poplars, aspens, and cottonwoods (genus *Populus*) play important roles in natural ecosystems as  
113 pioneer species ([25]; [26]) and are economically important for various industrial products with  
114 an increasing role as bioenergy crops ([27]; [28]; [29]; [30]). *Populus* species are still largely  
115 undomesticated with very low population differentiation indicative of extensive long-distance  
116 intraspecific gene flow [31]. In western North America, *P. trichocarpa* has an extensive  
117 cordilleran range (31-62°N), yet with no clear north-south differentiation in genetic diversity  
118 (and no decreasing genetic diversity with latitude), consistent with the species' colonization  
119 history from multiple potential glacial refugia [32]. Several studies have indicated subtle sub-  
120 structure in *P. trichocarpa* ([33]; [34]; [35]) relating to isolation-by-distance (IBD; *i.e.* the  
121 decrease of genetic similarity among populations with increasing geographical distance between  
122 these populations reflected in *continuous* patterns of genetic differentiation and allele frequency  
123 variation in the species [34] as opposed to natural barriers causing discrete local genetic  
124 clusters), introgression and adaptation [36]. We explored the extensive body of data on the  
125 genetics of *P. trichocarpa*, including genome-wide coverage of SNPs [35], and comprehensive  
126 GWAS results from wood characteristics [37], leaf rust fungus (*Melampsora xcolumbiana*)  
127 resistance [38], biomass, ecophysiology, leaf stomata and phenology traits [39]. We studied the  
128 divergence patterns of phenotypic variation and SNPs among distinct climate clusters in 433

129 unrelated *P. trichocarpa* genotypes originally collected throughout the northern two-thirds of the  
130 species' latitudinal range (excluding the highly diverged Californian population Tahoe: [34],  
131 [40]). We tested whether phenotypic variation in traits was diverged among the climatic regions  
132 (based on non-neutral  $Q_{ST}$ ), as would be expected of adaptive variation. We then predicted that  
133 SNPs that are most diverged among different climatic regions would be associated with mapped  
134 genes that underlie *adaptive* phenotypic variation [13].

135         In brief, we used an integrative analysis of quantitative traits and genetic markers to  
136 investigate climate adaptation in wild *P. trichocarpa* populations, we developed an integrative  
137 approach through merging genomic-based datasets and results. (1) The effects of individual loci  
138 were first separated from confounding population effects using spatial PCA (sPCA) to  
139 investigate the presence of local and global genetic structures. Following this assessment of  
140 population structure using genetic markers showing evidence of only one single genetic  
141 structure, distinct population clusters were generated based on climatic factors and this sub-  
142 population clustering was used in subsequent analyses (Fig. 1). (2) The genetic differentiation in  
143 quantitative traits (narrow-sense  $Q_{ST}$ ) among populations defined by climate clusters was  
144 calculated involving the estimation of relatedness based on genetic markers. (3) In parallel, the  
145 divergence of genetic markers ( $F_{ST}$  outlier analysis) among populations defined by climate  
146 clusters was assessed. (4) The significance of quantitative trait divergence among populations, as  
147 defined by climate clusters, was assessed by comparing the observed  $Q_{ST}$  values with the  
148 simulated distribution of  $Q_{ST}-F_{ST}$  for a neutral trait. If the null hypothesis was rejected, the trait  
149 was considered adaptive. (5) GWAS results identifying the SNP variants underlying adaptive  
150 traits were incorporated. If these SNP variants also corresponded to loci under selection  
151 (employing four different outlier detection methods), then, the SNP variants were considered



152 adaptive. This comprehensive analysis of genomic and phenotypic information underscores the

153 necessity of merging multiple datasets to more fully understand evolutionary genomics of *P.*

154 *trichocarpa*.

155

## 156 **Results**

### 157 **Population structure assessment**

158 Negative eigenvalues from sPCA were negligible (Fig. 2), suggesting no local genetic clusters.  
159 By comparison, the presence of IBD was verified by large positive eigenvalues (Fig. 2). These  
160 results were further confirmed by the local and global tests within the “adegenet” program (see  
161 Methods). While, again, we did not detect local genetic structure in *P. trichocarpa* (local test  
162  $P=0.937$ ), we did identify global genetic structure attributed to IBD (global test  $P=0.001$ ) that  
163 was observed across the entire population involving the 140 unique geographical locations  
164 represented by one randomly chosen genotype.

165

### 166 **Divergence of quantitative characters ( $Q_{ST}$ ) among climate clusters**

167 We calculated *narrow-sense*  $Q_{ST}$  values for 74 distinct field-assessed traits for the study  
168 population. Assessments included 16 wood, 12 biomass, 14 phenology, 18 ecophysiological, 13  
169 leaf stomata, and one rust resistance phenotype (Table S1). Observed  $Q_{ST}$  values for each trait  
170 were compared to the simulated distribution of  $Q_{ST}-F_{ST}$  values for a neutral trait (simulating a  
171 range of possible demographic scenarios, see Methods). Among all traits, 53% (39/74 traits) had  
172  $Q_{ST}$  values significantly different from zero and therefore were classified as *adaptive* (Table 1).  
173 The highest number of significant  $Q_{ST}$  values was observed among biomass traits (76%),  
174 phenology traits (70%), ecophysiology traits (64 %) and leaf rust resistance (100%). By  
175 comparison, only 25% of wood-based traits had significant  $Q_{ST}$  values.  $Q_{ST}$  values for traits that  
176 significantly diverged among the four climate clusters ranged from 0.03 (  $^{15}\text{N}$ , *i.e.* stable  
177 nitrogen isotope ratio) to 0.26 (bole biomass). Among all tested traits, the climatic clusters best  
178 explained the phenotypic variation in phenology based on the  $P_{ST}$  values, ranging from 17%

179 (100% leaf yellowing) to 24% (bud set). Among wood characteristics, two cell wall sugar traits  
180 (% galactose and % arabinose in dry wood) and two wood ultrastructure attributes (fiber length  
181 and microfibril angle) showed significant  $Q_{ST}$  values. The climatic clusters explained 13 and  
182 12% of the arabinose and galactose content, respectively.

183

#### 184 **Identification of SNPs under selection**

185 Using both unsupervised and climate-based SPA, a total of 1,468 SNPs were identified being  
186 under selection at a 5% cutoff for each method (Table S2). We also performed  $F_{ST}$  outlier  
187 analysis on climate clusters. While the mean  $F_{ST}$  value for the complete dataset (29,354 SNPs)  
188 was 0.0108, we obtained a mean neutral  $F_{ST}$  value (0.0078) after removing loci identified to be  
189 potentially under selection [41]. In the final analysis, all loci were tested against this neutral  
190 mean to identify a set of potential  $F_{ST}$  outliers relating to climate. Using 200k simulations in  
191 Fdist2, we identified 121 SNPs outside the 99% limits of the neutral distribution (Fig. S1) as  
192 potential candidates subjected to diversifying (positive) selection related to the four climate  
193 clusters. Among these, 88% of these climate-related ‘outliers’ were confirmed by allelic  
194 frequency correlation analysis with averages for climate variables within subpopulation (using  
195 multiple univariate logit regression models in SAM ( $\alpha=0.05$ , Table S2)), 77 of these loci  
196 persisted across different selection scan scenarios employed (unsupervised SPA, climate-based  
197 SPA, and  $F_{ST}$  analysis based on population subdivision [36]), and 48 SNPs were retrieved using  
198 association genetics (see below) (Table S2). A comparison between Fdist and SPA testing gene  
199 dispersal and employing Moran’s test for spatial autocorrelation (Fig. 3) indicates, in general, the  
200 higher effectiveness of SPA to identify genetic selection signals under patterns of IBD.

201 A significant accumulation of  $F_{ST}$  outliers was identified on chromosome 15 (Fig. S1).  
202 The extent of linkage disequilibrium (LD) between all 121 outlier loci is presented in Fig. S2. In  
203 general, we found that LD was not substantial between SNPs from different genes. Incomplete  
204 LD can be caused by the possibility that SNPs are close to but not in complete LD with the  
205 causal variants (here probably due to ‘tag SNP’ design of the SNP chip array [35]) explaining  
206 why the observed LD between diverged loci is generally low [42] One notable exception is two  
207 neighboring poplar genes (Potri.009G008600 and Potri.009G008500) initially annotated based  
208 on sequence homology to *Arabidopsis* genes as nitrate transporter types *ATNRT2:1* and  
209 *ATNRT2:4*, respectively. The allele frequencies of three SNPs and one SNP, respectively, in  
210 poplar orthologs of *ATNRT2:1* and *ATNRT2:4*, respectively, are strongly correlated to  
211 temperature ( $R^2 > 0.9$ ;  $P = 0.05$ ), while the remaining SNPs in both genes did not follow such a  
212 strong pattern (Fig. S2).

213

#### 214 **SNPs under diversifying selection and associated with quantitative traits**

215 To corroborate findings of candidate loci putatively under diversifying selection based on  
216 climate, we compared these results with SNPs uncovered by associations with adaptive traits  
217 (showing non-neutral  $Q_{ST}$ ). Among four GWAS studies in *P. trichocarpa*, a total of 619 SNPs  
218 had been identified with significant trait associations (at  $\alpha = 0.05$ ): 410 with biomass,  
219 ecophysiology and phenology [39], 141 with wood property traits [43], 40 with *Melampsora*  
220 *xcolombiana* resistance [38], and 28 SNPs related to leaf stomata variation [44].

221 We compared four different outlier analyses to identify selection signals in 29,354 SNPs.  
222 Most trait-associated SNPs for which we detected selection signals were associated with adaptive  
223 traits (89%, Table S2). The highest percentage of trait-associated SNPs in outlier analyses was

224 found for climate-based  $F_{ST}$  outlier analysis (40% of the total number of outliers identified by the  
225 method; 48 SNPs), followed by geography-based  $F_{ST}$  outlier analysis (8%; 75 SNPs that were  
226 reported in [36], unsupervised SPA (5%; 75 SNPs), and SPA with climate as a covariate (3%; 37  
227 SNPs). In total, selection signals were detected for 151 trait-associated SNPs with 44% overlap  
228 among evaluation methods. Interestingly, there was a lack of genome-wide correlation between  
229 selection and association signal (Fig. 4) and thus only dispersed association signals were detected  
230 among SPA selection signals (Fig. 5, Table S2). This result is probably a consequence of the  
231 structure correction methods employed in GWAS.

232 We retrieved a number of unique but also shared SNPs among the different analyses (Fig.  
233 6). Shared SNPs were highest for climate  $F_{ST}$  (75%) and geography-based  $F_{ST}$  (72%).  
234 Unsupervised SPA had the highest number of unique SNPs among the four methods (51%). We  
235 found 118 SNPs associated with adaptive traits (significant  $Q_{ST}$ ) including 59 SNPs under  
236 diversifying selection shared among at least two outlier detection methods and 59 unique SNPs  
237 detected by climate  $F_{ST}$ , climate SPA and unsupervised SPA, respectively (Table S3). A large  
238 number of SNPs (40%) that we identified as  $F_{ST}$  outliers using climate clustering were candidate  
239 SNPs from association studies (Table S2). The high number of trait-associated SNPs reflects  
240 both the polygenic nature of phenotypic traits (*e.g.*, c.200 for bud set, [39]) and linkage  
241 disequilibrium (LD) to a lesser extent. The highest number of climate-based  $F_{ST}$  outliers  
242 associated with adaptive traits was found on chromosome 15 (12 SNPs), identifying a genomic  
243 region where SNPs putatively under selection to local climate generally may be clustered (Fig.  
244 S1).

245 We found that SNPs under potential climate selection matching putative causal variants  
246 from association studies consistently mapped to non-neutral  $Q_{ST}$ , *adaptive* traits (Table S1, Table

247 S2). Only one SNP associated with wood traits (within Potri.009G006500 annotated as *FRA8*  
248 associated with fiber length, [43]) was among the  $F_{ST}$  outlier loci. Comparatively, phenology  
249 traits were the most complex *adaptive* traits from the high match between the total number of  
250 associated SNPs and the proportion of SNPs with allele frequencies significantly diverged  
251 among climate clusters (Table S2). In total, 118 SNPs were outliers under diversifying selection,  
252 associated with *adaptive* traits (significant  $Q_{ST}$ ), and with many SNPs putatively pleiotropic for  
253 functionally uncorrelated adaptive traits, such as autumn phenology, height, and disease  
254 resistance (Table S3). The 78 annotated poplar genes were largely derived from major gene  
255 functional group such as (1) transcription factors of several categories and (2) carbohydrate-  
256 related genes, but also transporters. Among these transporters, two poplar genes  
257 (Potri.009G008600 and Potri.009G008500) annotated based on sequence homology to  
258 *Arabidopsis* genes as nitrate transporter types *ATNRT2:1* and *ATNRT2:4*, respectively, were  
259 highly pleiotropic for several adaptive traits (Table S3).  
260

261 **Discussion**

262 **Evolutionary quantitative genomics**

263 The main focus of our work involved identifying adaptive traits and their genetic basis in forest  
264 trees by employing both a quantitative genetics approach ( $Q_{ST}$  analysis) and population genomics  
265 [16] to uncover SNPs under strong selection (among c.29k tested genetic polymorphisms). Our  
266 analyses revealed that 53% of these traits produced significant *narrow-sense*  $Q_{ST}$  (Table S1)  
267 underscoring that such quantitative traits are very likely related to adaption to local climatic  
268 conditions [45].

269 This study uses SNP marker-inferred relatedness estimation (*i.e.* the ‘animal model’) to  
270 obtain *narrow-sense* estimates of heritability and  $Q_{ST}$  in wild populations [24]. The quality of  
271 genetic estimates using the ‘animal model’ approach largely depends on the accuracy of  
272 relationship coefficient estimates and are affected by: 1) number and quality of markers [46], 2)  
273 variance in actual relatedness [47], and 3) how well the relationship estimates reflect the  
274 segregation of causal variants [48] Our present study is based on extensive, genome-wide SNPs  
275 [35] which can provide high accuracy for both the relationship coefficients and the estimated  
276 genetic parameters. However, samples from natural tree populations are subject to intensive gene  
277 flow (outcrossing) and generally show low levels of relatedness which can negatively affect  
278 heritability and  $Q_{ST}$  analyses.

279 Heritability is usually dependent on the population sampled (*i.e.* the observed allele  
280 frequency differences) and thus, can differ for smaller sampling sizes and/or specific sampling  
281 areas (*e.g.*, central vs. marginal regions of species distribution). Heritability estimates taken  
282 across a greater coverage of the species distribution are more likely to reflect evolutionary  
283 history of the traits (stabilizing vs. diversifying selection) rather than the effects of population

284 subsampling. Sufficient variance in the actual relatedness is also required to reveal heritability in  
285 wild populations [47], although heritability, and indirectly,  $Q_{ST}$  estimates, can suffer from the  
286 inability to separate the pure additive genetics from environmental effects, specifically when  
287 relatedness is lacking. Thus, the presence of LD between markers and causal variants (QTLs) is  
288 crucial to recover the genetic parameters with sufficient precision. In the case of traits under  
289 diversifying selection, the additive genetic variance estimates (such as *narrow-sense* heritability)  
290 may also include a substantial QTL covariance component, in addition to the pure genetic  
291 variance. This is especially the case when many QTLs follow the same cline, and can further  
292 extend the additive genetic variance when the QTLs interact (*i.e.*, epistasis) [49] unless the  
293 epistasis is accounted for in the model [50]. Thus, heritability estimates for traits under  
294 diversifying selection (Table 1) may be upwardly biased (see below).

295         Heritability estimates are often interpreted as the capacity for adaptive evolution. In  
296 addition, epistatic interactions, specifically, the directional epistasis, have major effects through  
297 altering the genetic background (both, the additive genetic variances and the covariances, *i.e.* the  
298 allelic frequencies but also their effects) [51]. Hemani *et al.* (2013) outlined that for traits under  
299 selection, high levels of genetic variation are maintained and the traits evolve more slowly than  
300 expected, yet this could be attributed to high epistasis in traits under strong diversifying selection  
301 [42].

302

### 303 **Selectively non-neutral genetic variants underlying traits adaptive to climate**

304 Overall, the number of  $F_{ST}$  outlier SNPs underlying an adaptive trait correlated well with the  
305 total number of candidate SNPs associated with that trait ( $r=0.625$ ,  $P=0.0005$ ). Yet, the majority  
306 of trait associated SNPs were not  $F_{ST}$  outliers (Table S2) and appeared to be unresponsive to



307 selection for different climatic conditions, especially for phenology traits such as bud set, leaf  
308 drop or growth period. A previous simulation study suggested that differentiation in candidate  
309 loci is limited for complex traits in forest trees (*i.e.*, their  $F_{ST}$  values are similar to neutral values),  
310 despite their strong adaptive divergence among local populations (high  $Q_{ST}$ ), due to large  
311 population sizes and high levels of gene flow [52]. Thus, highly polygenic adaptation (as  
312 observed in complex genetic traits) will not show sufficient allele frequency differentiation such  
313 that climatic clines in SNPs of candidate genes can be exhaustively detected.

314 We modelled the spatial structure of genetic variation using SPA (addressing gene flow  
315 under IBD), and SNPs identified via SPA were compared against GWAS-identified SNPs,  
316 climate-related  $F_{ST}$  outliers and geography-informed  $F_{ST}$  outliers. The majority of SNPs with  
317 steep allele frequency clines (based on unsupervised SPA) uncovered allele frequency  
318 correlations with the north-south cline (Table S2). We noted that enrichment for particular genes,  
319 such as circadian rhythm/clock genes, was found in PC1 (a north-south population structure) [45]  
320 and that SNPs of these genes were among the highest ranked in SPA. Nonetheless, associations  
321 of circadian rhythm clock genes with strong correlations to environment were largely missing  
322 among the identified genetic associations for phenology traits (discussed in McKown et al. [39]).  
323 The interplay of IBD and natural selection was lost by the necessary structure correction in  
324 GWAS, however, evidence from gene expression or gene regulation that is also highly correlated  
325 with the trait under question might be possible to retrieve such SNPs of putative importance  
326 (Anonymous, [53]).

327 The presence of IBD in *P. trichocarpa* underscores the larger issue for investigating wild  
328 populations with quantitative genetics and population genomics approaches as IBD can confound  
329 population structure, association mapping, and outlier analyses. The power to detect local

330 selection depends on several factors, including selection strength, the presence of distinct types  
331 of microenvironment heterogeneity, and the distance of gene dispersal compared to the overall  
332 spatial scale [54]. In our case, as the observed gene dispersal is ~500 km (Fig. 3) and sampling is  
333 also discontinuous (Fig. 1), this does not allow us to perform  $F_{ST}$  analysis on arbitrarily defined  
334 local populations because it will be more difficult to separate the stochastic noise (drift,  
335 migration) from the selection signal in smaller scale population subsampling leading to an excess  
336 of false positives [54]. Yet, selection pressures can differ along environmental clines. Thus,  $F_{ST}$   
337 outliers should be investigated on the largest scale possible following the spatial distribution of  
338 the environment in order to identify spatial genetic structure. Nevertheless, IBD in wild  
339 populations will create some compromised statistical power in detecting local adaptation using  
340 specific pairs of populations that is unavoidable (Fig. 3).

341

### 342 **Polygenic and pleiotropic adaptation relating to climate**

343 Our climate clustering partitioned the study population into four large, evenly-sized groups of  
344 individuals lending robustness to SNP detection even for lower frequency (recent) variants. In  
345 our study, the top two SNPs among climate related  $F_{ST}$  outliers showed strongest associations to  
346 climate partitions according to SAM analysis [Potri.010G250600 (*MSR2/MANNAN SYNTHESIS*  
347 *RELATED 2* implicated in carbohydrate metabolism) and Potri.010G254400 (transporter  
348 *ATGCN4*) (Table S2)]. In addition, six genes that harboured climate-related  $F_{ST}$  outlier SNPs  
349 have been identified as candidates for bud set in previous studies ([55]; [56]), yet these loci were  
350 not associated with bud set in our GWAS study ([39]; Table S2), possibly through implementing  
351 the conservative population structure correction term in GWAS. Nevertheless, these genes may  
352 represent additional candidates for bud set, including Potri.003G218900 (*ACDI-LIKE*),

353 Potri.009G015100 (senescence-associated family protein), Potri.014G170400 (*XERICO*),  
354 Potri.015G012500 (*IQ-domain 21*), Potri.018G015100 (chloroplast nucleoid DNA-binding  
355 protein), and Potri.019G078400 (leucine-rich repeat transmembrane protein kinase) (Table S2).

356 Evidence is emerging that for perennial trees to effectively sense short day signals, *i.e.*  
357 critical day length in autumn phenology [57], a temperature optimum is required and genetically  
358 pre-determined by the local climate of the individual's origin [58]. Allele frequencies for most of  
359 the SNPs that both associated with bud set and diverged among the climate clusters showed  
360 strong regression on the mean temperature variation of the climatic clusters ( $R^2$  up to 0.94; Table  
361 S2). A critical role for temperature, rather than precipitation, on bud set has also been found in  
362 *Picea* [12]. For autumn phenology, elevated temperatures can either accelerate or delay growth  
363 cessation depending on species or ecotype ([59]; [60]), but under climate warming, the overall  
364 effects on phenological timing in forest trees is unknown.

365 SNP allelic frequencies within both nitrate transporter genes *ATNRT2:4* and *ATNRT2:1*  
366 were strongly aligned with temperature variation ( $R^2 \sim 90\%$ ) in *P. trichocarpa*. Moreover, these  
367 SNPs were pleiotropic for multiple autumn phenology traits, height, and leaf rust resistance  
368 (Table S3). Nitrate transporters are generally important in plants, as nitrate is the main nitrogen  
369 source required for synthesis of nucleic and amino acids. Therefore, a regulation of nitrate  
370 distribution is crucial to modulate growth (biomass acquisition) in response to temperature or  
371 light conditions ([61]; [62]). Interestingly, there are only two poplar representatives within a  
372 phylogenetic sub-clade of NRT2 that is populated by as many as five *Arabidopsis* sequences  
373 (*ATNRT2.1/2.2/2.3/2.4/2.6*). This implies that a deletion event occurred in this clade whose  
374 functional significance remains elusive to date [62]. Phylogenetic reconstruction coupled with  
375 gene expression analysis point at neo/subfunctionalisation of the two poplar nitrate transporters

376 for long distance nitrate transport from roots, wood to leaves [62]. This acquisition of novel  
377 expression pattern and loss of the ancestral expression pattern demonstrates the signature of  
378 adaptive evolution in functional diversification in paralogous gene pairs [63].

379 In addition, our results revealed that adaptive genetic variants within both poplar nitrate  
380 transporters were also associated with leaf rust resistance ([38]; Table S3). In *Arabidopsis*, loss  
381 of function of *ATNRT2.1* primes salicylic acid signaling and *PR1* up-regulation [64]. In poplar  
382 leaf rust inoculations, both *PTNRT2.4* and *PTNRT2.1* are strongly down-regulated in  
383 incompatible interactions, while no expression change is apparent in compatible interactions (J.  
384 La Mantia, personal observation). The identified nitrogen transporters might be important in  
385 nitrogen storage and nitrogen remobilization to recycle nutrients during the progression of leaf  
386 senescence [65]. They may also function in down-regulation of nitrogen assimilation during  
387 seasonal remodeling of tree phenology related to growth cessation induced by short photoperiods  
388 ([66]; [67]) and/or temperature [58]. The effect of temperature on rust aggressiveness is noted  
389 [68] and the climatic conditions which form a conducive environment for rust infection and  
390 disease duration likely provide a strong adaptive selection toward resistance.

391 Pectin esterase gene Potri.012G014500 (SNP scaffold\_12\_1811250) represents another  
392 example for which significant associations with climate (here: temperature) and several adaptive  
393 traits were found (Table S2, Table S3). In fact, the allelic effects of this SNP related to  
394 hypostomaty also related to less rust infection ([45]). This is an illustrative example regarding  
395 the tradeoff between carbon gain and pest resistance under favourable climatic conditions  
396 relating to pathogen pressure ([45]).

397

398 **Conclusions**

399 The high adaptive potential of tree populations is considered the result of positive effects of  
400 long-distance gene flow based on its interactions with divergent selection across the contrasting  
401 environments [69], while local adaptation in forest trees with regards to climate-related traits is  
402 polygenic and recent [70]. For instance, interactions between temperature and photoperiodic cues  
403 were shown to influence bud set for short-term acclimation in poplar [58]. By combining  
404 quantitative genetics and population genomics analyses, our study contributes to an enhanced  
405 understanding of the molecular basis of adaptation to different local climate in an  
406 undomesticated perennial species (*P. trichocarpa*). The key findings provided SNPs whose  
407 allelic frequencies were most diverged among populations from different climate clusters and  
408 these SNPs tended to be associated with mapped genes underlying phenotypic variation. This  
409 phenotypic variation itself diverged among the different climate clusters. Our study dissected the  
410 influence of climate (specifically, temperature and precipitation), yet much of the variation in  
411 phenology is also attributed to photoperiod ([71]; [72]; [45]). The tight photoperiodic control of  
412 traits such as bud set, height growth cessation, and leaf senescence ([73]; [74]; [59]) is crucial  
413 both for resistance to cold temperatures and maximization of the growing season, particularly in  
414 trees originating from high-latitude and/or high elevation provenances ([75]; [56]). While we  
415 tested the influence of climate on the variation of other traits in *P. trichocarpa*, such as wood and  
416 biomass, we consider other local factors, such as soil condition (pH and minerals), soil/root  
417 microbial diversity, groundwater, and other ecological interactions also of potential importance.  
418 Reciprocal transplants will be necessary to elucidate the effects of gene  $\times$  environment plasticity  
419 on the expression of traits with spatially heterogeneous selection [76], but can focus on specific  
420 genes identified through a combined quantitative genomics analysis, such as the one proposed  
421 here. Forthcoming research can also scale trait-to-performance mapping in known pedigrees for

422 the assessment of SNP effects on fitness [77]. These findings will have important implications  
423 for the future management of natural forests, acting to guide efforts in facilitated adaptation to  
424 climate change via measure such as assisted gene flow [78].

425

## 426 **Materials and Methods**

### 427 **Collection, genotyping, and phenotyping of *P. trichocarpa***

428 Plant material was collected from a population of 433 *P. trichocarpa* Torr. & A. Gray genotypes  
429 growing in a common garden. These genotypes came from 140 unique geographic locations  
430 spanning two thirds of the species' range (44-60°N, 121-138°W) ([79], Fig. 1). Originally  
431 collected by the BC Ministry of Forests, Lands and Natural Resource Operations, individual  
432 genotypes were grown in two common gardens, Surrey, BC and Totem Field, University of  
433 British Columbia, BC. Genotypes were replicated across the two field gardens and the Totem  
434 Field individuals (established in 2008 [80]) were clonal propagations from Surrey site  
435 individuals (established in 2000 [79]).

436 Trees were genotyped using an Illumina iSelect array with 34,131 SNPs from 3,543  
437 candidate genes designed for *P. trichocarpa* [35]. The characteristics of the poplar genome and  
438 array development are outlined in [35]). Briefly, the SNP array was designed to include genes of  
439 known importance (*i.e.* candidate genes) or genes based on expression analyses. Because of the  
440 rate of linkage disequilibrium (LD) decay in *P. trichocarpa*, between 67 – 134k SNPs would be  
441 required to include all common variants throughout the genome at LD=0.2 (assuming a 403 Mb  
442 assembled genome length and an average of 3–6 kb for  $r^2$  between common variants to drop to  
443 0.2). Therefore, some SNPs were selected as representative SNPs to “tag” genes and genetic  
444 regions with high LD, and thus represent a group of SNPs (the haplotype). For this study, we  
445 further filtered array SNPs for: i) minor allele frequency (MAF) <0.05, ii) >10% missing data,  
446 and iii) Illumina's GenTrain score <0.5, thereby reducing SNP numbers to 29,354. This filtering  
447 is not biased towards higher frequency SNPs (*i.e.* older variants established at much higher

448 frequencies within the population over time) as a wide distribution of allele frequencies  
449 (MAF>0.05) was considered for the analysis.

450 Phenotyping of genotype accessions within the common gardens and climate of origin  
451 data were obtained from previously published work (for full phenotyping details, see [38]; [37],  
452 [45]). In brief, phenology, ecophysiology, biomass [45], leaf stomatal anatomy [44] and leaf rust  
453 (*Melampsora xcolumbiana*) resistance traits [38] were repeatedly measured from accessions  
454 planted at the University of British Columbia's research field through replication in space (clonal  
455 ramets) and in time (measurements across years). Wood chemistry and ultrastructure traits were  
456 measured from wood cores of the nine-year-old ortets representing the same genotypes and  
457 growing in Surrey [37].

458

#### 459 **Assessment of population structure**

460 Since forest tree species usually have extensive geographic ranges, exhibit extensive gene flow  
461 and have low levels of population stratification [81], we investigated whether the genetic  
462 variability due to non-random mating in our population was caused solely by isolation-by-  
463 distance (IBD), reflecting the large geographical distribution of our sample (cf. [36]), or also by  
464 natural barriers causing local genetic clusters. We performed spatial principal component  
465 analysis (sPCA) by using the “spca” function implemented in the R package “adegenet” [82]  
466 which is a spatially explicit multivariate analysis accounting for spatial autocorrelation processes  
467 and patterns of genetic variation. A K-nearest neighbours method with K = 10 was used as  
468 connection network. Positional information for each genotype were transformed into Universal  
469 Transverse Mercator (UTM) coordinates using “convUL” in the R package “PBSmapping” [83].  
470 Due to the occurrence of multiple genotypes with identical geographical coordinates (*i.e.* trees



471 collected at the same latitude/longitude), we randomly selected a single genotype representing a  
472 geographical region (out of the total 140 locations). Eigenvalues for principal components from  
473 sPCA provided a cumulative picture about contributing factors, including the genetic variance  
474 and the spatial autocorrelation (through Moran's I, see below). Large positive eigenvalues reflect  
475 the importance of the proportion of the genetic variance along with a strong positive  
476 autocorrelation in the global pattern (*i.e.* IBD), while large negative eigenvalues indicate the  
477 importance of the proportion of the genetic variance along with negative autocorrelation  
478 indicating the existence of discrete local genetic clusters.

479 We used the "global.test" and "local.test" functions in the "adegenet" package to infer the  
480 statistical significance of each type of genetic structure. These functions are based on a spectral  
481 decomposition of the connection matrix into Moran's eigenvector map and test for association of  
482 those eigenvectors from Moran's eigenvector map with Moran's I [82]. To investigate gene  
483 dispersal, we employed a Moran I test for spatial autocorrelation ([84]; [54]). Moran's I  
484 coefficients were investigated in 200 km spatial lags and the analysis was performed using  
485 "moran.test" in the "spdep" R package [85]. Moran's I coefficients were estimated as follows:

$$486 \quad I = \frac{n}{\sum_{i=1}^n \sum_{j=1}^n w_{ij}} * \frac{\sum_{i=1}^n \sum_{j=1}^n w_{ij} (x_i - \bar{x})(x_j - \bar{x})}{\sum_{i=1}^n (x_i - \bar{x})^2} \quad [1]$$

487 where  $n$  is the number of populations (*i.e.* unique geographical locations),  $w_{ij}$  is weight set at 0  
488 or 1 depending on whether populations are considered neighbours in each 200 km lag test,  $x_i$  is  
489 the allele frequency in the  $i^{\text{th}}$  population, and  $\bar{x}$  is the allele frequency across all populations.

490

#### 491 **Climatic zone clustering of *P. trichocarpa***

492 Since our initial investigation of population structure with sPCA indicated the presence of only  
493 one global structure consisting of IBD and lack of local discrete clusters, any marker-based

494 inference about genetic clusters might be highly unreliable [86]. Therefore, we established  
495 population differentiation on the basis of climate envelopes ([12]). Clusters of individual  
496 genotypes were defined using climate of origin measures (*i.e.* independently of the genetic data).  
497 Climate variables were obtained using ClimateWNA [87] and included mean annual temperature  
498 (MAT; °C), number of frost-free days (NFFD), and mean annual precipitation (MAP; mm).  
499 Climate data were based on positional information (latitude, longitude, elevation) and 1971-2002  
500 Canadian Climate Normals [45]. Using K-medoids clustering and the Calinski-Harabasz  
501 criterion [88], we split the study population into four groups with relatively balanced sample  
502 sizes of 87, 103, 142, and 101 representing climate classes #1-4, respectively. Clusters generally  
503 followed the western North American coastline inwards (Fig. 1a & b).

504

### 505 **Genetic differentiation in quantitative characters among populations defined by climate** 506 **clustering**

507 We tested phenotypic characteristics in *P. trichocarpa* for their adaptive potential (Table S1).  
508 For  $Q_{ST} - F_{ST}$  comparisons,  $Q_{ST}$  values among the identified climate-related population groups  
509 were first estimated for each trait following [89] and [24], respectively.

510 The *narrow-sense*  $Q_{ST}$  was estimated by computing the variance components using the  
511 ‘animal model approach’ [90] following:

$$512 \quad y = \mathbf{X}\beta + \mathbf{Z}p + \mathbf{Z}a + e \quad [2]$$

513 where  $\beta$  is a vector of fixed effects (intercept),  $p$  and  $a$  are vectors of random climate cluster and  
514 individual tree additive genetic effects,  $\mathbf{X}$  and  $\mathbf{Z}$  are incidence matrices assigning fixed and  
515 random effects to measurements in vector  $y$ , the cluster effects are following  $p \sim N(0, \sigma_p^2)$  where  
516  $\sigma_p^2$  is the cluster variance, individual tree additive effects are following  $a \sim N(0, \sigma_a^2 \mathbf{G})$  where  $\sigma_a^2$  is

517 the additive genetic variance and  $\mathbf{G}$  is the realized relationship matrix [91], using 29,354 SNPs  
518 estimated in R package “synbreed” [92] as follows:

$$519 \quad \mathbf{G} = \frac{\mathbf{Z}\mathbf{Z}'}{2\sum p(1-p)} \quad [3]$$

520 where  $\mathbf{Z}$  is  $\mathbf{M}-\mathbf{P}$ , with  $\mathbf{M}$  the marker matrix with genotypes recoded into 0, 1 and 2 for the  
521 reference homozygote allele, the heterozygote and the alternative homozygote allele,  
522 respectively, and with  $\mathbf{P}$  the vector of doubled allele frequency;  $\mathbf{e}$  is the vector of random residual  
523 effects following  $\mathbf{e} \sim \mathbf{N}(0, \sigma_e^2 \mathbf{I})$  where  $\sigma_e^2$  is the residual variance and  $\mathbf{I}$  is the identity matrix. The  
524 *narrow sense*  $Q_{ST}$  was estimated as follows:

$$525 \quad Q_{ST} = \frac{\hat{\sigma}_p^2}{(\hat{\sigma}_p^2 + 2\hat{\sigma}_a^2)} \quad [4]$$

526 where  $\hat{\sigma}_p^2$  and  $\hat{\sigma}_a^2$  are the estimates of cluster and additive genetic variance representing among-  
527 and within-group trait variances attributable to additive effects.

528         The measurements of all ecology and disease traits using clonal ramets (*i.e.* replication)  
529 enable estimating *broad-sense*  $Q_{ST}$  directly without the use of any relationship matrix, while  
530 *narrow-sense*  $Q_{ST}$  estimation was based on variance components estimated in the mixed linear  
531 model considering the realized relationship matrix [91] as in equation 2. The model is identical  
532 to equation 2 where the variance components for *broad-sense*  $Q_{ST}$  were estimated in the model  
533 considering  $\mathbf{a}$  as the vector of clonal genotypic values following  $\mathbf{a} \sim \mathbf{N}(0, \sigma_a^2 \mathbf{I})$  where  $\sigma_a^2$  is the total  
534 genetic variance (including both additive and non-additive component) and  $\mathbf{e}$  as the vector of  
535 ramet within clone effects following  $\mathbf{e} \sim \mathbf{N}(0, \sigma_e^2 \mathbf{I})$ . Then, the computed  $Q_{ST}$  values for each trait  
536 were compared to the average population differentiation estimate ( $F_{ST}$ ) strictly based on neutral  
537 markers (see below) allowing inferences about trait evolution based on selection or genetic drift  
538 (neutral trait), [93].

539 *Narrow-sense* heritability ( $h^2$ ) was based on variance components estimated in the mixed  
540 model as follows:

$$541 \quad y = \mathbf{X}\beta + \mathbf{Z}a + e \quad [5]$$

542 where  $\beta$  is the vector of fixed effects (intercept and cluster) and  $a$  is the random vector of  
543 additive genetic effects following the description of equation 2. The *narrow-sense* heritability  
544 was estimated as follows:

$$545 \quad \hat{h}^2 = \frac{\hat{\sigma}_a^2}{\hat{\sigma}_a^2 + \hat{\sigma}_e^2} \quad [6]$$

546 where  $\hat{\sigma}_a^2$  and  $\hat{\sigma}_e^2$  are estimates of additive genetic and residual variance, respectively. The  
547 phenotypic  $Q_{ST}$  (i.e.  $P_{ST}$ ) ([89]; [24]) was estimated as follows:

$$548 \quad P_{ST} = \frac{\hat{\sigma}_p^2}{(\hat{\sigma}_p^2 + 2\hat{h}^2\hat{\sigma}_e^2)} \quad [7]$$

549 where  $\hat{\sigma}_p^2$  and  $\hat{\sigma}_e^2$  are estimates of cluster and residual variance representing among- and within-  
550 population variances, respectively, and  $\hat{h}^2$  is the heritability estimated according to [37]. The  
551 variance components were estimated in ASReml software [94] using the mixed linear model  
552 following:

$$553 \quad y = \mathbf{X}\beta + \mathbf{Z}p + e \quad [8]$$

554 where  $\beta$  is the vector of fixed effects (intercept) and  $p$  is the vector of random cluster effects, the  
555 effect of individuals within cluster is found within the error variance.

556

### 557 **Identification of non-neutral SNPs and quantitative traits divergent among climate clusters**

558 To identify SNPs putatively under selection and also associated with adaptive traits ([38]; [43];  
559 [39]), we performed: 1)  $F_{ST}$  outlier analysis (using Fdist2) employing the same climate clusters  
560 as for  $Q_{ST}$  analysis, 2) unsupervised spatial ancestral analysis (SPA), and 3) SPA with climate as

561 a covariate. Additionally, we compared our results with  $F_{ST}$  outlier analysis (using Fdist2 and  
562 BayeScan) that were reported in [36] using 25 topographic units separated by watershed barriers  
563 within the geographic area from Central Oregon, USA (44.3°N) to northern BC, Canada  
564 (59.6°N)).

565  $F_{ST}$  values for SNPs were calculated among the four climate clusters (for definition and  
566 calculation, see above). We implemented the Fdist2 program within the LOSITAN project [41]  
567 for SNP  $F_{ST}$  outlier detection. Fdist2 compares the distribution of  $F_{ST}$  values of sampled loci to  
568 the modeled neutral expectation of  $F_{ST}$  distribution using coalescent simulations [9]. We  
569 employed the infinite alleles mutation model (as we investigated SNPs), a subsample size of 50,  
570 and ran 200k simulations.  $F_{ST}$  values conditioned on heterozygosity and outside the 99%  
571 confidence interval were considered candidate outliers.

572 Since *P. trichocarpa* populations have known structure related to IBD ([36] and this  
573 study), we applied spatial ancestral analysis (SPA), a logistic regression-based approach [86], to  
574 detect SNPs with sharp allelic frequency changes across geographical space (implying  
575 candidates under selection). The unsupervised learning approach (using only genomic data) was  
576 employed to obtain SPA statistics. In addition, we tested SPA including the first two principal  
577 components (PCs) based on climate variables (explaining 91% of the variance) as covariates to  
578 determine individuals' location based on allele frequencies related to MAT, NFFD, and MAP  
579 climate components.

580 We investigated correlations between the outlier SNPs (based on climate clusters) and the  
581 environmental variables that defined the established climatic clusters (Fig. 1). Subpopulation  
582 averages for MAT, NFFD, and MAP were tested for correlations with SNP allele frequencies  
583 employing multiple univariate logistic regression models with the spatial analysis method (SAM;

584 [95]). The significance of correlations was assessed using three independent statistical tests  
585 (likelihood ratio and two Wald tests) implemented in SAM and applying an initial 95%  
586 confidence interval for the statistical tests. We used the Bonferroni correction method ( $\alpha=0.05$ )  
587 for multiple testing resulting in  $p < 6.887052 \times 10^{-5}$  for 726 tested models (242 alleles, three  
588 variables). Only those correlations that remained significant after Bonferroni correction for each  
589 of the three test statistics (*i.e.* the likelihood ratio and the two Wald tests) were retained.

590 Finally, we compared observed  $Q_{ST}$  values with the simulated distribution of  $Q_{ST}-F_{ST}$   
591 values for a neutral trait using previously provided R scripts [96]. In brief, a range of possible  
592 demographic scenarios was tested simulating the distribution of  $Q_{ST}$  values based on mean  $F_{ST}$   
593 for neutral markers and mean  $Q_{ST}$  for neutral traits ([97]; [98]). For a neutral trait, the expected  
594  $Q_{ST}$  was estimated based on  $\hat{\sigma}_p^2$  (*i.e.*, measured within-population variance; see above) and  $\hat{\sigma}_a^2$   
595 (*i.e.*, expected between-population variance) given in equation 4. The distribution of  $\sigma_p^2$  values  
596 was based on  $\sigma_a^2$  and the observed  $F_{ST}$  values of 29,233 SNPs present (total number reduced by  
597 removing outliers) within the simulated *neutral* envelope of  $F_{ST}$  values ( $F_{ST}$  outlier analysis) with  
598  $Q_{ST}$  replaced by the  $F_{ST}$  in equation 4. *P*-values were obtained by testing whether the null  
599 hypothesis that the estimated *narrow-sense*  $Q_{ST}$  for each tested trait is statistically equal to the  
600 expected  $Q_{ST}$  for a neutral trait [96].

601

## 602 **Marker-trait association mapping**

603 In previous analyses of marker-trait associations in *P. trichocarpa*, confounding effects of  
604 population stratification were adjusted using principal component analysis ([38]; [43]; [39] and a  
605 **Q** matrix population structure correction [39]. Phenological mismatch within the common garden  
606 can confound trait values [45], thus, association analyses included “area under the disease curve”

607 resistance measures with adjustment for bud set [38] and all ecophysiological traits that were  
608 measured prior to bud set [39]. The Unified Mixed Model (a modification of the generalized  
609 linear model) was employed for marker-trait association mapping and is fully described ([38];  
610 [43]; [39]). While necessary, the adjustment for confounding, cryptic genetic structure in the  
611 association analyses may have reduced the statistical power to detect associations. This is  
612 particularly problematic in species whose distribution is mainly along a one-dimensional cline or  
613 for which differentiation in ecological traits covaries with the species demographic history ([13];  
614 [45]). Furthermore, the GWAS results may be biased towards common variants or variants with  
615 the greatest effects. This is related to the size of the SNP discovery panel (34k) [99] and the  
616 power to detect significant associations given the tested population sizes (334-448 individuals).  
617 As whole genome sequencing and phenotyping of thousands of genotypes would be required to  
618 comprehensively uncover the genetic architecture of complex traits, we consider the GWAS  
619 results informative but not exhaustive.

620

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626

## 627 **Author contributions**

628 Conceived and designed the experiments: YE-K, RDG, RCH, JE, SDM, CJD, Performed the  
629 experiments: IP, ADM, JL, Analyzed the data: JK, IP, Contributed reagents/materials/analysis  
630 tools: PI, Wrote the paper: IP, JK, YE-K.

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877 **Supporting table captions**

878

879 Table S1. Comprehensive population differentiation estimates and  $h^2$  corrected  $P_{ST}$  for *P.*

880 *trichocarpa*: broad-sense and narrow-sense  $Q_{ST}$  for 58 distinct field traits;  $Q_{ST1}$  and narrow-

881 sense  $Q_{ST}$  ( $Q_{ST2}$ ) estimates for 16 wood traits.

882 (XLS)

883

884 Table S2. Comprehensive summary table of all SNP detection results from GWAS [ecology

885 [39]; rust [38]; stomata [44]; wood [43]] and outlier analysis (geographic  $F_{ST}$  [36], this study:

886 climate  $F_{ST}$ , unsupervised SPA, climate SPA) for the black cottonwood population (presented in

887 Fig. 1) and using the 34k SNP chip [35]; adaptive traits (significant  $Q_{ST}$ ) are in bold. In red and

888 dark blue are 1% cutoffs ( $spa=2.78025$  and  $spa=1.50795$ ), in orange and light blue are 5%

889 cutoffs ( $spa=2.12467$  and  $spa=1.08868$ ) in unsupervised SPA and climate SPA analyses,

890 respectively.

891 (XLSX)

892

893 Table S3. List of 118 SNPs associated with *adaptive* traits (significant  $Q_{ST}$  for at least one

894 associated trait) including 59 SNPs under diversifying selection shared among at least two outlier

895 detection methods and 59 unique SNPs detected by climate  $F_{ST}$ , climate SPA and unsupervised

896 SPA, respectively. Comprehensive results are provided in Table S2.

897 (XLS)

898 **Tables**

899

900 Table 1.  $h^2$ ,  $Q_{ST}$ , and  $h^2$  corrected  $P_{ST}$  of adaptive traits ( $P < 0.05$ )

901 Summary of 39 distinct *adaptive* traits of *P. trichocarpa* that diverged among different climate clusters (displayed are 59 tests for

902 adaptation including tests for traits replicated in time, comprehensive results shown in Table S1)

#	Trait	<i>narrow-sense</i> $h^2$	S.E.	<i>narrow-sense</i> $Q_{ST}$	S.E.	Variance		
						explained by	S.E.	$P$ -value
						partitions		
1	Bole density_2012 <sup>a</sup>	0.4040	0.0402	0.0482	0.0522	0.0397	0.0429	0.0017
2	Bole mass_2012 <sup>a</sup>	0.1758	0.0430	0.2584	0.1788	0.1109	0.0877	0.0000
3	*Branches_2009 <sup>a</sup>	0.4898	0.0245	0.1567	0.1151	0.1541	0.1131	0.0000
4	H:D2+_2011 <sup>a</sup>	0.3753	0.0254	0.0321	0.0352	0.0243	0.0268	0.0178
5	*Height_2008 <sup>a</sup>	0.4540	0.0260	0.1133	0.0905	0.1040	0.0835	0.0000
6	*Height_2009 <sup>a</sup>	0.6543	0.0200	0.1132	0.0893	0.1432	0.1088	0.0000
7	*Height_2010 <sup>a</sup>	0.7378	0.0165	0.0900	0.0743	0.1274	0.1006	0.0000
8	*Height_2011 <sup>a</sup>	0.7092	0.0178	0.0792	0.0673	0.1087	0.0892	0.0000
9	*Height gain_2009 <sup>a</sup>	0.7504	0.0163	0.0952	0.0777	0.1364	0.1061	0.0000
10	*Height gain_2010 <sup>a</sup>	0.6217	0.0212	0.0477	0.0455	0.0586	0.0551	0.0019
11	*Height gain_2011 <sup>a</sup>	0.3372	0.0250	0.0490	0.0483	0.0337	0.0335	0.0016
12	Whole tree mass_2012 <sup>a</sup>	0.2279	0.0434	0.2323	0.1634	0.1225	0.0953	0.0000
13	*Volume_2009 <sup>a</sup>	0.3663	0.0256	0.1159	0.0925	0.0877	0.0718	0.0000
14	*Volume_2010 <sup>a</sup>	0.4519	0.0253	0.0945	0.0783	0.0862	0.0718	0.0000
15	*Volume_2011 <sup>a</sup>	0.5091	0.0243	0.0900	0.0751	0.0915	0.0760	0.0000
16	*Volume gain_2010 <sup>a</sup>	0.4441	0.0254	0.0913	0.0763	0.0820	0.0689	0.0000



17	*Volume gain _2011 <sup>a</sup>	0.4396	0.0253	0.0923	0.0771	0.0822	0.0691	0.0000
18	Amax/mass_2009 <sup>b</sup>	0.1349	0.0264	0.1822	0.1396	0.0579	0.0493	0.0000
19	Amax_2009 <sup>b</sup>	0.1916	0.0261	0.0596	0.0604	0.0240	0.0248	0.0007
20	Chlsummer _2009 <sup>b</sup>	0.2692	0.0292	0.1160	0.0968	0.0663	0.0577	0.0000
21	Chlsummer _2011 <sup>b</sup>	0.3078	0.0288	0.1438	0.1135	0.0939	0.0777	0.0000
22	C:N_2009 <sup>b</sup>	0.1631	0.0270	0.1423	0.1156	0.0518	0.0454	0.0000
23	d15N_2009 <sup>b</sup>	0.0882	0.0232	0.0257	0.0395	0.0047	0.0072	0.0446
24	Dleaf_2009 <sup>b</sup>	0.4872	0.0272	0.0269	0.0299	0.0263	0.0291	0.0371
25	gs_2009 <sup>b</sup>	0.4243	0.0279	0.0402	0.0401	0.0344	0.0343	0.0055
26	Leaves per bud _2011 <sup>b</sup>	0.3307	0.0310	0.0767	0.0695	0.0523	0.0482	0.0001
27	Leaves per bud _2012 <sup>b</sup>	0.4786	0.0297	0.0910	0.0765	0.0875	0.0735	0.0000
28	*LMAsummer _2010 <sup>b</sup>	0.2360	0.0281	0.0628	0.0644	0.0307	0.0322	0.0000
29	Narea_2009 <sup>b</sup>	0.1907	0.0278	0.0479	0.0525	0.0189	0.0211	0.0028
30	Nmass_2009 <sup>b</sup>	0.1592	0.0271	0.1409	0.1150	0.0500	0.0441	0.0000
31	WUE_2009 <sup>b</sup>	0.2457	0.0274	0.0731	0.0667	0.0373	0.0350	0.0000
32	AUDPC-2009 <sup>c</sup>	0.5322	0.0245	0.0490	0.0470	0.0521	0.0495	0.0017
33	AUDPC-2010 <sup>c</sup>	0.3937	0.0260	0.0723	0.0646	0.0579	0.0523	0.0002
34	AUDPC-2011 <sup>c</sup>	0.3132	0.0251	0.0848	0.0740	0.0551	0.0492	0.0001
35	*Active growth rate _2009 <sup>d</sup>	0.6094	0.0222	0.0390	0.0393	0.0471	0.0469	0.0083
36	*Bud set _2008 <sup>d</sup>	0.5970	0.0224	0.1390	0.1051	0.1617	0.1186	0.0000
37	*Bud set _2009 <sup>d</sup>	0.7390	0.0165	0.1790	0.1262	0.2438	0.1580	0.0000
38	*Bud set _2010 <sup>d</sup>	0.6483	0.0200	0.1708	0.1224	0.2108	0.1434	0.0000

39	Bud set186_2009 <sup>d</sup>	0.5247	0.0234	0.1988	0.1368	0.2067	0.1403	0.0000
40	Bud set186_2010 <sup>d</sup>	0.4041	0.0268	0.2125	0.1444	0.1792	0.1261	0.0000
41	*Height growth cessation _2009 <sup>d</sup>	0.7114	0.0178	0.1434	0.1072	0.1923	0.1354	0.0000
42	*Leaf drop _2008 <sup>d</sup>	0.5175	0.0244	0.1533	0.1137	0.1579	0.1160	0.0000
43	*Leaf drop _2009 <sup>d</sup>	0.5168	0.0237	0.2335	0.1525	0.2396	0.1547	0.0000
44	*Leaf drop _2010 <sup>d</sup>	0.5965	0.0214	0.1453	0.1088	0.1687	0.1225	0.0000
45	*Leaf lifespan_2010 <sup>d</sup>	0.6278	0.0208	0.0432	0.0419	0.0537	0.0514	0.0039
46	Canopy duration _2009 <sup>d</sup>	0.2409	0.0253	0.0944	0.0809	0.0480	0.0428	0.0000
47	*Canopy duration _2010 <sup>d</sup>	0.8119	0.0126	0.0462	0.0438	0.0729	0.0671	0.0024
48	Growth period _2009 <sup>d</sup>	0.3176	0.0255	0.1046	0.0862	0.0693	0.0589	0.0000
49	*Growth period _2010 <sup>d</sup>	0.7095	0.0176	0.1365	0.1032	0.1833	0.1308	0.0000
50	*Post-bud set period _2009 <sup>d</sup>	0.4222	0.0260	0.0332	0.0352	0.0282	0.0299	0.0187
51	*Post-bud set period _2010 <sup>d</sup>	0.5230	0.0237	0.1432	0.1075	0.1489	0.1106	0.0000
52	*100% Yellowing _2010 <sup>d</sup>	0.5886	0.0220	0.1498	0.1113	0.1718	0.1240	0.0000
53	*75% Yellowing _2010 <sup>d</sup>	0.5640	0.0227	0.0638	0.0571	0.0714	0.0632	0.0002
54	Arabinose <sup>e</sup>	0.8786	0.2227	0.0749	0.0707	0.1276	0.1079	0.0002
55	Fiber <sup>e</sup>	0.3027	0.2423	0.0825	0.1135	0.0446	0.0515	0.0000
56	Galactose <sup>e</sup>	0.9327	0.2089	0.0663	0.0621	0.1167	0.1002	0.0000
57	MFA1 <sup>e</sup>	0.4074	0.2383	0.0403	0.0539	0.0355	0.0419	0.0054
58	Ad_StomataNUM1 <sup>f</sup>	0.3165	0.0266	0.1229	0.0984	n.d.	n.d.	0.0129
59	Ad_STM_distribution <sup>f</sup>	0.1779	0.0351	0.1050	0.1041	n.d.	n.d.	0.0357

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903 Note:  $P$ -value obtained by comparison of the observed  $Q_{ST} - F_{ST}$  to the quantile of the simulated  $Q_{ST} - F_{ST}$  distribution for a neutral  
904 trait [96].  
905 <sup>a</sup>biomass trait [45]  
906 <sup>b</sup>ecophysiology trait [45]  
907 <sup>c</sup>leaf rust resistance trait [38]  
908 <sup>d</sup>phenology trait [45]  
909 <sup>e</sup>wood trait [37]  
910 <sup>f</sup>leaf stomata traits [44]  
911 \*spatially adjusted trait [45]  
912 the variance explained by climate clusters compared to the total variance was estimated as  $h^2$  corrected  $P_{ST}$   
913 S.E. refers to standard errors  
914 Active growth rate (cm day<sup>-1</sup>)  
915 Ad\_StomataNUM1: Adaxial stomata numbers  
916 Ad\_STM\_distribution: Adaxial stomata distribution  
917 Amax/mass = photosynthetic rate per unit dry mass ( $\mu\text{mol CO}_2 \text{ mg}^{-1} \text{ s}^{-1}$ )  
918 Arabinose in dry wood (%)  
919 AUDPC = (calculated) area under the disease curve, based on *M. xcolombiana* infection rating  
920 Bole density (kg/m<sup>3</sup>)  
921 Bole mass (kg)  
922 Branch #  
923 Bud set (day)  
924 Bud set (day): bud set dates considered only after summer solstice

925 C:N = carbon:nitrogen ( $\text{mg mg}^{-1}$ )  
926 Canopy duration (days)  
927 Chlsummer = chlorophyll content index (CCI)  
928 D15N = stable nitrogen isotope ratio (‰)  
929 Dleaf = net discrimination (‰)  
930 Fiber: fiber length Lw (mm)  
931 Galactose in dry wood (%)  
932 Growth period (days)  
933  $g_s$  = stomatal conductance ( $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$ )  
934 H:D = height to diameter (cm:cm)  
935 Height (cm)  
936 Height gain (cm)  
937 Height growth cessation (day)  
938 Leaf drop (day)  
939 Leaf lifespan (days)  
940 Leaves per bud (#)  
941 LMA = leaf mass per unit area ( $\text{mg mm}^{-2}$ )  
942 MFA1: microfibril angle at most recent growth ring ( $^\circ$ )  
943 Narea = nitrogen ( $\text{mg mm}^{-2}$ )  
944 Nmass = nitrogen ( $\text{mg mg}^{-1}$ )  
945 Post-bud set period (days)  
946 Volume ( $\text{cm}^3$ )

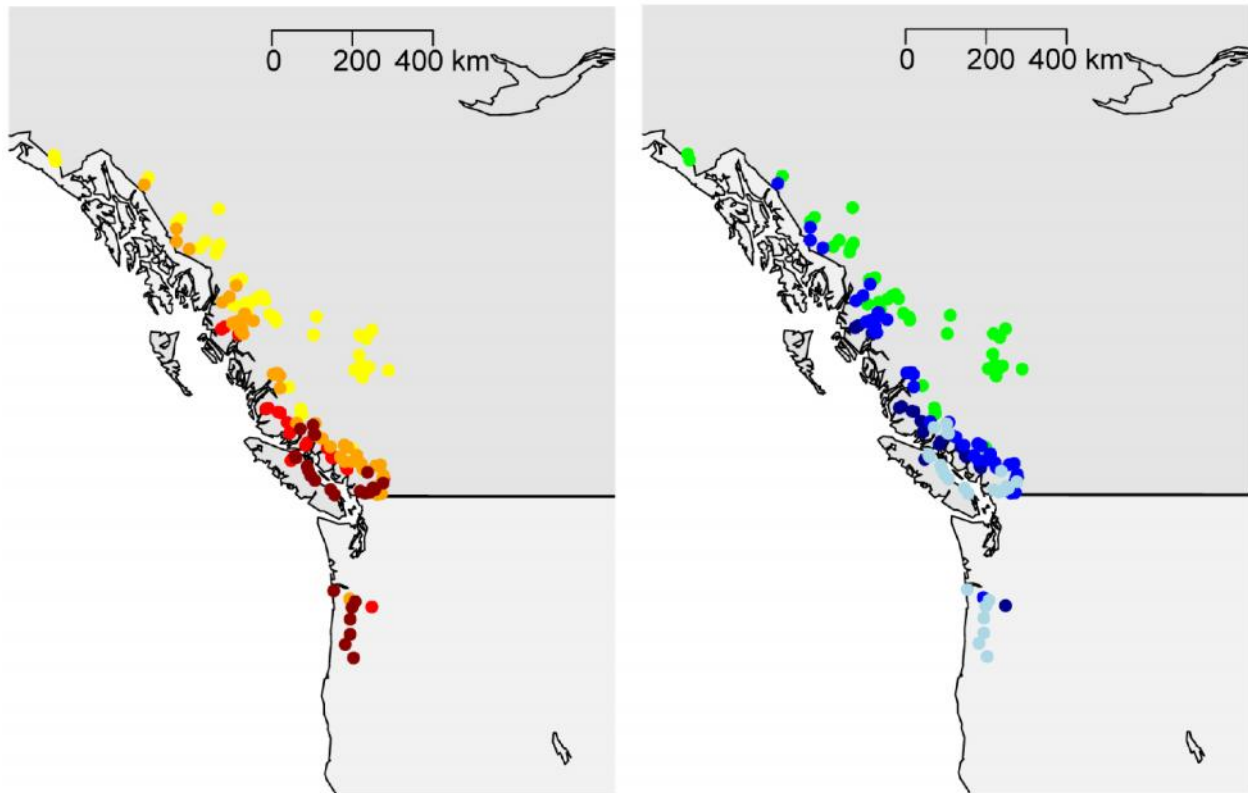
- 947 Volume gain (cm<sup>3</sup>)
- 948 Whole tree mass (kg)
- 949 WUE = instantaneous water-use efficiency ( $\mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$ )
- 950 Yellowing, 100% (day)
- 951 Yellowing, 75% (day)

952 **Figures**

953

(a) Temperature

(b) Precipitation



954

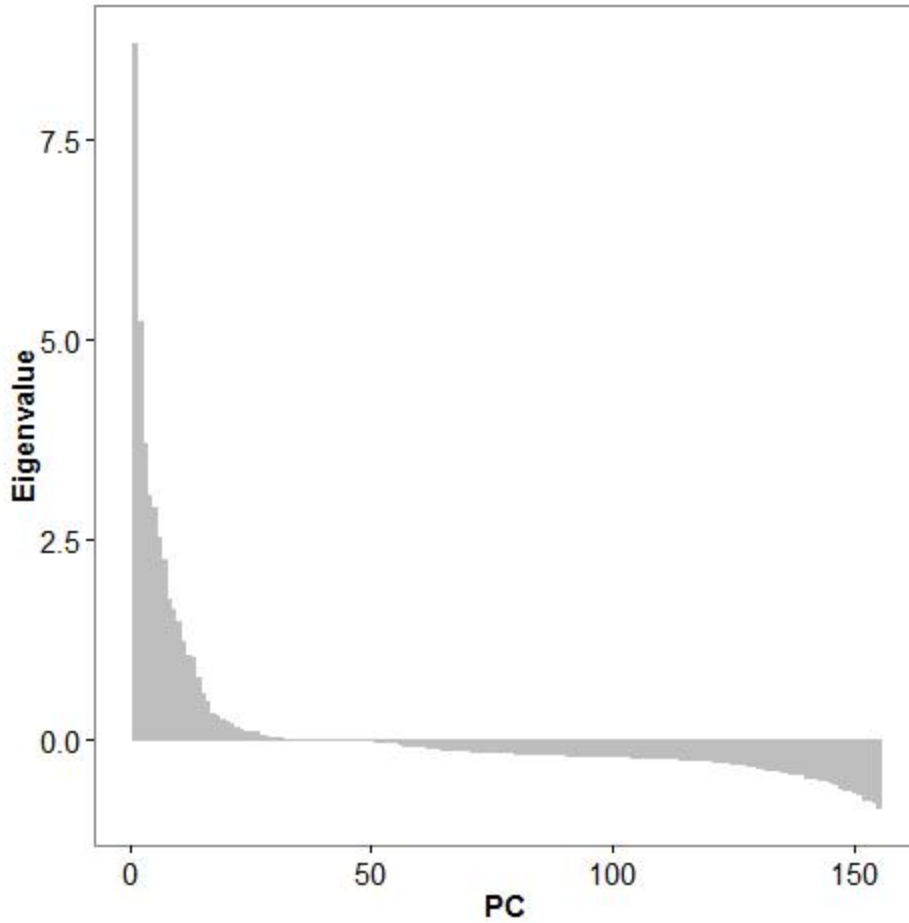
955 Fig. 1. Geographical origins of 433 *P. trichocarpa* genotypes collected across 140 unique locations within the  
956 Pacific Northwest (British Columbia, Canada; Oregon, USA) and grouped into four distinct climate clusters using  
957 local temperature and precipitation records for location of origin.

958 The climate regions were identified based on K-medoids clustering using the mean annual temperature (°C) between  
959 yrs 1971-2002 (MAT\_1971-2002), the number of frost-free days (NFFD\_1971-2002), and the mean annual  
960 precipitation (mm), observed between yrs 1971-2002 (MAP\_1971-2002). Color coding is as follows: (a) population  
961 averages for MAT\_1971-2002; NFFD\_1971\_2002: dark red (9.5°C; 287.1d); red (8.1°C; 267.2d); orange (6.4°C;  
962 215.2d); yellow (4.2°C; 175.4d); (b) population average for MAP\_1971-2002: dark blue (2805.9mm); blue  
963 (1571.8mm); light blue (1517.0mm); green (744.2mm).

964 We note here that canonical correlations between geography and ecology were high ( $r=0.9$  for the first canonical  
965 variable component).

966 (TIFF)

967



968

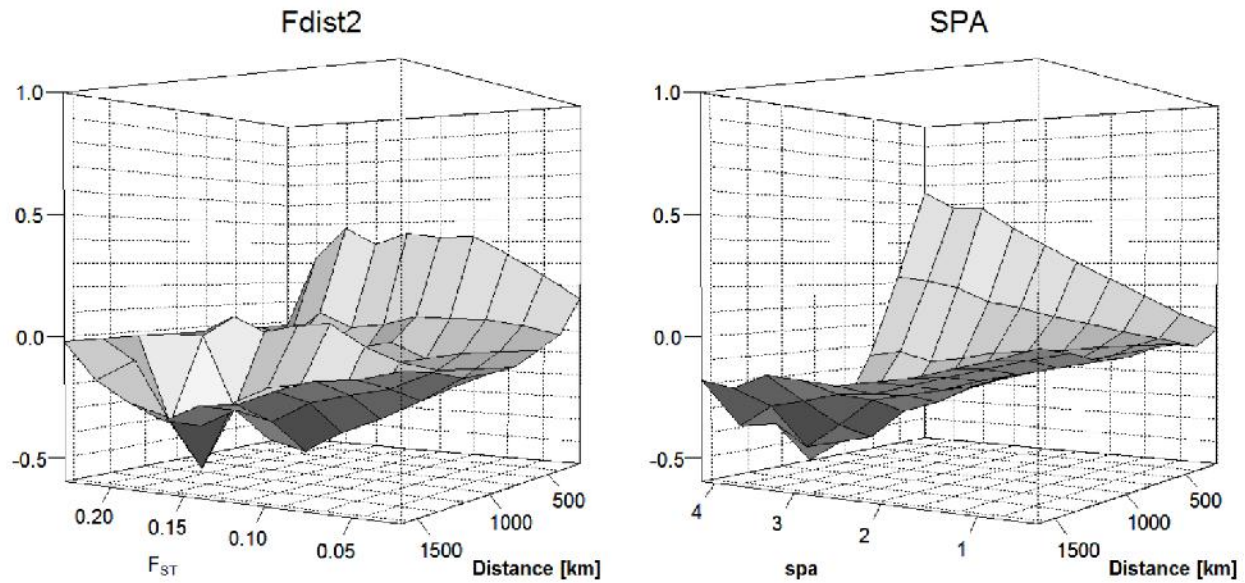
969 Fig. 2. Identification of isolation-by-distance (IBD) among 433 *P. trichocarpa* genotypes based on spatial PCA.

970 Large positive eigenvalues were indicative of IBD.

971 (TIFF)

972





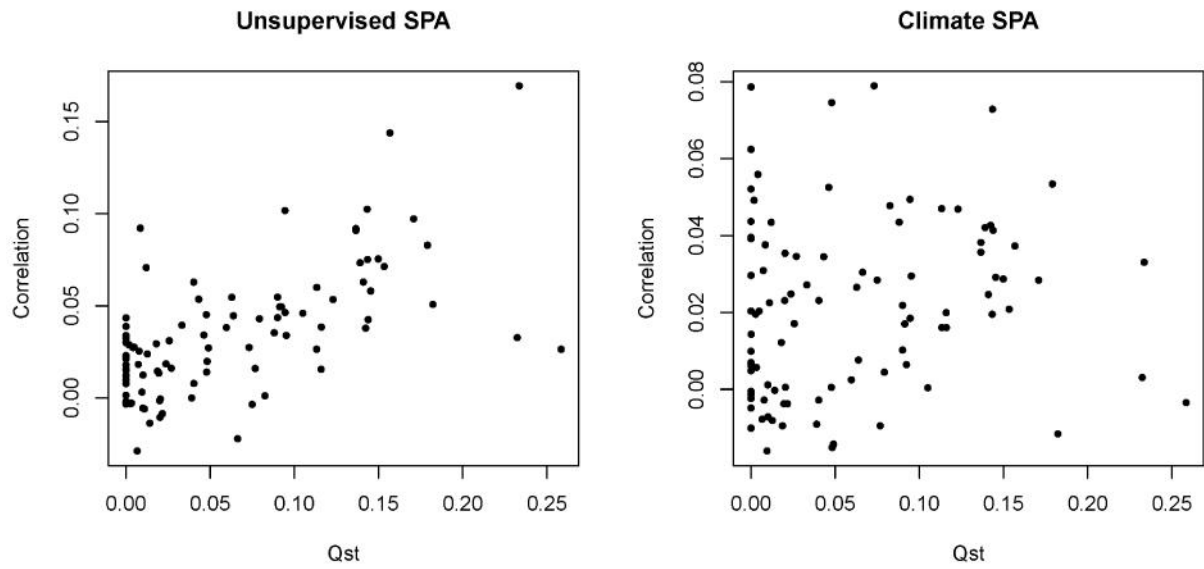
973

974 Fig. 3. Comparison of two outlier detection methods ( $F_{ST}$ , SPA) for their efficiency to identify genetic selection  
975 signals under isolation-by-distance (IBD).

976 Gene dispersal was tested employing Moran's test for spatial autocorrelation using 200km lags.

977 (TIFF)

978



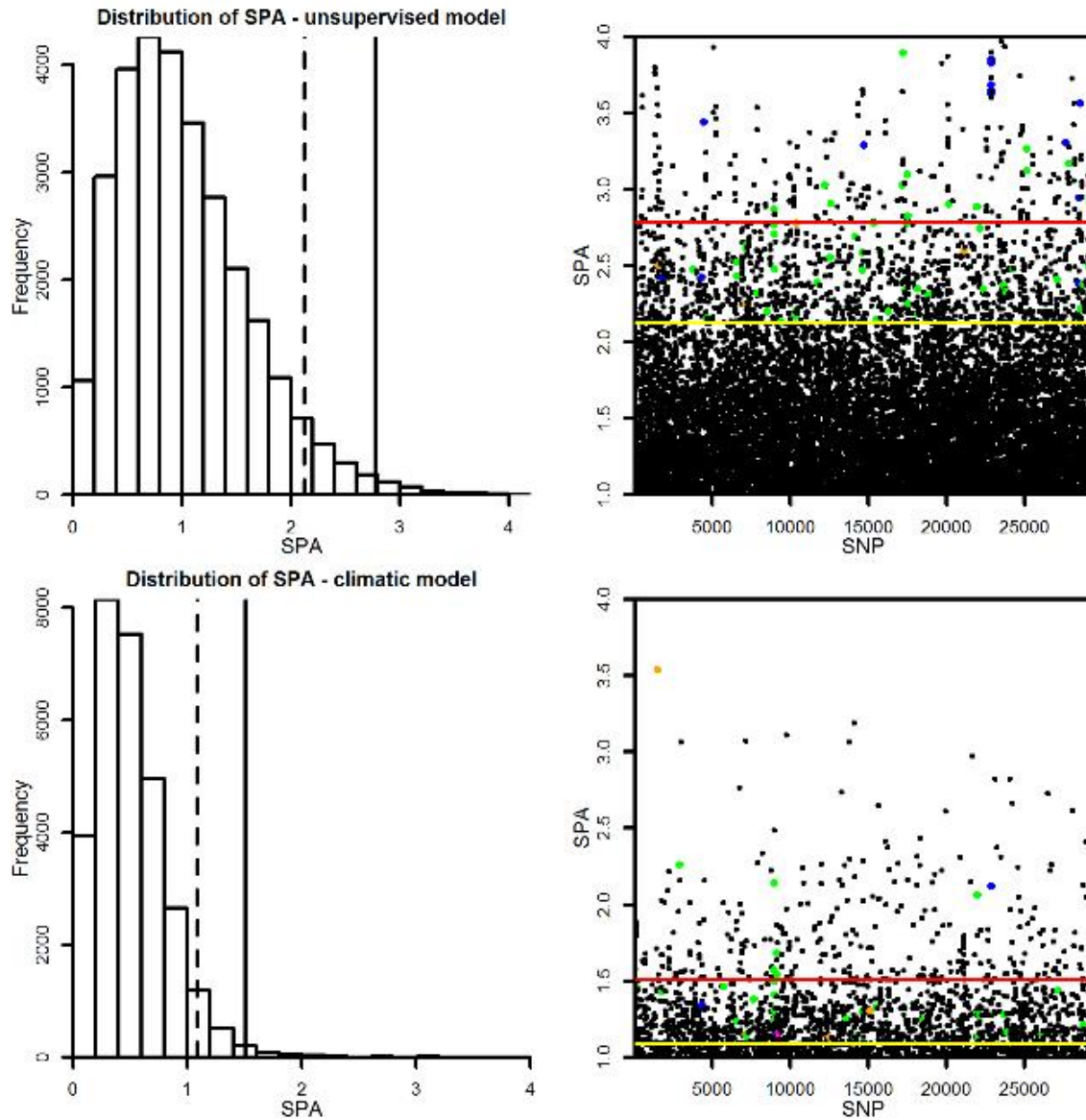
979

980 Fig. 4. Genome-wide correlations between selection outliers and association signals based on 29k SNPs.

981 Correlation of  $-\log(P)$  versus spa was plotted against the trait's  $Q_{ST}$ .

982 (TIFF)

983



984

985

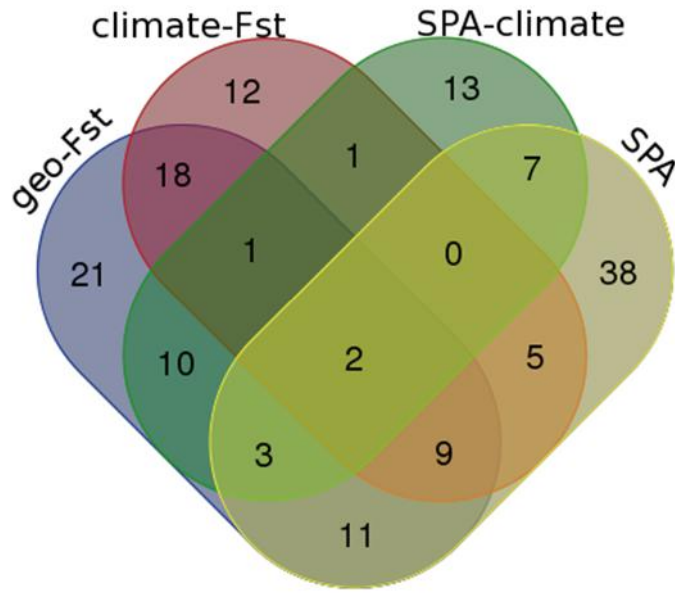
986 Fig. 5. Individual SNPs under diversifying selection within genes mapping to quantitative trait variation.

987 5% cutoff: dashed and yellow lines; 1% cutoff: solid and red lines; ecology (biomass, ecophysiology, phenology,

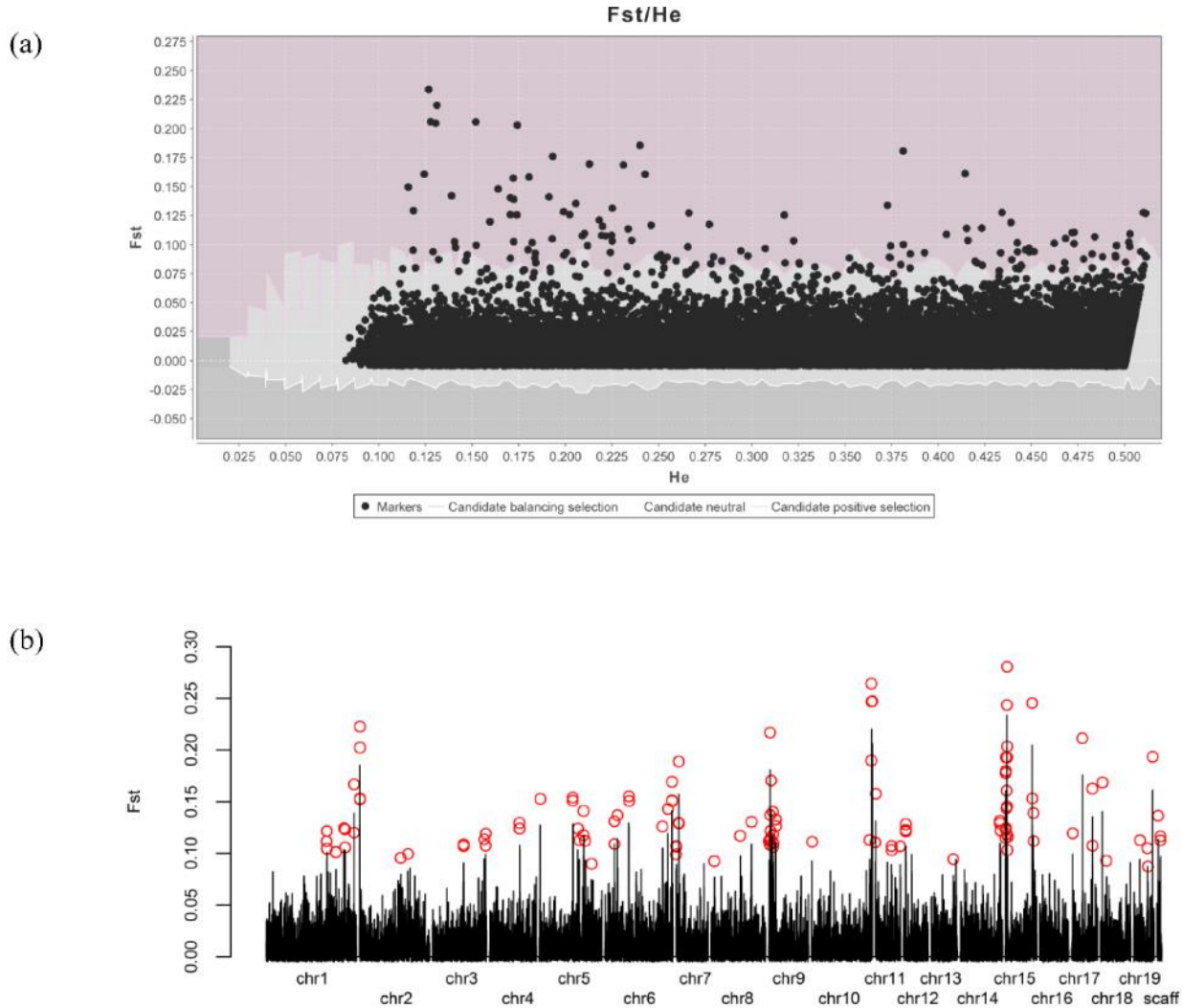
988 stomata) - green dots; wood properties (orange); rust resistance (blue)

989 (TIFF)

990



991  
992 Fig. 6. Venn diagram showing the numbers of unique and shared SNPs (totaling 151 trait-associated SNPs) among  
993 four different outlier detection approaches.  
994  $F_{ST}$  using climate clusters,  $F_{ST}$  using geographical grouping, SPA analyses - with climate-based PCs incorporated as  
995 covariates and unsupervised, respectively. A subset of this information (118 SNPs) related to genetic  
996 polymorphisms associated solely with *adaptive* trait variation is provided in Table S3.  
997 (TIFF)  
998



999

1000 Fig. S1.  $F_{ST}$  outlier loci detection in *P. trichocarpa* and distribution of outliers along the poplar chromosomes.

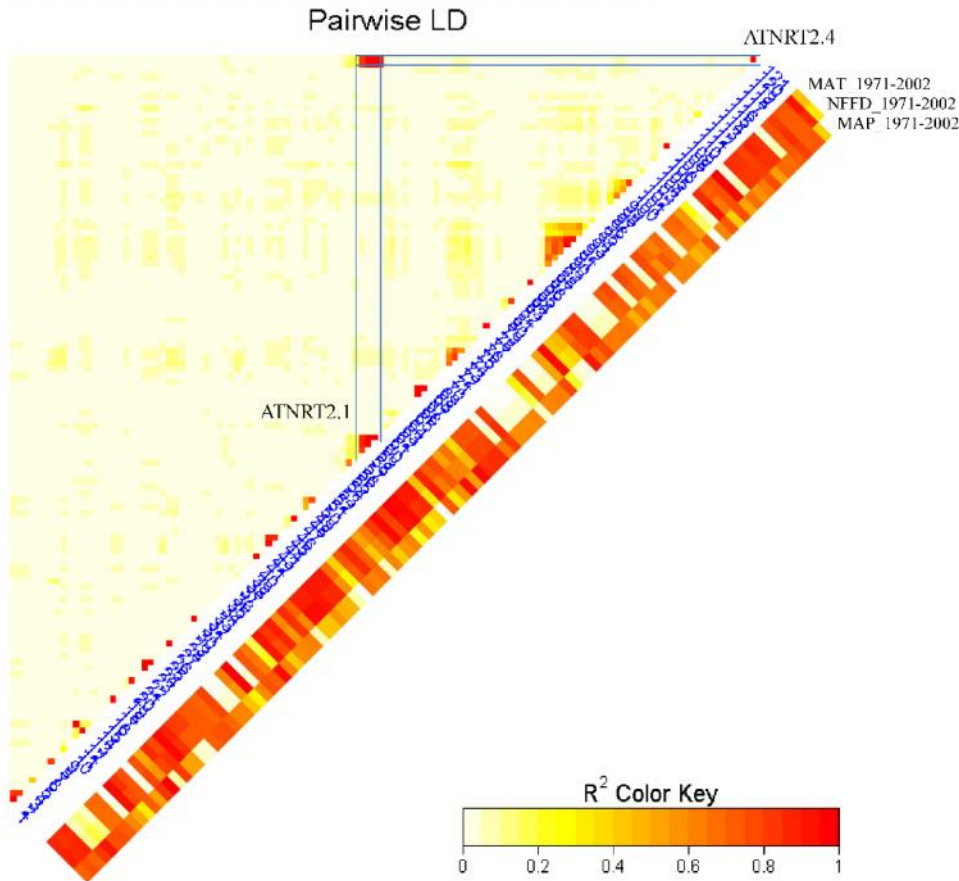
1001 Caption: (a)  $F_{ST}$  outlier loci detection and distribution of empirical  $F_{ST}$  estimates conditioned on expected  
1002 heterozygosity ( $H_E$ ).

1003 The envelope of values corresponding to neutral expectations at 99% CI level (with mean  $F_{ST}=0.0078$ ), solid line,  
1004 was constructed with the infinite allele model according to (Beaumont & Nichols, 1996)

1005 (b) Distribution of the empirical  $F_{ST}$  estimates along the 19 poplar chromosomes and additional scaffolds (abbrev: scaff); the 121 identified  
1006 outlier loci are indicated by red circles above their  $F_{ST}$  value bars.

1007 A goodness-of-fit test assuming a uniform distribution was performed to test whether the observed frequencies of  
1008 'outlier loci' along the 19 poplar chromosomes differed significantly from the expected value. Following the  
1009 rejection of the null hypothesis (chi-square = 81.98 df = 18,  $p$ -value = 3.85e-10), we declared 'outlier loci hotspots'  
1010 if the number of outliers at a given chromosome was equal or above the maximum value (*i.e.*, 20) for assessed  
1011 outlier clusters from a randomly generated data set using the 118 outliers found across the 19 chromosomes, and  
1012 running 1,000 replicates, which identified significant clustering of outliers on chromosome 15.

1013



1014

1015

1016 Fig. S2. Linkage disequilibrium between 121 identified  $F_{ST}$  outlier loci and relationship between  $F_{ST}$  outlier allele  
1017 frequencies and climate variables in *P. trichocarpa*.

1018 Simple linear regression ( $R^2$ ) of allelic frequencies (following arcsine transformation) on temperature and  
1019 precipitation, respectively (mean annual temperature in °C: MAT\_1971-2002; number of frost-free days:  
1020 NFFD\_1971-2002 and mean annual precipitation in mm: MAP\_1971-2002, observed between yrs 1971-2002)  
1021 calculated among the four distinct climate clusters (Fig. 1); Note: POPTR\_0143s00200 was recently re-annotated to  
1022 Potri.009G008500 and both genes are now assembled on chromosome 9 within 50kb of each other (new poplar  
1023 genome assembly Phytozome v3). Both sequences are now described as tandem gene pair *PTNRT2.4A* (alias  
1024 Potri.009G008600) and *PTNRT2.4B* (alias Potri.009G008500) with 97% DNA sequence similarity (Bai *et al.*,  
1025 2013).

1026

1027 The order of loci follows:

1028 1 scaffold\_1\_27485620

1029 2 scaffold\_1\_27487874

1030 3 scaffold\_1\_27488119

1031 4 scaffold\_1\_33628533

1032	5	scaffold_1_33632379
1033	6	scaffold_1_37065304
1034	7	scaffold_1_37410840
1035	8	scaffold_1_37410856
1036	9	scaffold_1_45757179
1037	10	scaffold_1_45758739
1038	11	scaffold_2_127966
1039	12	scaffold_2_128416
1040	13	scaffold_2_128432
1041	14	scaffold_2_130506
1042	15	scaffold_2_10949533
1043	16	scaffold_2_13035475
1044	17	scaffold_3_14135487
1045	18	scaffold_3_14135542
1046	19	scaffold_3_19339785
1047	20	scaffold_3_19747482
1048	21	scaffold_3_19750521
1049	22	scaffold_4_17161026
1050	23	scaffold_4_17161413
1051	24	scaffold_4_17162655
1052	25	scaffold_5_88127
1053	26	scaffold_5_12339685
1054	27	scaffold_5_12344723
1055	28	scaffold_5_16487025
1056	29	scaffold_5_16811923
1057	30	scaffold_5_19211088
1058	31	scaffold_5_19211834
1059	32	scaffold_5_19953723
1060	33	scaffold_5_22633044
1061	34	scaffold_6_2485373
1062	35	scaffold_6_2489698
1063	36	scaffold_6_3249232
1064	37	scaffold_6_6390362
1065	38	scaffold_6_6436509
1066	39	scaffold_6_23299767
1067	40	scaffold_6_24631540
1068	41	scaffold_6_24634215

1069	42	scaffold_6_25893186
1070	43	scaffold_6_25893407
1071	44	scaffold_6_25893900
1072	45	scaffold_7_74879
1073	46	scaffold_7_178643
1074	47	scaffold_7_179188
1075	48	scaffold_7_808919
1076	49	scaffold_7_809632
1077	50	scaffold_7_811143
1078	51	scaffold_8_805284
1079	52	scaffold_8_6567373
1080	53	scaffold_8_9267412
1081	54	scaffold_9_1379696
1082	55	scaffold_9_1599746
1083	56	scaffold_9_1606213
1084	57	scaffold_9_1676227
1085	58	scaffold_9_1676590
1086	59	scaffold_9_1678624
1087	60	scaffold_9_1678826
1088	61	scaffold_9_2160922
1089	62	scaffold_9_2563600
1090	63	scaffold_9_2677917
1091	64	scaffold_9_2679340
1092	65	scaffold_9_2687811
1093	66	scaffold_9_3795784
1094	67	scaffold_9_3798176
1095	68	scaffold_9_3800384
1096	69	scaffold_10_255159
1097	70	scaffold_10_20168770
1098	71	scaffold_10_21246081
1099	72	scaffold_10_21249991
1100	73	scaffold_10_21253673
1101	74	scaffold_10_21451968
1102	75	scaffold_11_145058
1103	76	scaffold_11_295988
1104	77	scaffold_11_15084939
1105	78	scaffold_11_15084942



1106	79	scaffold_11_18477497
1107	80	scaffold_12_1811250
1108	81	scaffold_12_1811719
1109	82	scaffold_12_1812031
1110	83	scaffold_13_14296993
1111	84	scaffold_14_12173467
1112	85	scaffold_14_12173560
1113	86	scaffold_14_12927245
1114	87	scaffold_15_133408
1115	88	scaffold_15_247054
1116	89	scaffold_15_247527
1117	90	scaffold_15_247811
1118	91	scaffold_15_267849
1119	92	scaffold_15_268612
1120	93	scaffold_15_342410
1121	94	scaffold_15_382827
1122	95	scaffold_15_512479
1123	96	scaffold_15_630677
1124	97	scaffold_15_703349
1125	98	scaffold_15_704562
1126	99	scaffold_15_718240
1127	100	scaffold_15_719540
1128	101	scaffold_15_719682
1129	102	scaffold_15_910808
1130	103	scaffold_15_1006871
1131	104	scaffold_15_13596400
1132	105	scaffold_15_13618770
1133	106	scaffold_15_13808656
1134	107	scaffold_15_13808709
1135	108	scaffold_15_13889772
1136	109	scaffold_17_724384
1137	110	scaffold_17_5220579
1138	111	scaffold_17_12392905
1139	112	scaffold_17_12436896
1140	113	scaffold_18_1110947
1141	114	scaffold_18_2565040
1142	115	scaffold_19_5985766

1143	116	scaffold_19_12221032
1144	117	scaffold_19_12484019
1145	118	scaffold_19_15299925
1146	119	scaffold_21_280997
1147	120	scaffold_143_2955
1148	121	scaffold_143_3026