Demographic history shapes forest tree vulnerability to climate change

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Abstract

Demographic history is expected to play a central role in shaping population vulnerability to climate change through its lasting effects on effective population sizes and genetic connectivity. However, existing studies report contrasting outcomes, and the consequences of alternative demographic histories have seldom been assessed concurrently across multiple taxa. Here, we analysed population genomic data from six of the major European forest tree species to address this gap. Across species, genetic isolation reduced adaptive potential, as reflected in lower standing genetic diversity. Greater genetic differentiation was also associated with increased maladaptation to climate, whereas the purge or accumulation of deleterious mutations depended on the severity of past demographic events. Finally, the beneficial effects of gene flow were evidenced across the six species by more optimal climate adaptation in highly connected populations. Altogether, our results provide valuable insights into how genetic differentiation, reflecting the combined effects of genetic drift and limited historical gene flow, influences current vulnerability of forest tree populations to climate change.

Introduction

Trees are foundation species in many terrestrial ecosystems, supporting remarkable biodiversity, regulating the climate and providing other vital ecosystem services on a global scale[1]. Due to their long generation time and sessile nature, trees are particularly exposed to environmental hazards and gradual changes. An increasing body of literature indicates that they will not be able to adapt and/or disperse at rates matching projected climate change[2-4] and forests worldwide are already displaying signs of stress and decline[5,6]. The way in which populations respond to environmental changes depends on the intensity of the stress they experience, their sensitivity to this stress and their capacity to adapt to the new conditions, which together determine their vulnerability to a changing environment[7,8].

Genetic metrics are increasingly being used to assess vulnerability to climate change, as they capture impacts and responses at the intraspecific level[9-11]. Genetic diversity originates diverse phenotypes, some of which may confer advantages in shifting climate, thus forming the basis of the evolutionary potential of populations and, possibly, different patterns of local adaptation[12]. Although local adaptation is common in plants[13], the degree of local adaptation can be population-specific[14]. Both adaptation lags to current climate and pre-adaptation to future climate can influence the extent to which a population will be affected by climate change[15,16]. Recent advances in ecological genomics have introduced new metrics to quantify the extent of climate adaptation in populations (e.g., the genomic discrepancy index, GDI[17]). Genetic variation between populations resulting from different rates of accumulation and purging of deleterious mutations, can also shape their sensitivity to climate change. Indeed, levels of genetic load, defined as the reduction of fitness in a population due to deleterious mutations, can further constrain a population's ability to persist under shifting climatic pressures[18]. Additionally, inbreeding depression has been reported to be particularly high in gymnosperms compared to angiosperms[19,20], as evidenced, for example, by the high number of lethal equivalents affecting tree fitness observed in conifers[20-24]. Overall, regardless of their impact on

current fitness or adaptive potential, population attributes such as genetic diversity, climate adaptation, or genetic load are shaped by demographic processes, and are therefore neither static through time nor uniform across populations[23-27].

Over evolutionary timescales, tree populations have been profoundly shaped by repeated glacial cycles, large-scale environmental shifts and, more recently, land-use changes. These events triggered large distributional shifts that came with important variation in population size and connectivity, and thereby shaped the genetic makeup of populations. The impact of demographic history on wild populations is widely recognised, primarily through its effects on effective population sizes, gene flow and range limits[26,28,29]. However, its overall impact on tree species' genetic diversity remains unclear and requires broader investigation across species with contrasting demographic histories[30]. Demographic history can also affect a population's potential for climate adaptation by influencing the amount of standing genetic variation available for natural selection and the patterns of adaptive or maladaptive gene flow[31-33]. Furthermore, genetic load is also impacted by demographic historical events, through the purging or accumulation of deleterious mutations[24,25]. Although well-established theoretical predictions on the link between demographic history and genetic load exist[34,35], empirical studies have reported contrasting results[36-39], highlighting the need for further research, particularly in forest trees.

In this study, we investigated how historical demographic processes may have shaped key components of population vulnerability, namely sensitivity and adaptive potential, in six ecologically and economically important tree species: four conifers (*Taxus baccata* L., *Pinus pinea* L., *Pinus pinaster* Ait. and *Pinus sylvestris* L.) and two angiosperms (*Fraxinus excelsior* L. and *Fagus sylvatica* L.). By integrating genomic data from 6,435 trees across 326 populations spanning most of the species' distributions in Europe and surrounding regions, we estimated genetic diversity, genetic load, and current climate adaptation, and related these metrics to population-level genetic differentiation, used here as a proxy for demographic history. Finally, we interpreted these associations in the light of how historical demographic processes, including genetic drift and population isolation, may have

influenced present-day sensitivity and adaptive potential to ongoing climate change.

Results

Population genetic differentiation reflects the species' demographic history

The six tree species included in this study were chosen to follow a range of typical population sizes and levels of connectivity, likely resulting from the interplay between life-history traits and contrasting demographic histories. Population-level genetic differentiation (F_{ST}) reflected well these patterns (Fig. 1). Populations of *T. baccata* and *P. pinea* consistently showed intermediate to high F_{ST} (median values of 0.151 and 0.380, respectively; higher than any other species), which may have arisen from their known reduced effective population size and connectivity, resulting from recurrent historical bottlenecks and long-term habitat contraction[40-42]. In contrast, *P. pinaster*, displayed greater variability in F_{ST} across populations, reflecting the presence of continuous and well-connected populations in parts of its range, as well as smaller and fragmented ones in other parts, likely resulting from contractions during glacial periods followed by post-glacial range expansions and reduced gene flow across gene pools[30,43]. Finally, *F. excelsior*, *F. sylvatica* and *P. sylvestris* populations displayed very low F_{ST}, with only a few populations showing higher genetic differentiation (e.g., F_{ST} > 0.2 only in two *F. excelsior* and one *P. sylvestris* populations). Most populations of these species are known to be well-connected due to a combination of factors such as multiple glacial refugia, extensive post-glacial expansion and admixture, notable cold tolerance and efficient long-distance gene flow[19,30,43-51]. Nevertheless, smaller, fragmented populations can sporadically be found in few, mainly southern, parts of the range of these species due to environmental constraints or geographical barriers (*F. excelsior*[48]; *F. sylvatica*[52]; reviewed in Pyhäjärvi *et al*.[19] for *P. sylvestris*).

In addition, the geographical distribution of population-level F_{ST} varied among species. Highly differentiated populations of *T. baccata* and *P. pinea* showed no clear spatial pattern (Fig. 1A, B),

140 with high F_{ST} even in populations located within the species' core range. In contrast, the other species
141 exhibited higher F_{ST} towards the range edges compared to their core populations (Fig. 1C-F; see also
142 Theraroz *et al*.[39] and Guadaño-Peyrot *et al*.[53]).

143 *Key intraspecific components of climate change vulnerability vary across populations and*
144 *species*

145 Genetic diversity (*H*s) varied considerably within species, with distinct patterns emerging across
146 species (Fig. 2A; but notice that direct comparisons of genetic diversity among species are not
147 appropriate due to the use of different genotyping platforms; see Methods). At the within-species
148 level, *P. pinea* exhibited the greatest among-population variability in *H*s, as evidenced by its relatively
149 flat cumulative curve compared with the other species. The other two pine species also had higher
150 among-population variability in *H*s than *T. baccata* and the two broadleaves, for which the cumulative
151 curves were steeper. *H*s changed significantly with longitude in most species, increasing eastward in
152 *T. baccata*, *F. sylvatica* and *P. sylvestris*, decreasing in *P. pinea* and *P. pinaster* and showing no
153 significant trend in *F. excelsior* (Fig. S2A; Table S1). Additionally, *H*s increased significantly with
154 latitude in *P. pinaster* and *P. sylvestris*, with southern range-edge populations of both species
155 showing lower genetic diversity (Fig. S2B). These patterns likely reflect known glacial and post-glacial
156 forest dynamics as reconstructed from genetic data and pollen records (e.g.,[39,42,44,54-58]).

157 We also observed marked differences in realised genetic load (i.e., estimated by the number of
158 deleterious mutations in the homozygous state; hereafter referred to as 'genetic load'; see Methods)
159 among species (Fig. 2B). Interestingly, *T. baccata* and *P. pinea* exhibited the highest genetic load
160 values, whereas *P. pinaster* and *P. sylvestris* had comparable lower medians. Furthermore, among-
161 population variability in genetic load was greatly reduced in *P. sylvestris* compared to the other
162 species (e.g., 0.101 to 0.111 in *P. sylvestris* compared to 0.074 to 0.140 in *P. pinaster*).

163 Using the best genotype-environment association (GEA) models, we selected candidate single
164 nucleotide polymorphisms (SNPs) to estimate climate adaptation via the genomic discrepancy index

165 (GDI; corresponding to the deviation of a population from the expected GEA models; see Methods),
166 retaining between 100 (*T. baccata*) and 2,957 (*F. excelsior)* SNPs depending on the species (Figs. 2C,
167 S3; Table S2). The distribution of GDI (Fig. 2D) showed considerable variability within and among
168 species. The distributions for *F. sylvatica* and *P. pinaster* were nearly Gaussian, whereas *F. excelsior*
169 *and P. sylvestris* were still roughly Gaussian but exhibited long tails with higher GDI values. In
170 contrast, *T. baccata* and *P. pinea* displayed very long tails of higher GDI values, approaching a
171 bimodal distribution, with many populations exhibiting elevated GDI. Populations of *T. baccata* with
172 high GDI are primarily found in southern regions, including Greece, Bosnia-Herzegovina, Spain and
173 Italy[17].

174 *Population genetic differentiation as a major factor explaining key components of climate*
175 *change vulnerability*

176 Genetic diversity (*H*s) decreased with increasing population-level genetic differentiation (F_{ST}) in all
177 species (Table 1), a pattern that was further confirmed by using unbiased Jost's D (Table S3; see
178 Methods). Associations remained highly significant and consistent across species when atypical
179 population-level F_{ST} values were removed and when geography was considered in the models (except
180 for *F. excelsior*; Table 1; Fig. S4; see Methods).

181 All conifer species, except *P. pinea*, showed a lower genetic load (i.e., a reduction of deleterious
182 mutations in homozygous form) in populations with higher F_{ST}. In contrast, the increase in genetic
183 load with F_{ST} observed in *P. pinea* is likely linked to its unique demographic history, characterised by a
184 relatively recent population collapse[42]. These relationships were consistent whether populations with
185 atypically high population-level F_{ST} values were included or not in the analysis (Figs. 3A, S5A). Genetic
186 load was not computed for the two broadleaves (*F. excelsior* and *F. sylvatica*) as its impact is
187 expected to be lower in angiosperm species (see Introduction).

188 Climate adaptation, assessed via GDI, also varied among species in ways that reflected the
189 population size and connectivity gradient illustrated in Fig. 1G, with higher genetic differentiation

190 having from negative to negligible effects on climate adaptation along this gradient (Table 1; Fig. 3B,
191 S5B). In *T. baccata* and *P. pinea*, GDI was higher in populations exhibiting greater F_{ST}. A similar
192 pattern was observed in *P. pinaster*, *F. excelsior* and *F. sylvatica*, although for the latter two species it
193 was only evident when populations with atypically high population-level F_{ST} were included, likely
194 reflecting higher thresholds of isolation required to affect climate adaptation. Finally, no significant
195 association was detected between population-level F_{ST} and GDI in the widespread, weakly
196 differentiated *P. sylvestris*.
197

198 ## *Discussion*

199 Our study suggests that, beyond macro-glacial dynamics, local historical events and demography, as
200 reflected in present-day genetic differentiation, explain a substantial portion of the variability in
201 genetic diversity among populations. We consistently observed a reduction in genetic diversity with
202 increasing population-level F_{ST}. This result remained robust after accounting for geographic effects
203 (Table 1), except for *F. excelsior*. Since geography has previously been linked to glacial-postglacial
204 dynamics[59], these findings support the additional role of local genetic drift and limited historical gene
205 flow in shaping the observed patterns. Overall, our work suggests that even species capable of long-
206 distance dispersal and therefore substantial gene flow may still experience long-term isolation. This
207 isolation likely led to reduced genetic diversity through a combination of ecological or environmental
208 specialisation, genetic drift, and inbreeding[30,60,61]. Since rapid adaptation mostly relies on standing
209 genetic variation[62], the general reduction in genetic diversity in highly differentiated populations
210 could limit their adaptive potential, making them particularly vulnerable to climate change[12,39].

211 Genetic load varied among species, reflecting differences in their life-history traits and demographic
212 history. A reduction in deleterious mutations with increasing genetic differentiation was observed
213 across all species except *P. pinea*. Historical isolation is expected to intensify genetic drift, reducing
214 effective population size and increasing inbreeding. This can increase the fixation of deleterious

215 alleles, thereby strengthening purifying selection and ultimately reducing realised genetic load[63,65].
216 Similar patterns have been reported in other long-lived organisms, where increased genetic drift and
217 isolation have led to the purging of deleterious mutations, likely increasing their adaptive
218 potential[37,66]. The unique pattern observed in *P. pinea* (positive association with genetic
219 differentiation) can also be explained by demographic history. Present-day gene pools of this species
220 likely persisted through near-extinction in a small number of Iberian populations with fossil records
221 dating back to the last Neanderthals (c. 49 kyr BP)[42,67]. Under such prolonged and extreme isolation,
222 reduced effective population size and limited gene flow likely promoted the accumulation, rather
223 than the purging, of deleterious mutations[65,68].

224 Across species, genetic load tracked gradients of population size and connectivity (Fig. 1G), with
225 higher values observed in *T. baccata* and *P. pinea* than in *P. pinaster* and *P. sylvestris*. This pattern
226 suggests that species with smaller and more fragmented populations tend to accumulate more
227 deleterious mutations, regardless of within-species dynamics. Consistent with this, demographic
228 history and the resulting patterns of population differentiation account for much of the variation in
229 genetic load across conifer populations. In particular, the accumulation of deleterious mutations
230 appears to be shaped by historical effective population size and connectivity, underscoring the
231 importance of considering these factors when assessing the consequences of genetic load for
232 population persistence under climate change.

233 Increasing genetic differentiation was associated with suboptimal climate adaptation in all species
234 except *P. sylvestris*. Alongside the parallel reduction in genetic diversity with increasing
235 differentiation, these results highlight the potential beneficial role of gene flow in enhancing
236 adaptation by maintaining standing genetic variation, a key driver of adaptive evolution[33,69].
237 Furthermore, both theoretical predictions and empirical evidence also showed that, by introducing
238 pre-adapted alleles, gene flow can further facilitate climate tracking, particularly in leading-edge
239 populations[32,70,71]. We observed marked interspecific differences in climate adaptation in our study.
240 Species with a prevalence of small and fragmented populations (e.g., *T. baccata* and *P. pinea*)

241 showed stronger negative associations between population genetic differentiation and climate
242 adaptation than species with larger, more continuous populations (e.g., *F. sylvatica* and *P. sylvestris*).
243 These differences may, at least in part, reflect variation in historical effective population size, as the
244 benefits of increased standing genetic diversity and enhanced climate tracking via gene flow are
245 likely to be more pronounced in smaller populations[32,71,72].

246 Interestingly, across the six tree species, populations with low genetic differentiation consistently
247 showed no signs of reduced climate adaptation. These results provide further empirical support for
248 the positive role of genetic connectivity in enhancing climate adaptation in small, fragmented
249 populations, while showing no detectable negative effects in larger, more continuous populations.
250 However, while biologically meaningful, these insights need to be taken with caution. For instance,
251 climate adaptation models used here rely on several assumptions, including that genotype-
252 environment associations accurately capture adaptive signals[73]. Furthermore, higher Euclidean
253 distances between observed and predicted genomic compositions (GDI) were interpreted here as
254 signatures of suboptimal climate adaptation, therefore neglecting other potential explanations, such
255 as alternative adaptive pathways or pre-adaptation to future climate.

256

257 *Conclusion*

258 Genomic analyses of 6,435 trees from 326 populations across six ecologically and economically
259 important forest tree species revealed major effects of demographic history, as summarised by
260 population-level genetic differentiation, on shaping population vulnerability to climate change.
261 Population adaptive potential, reflected in within-population genetic diversity, declined consistently
262 with historical isolation. At the same time, sensitivity to climate change was likely shaped by reduced
263 effective population size and limited historical gene flow, leading either to the purging or the
264 accumulation of deleterious mutations, and increasing maladaptation to contemporary climates.
265 Overall, this work provides valuable insights into how genetic differentiation, reflecting genetic drift

266 and limited historical gene flow, influences current vulnerability of forest tree populations to climate
267 change. Future empirical work could aim to link genetic metrics to effective population size,
268 phenotypic performance, or reproductive fitness, to better assess the contribution of each factor to
269 climate vulnerability. Our findings can also inform management strategies, including assisted gene
270 flow, by helping to identify populations most at risk from climate change and those likely to benefit
271 from increased genetic variance in fitness-related traits[74]. Assessing current gene flow dynamics will
272 be crucial to determine whether rapid environmental change is already reshaping historical patterns
273 of connectivity and genetic differentiation.

274 *Materials and methods*

275 *Sampling and genotyping*

276 Our study focused on six European tree species, namely *Taxus baccata*, *Pinus pinea*, *Pinus pinaster*,
277 *Fraxinus excelsior, Fagus sylvatica* and *Pinus sylvestris.* Species were selected to represent
278 contrasting demographic histories, including different phylogenetic groups, stand formation and
279 population sizes, as well as levels of connectivity and population genetic structure[30,43] (Fig. 1G; see
280 Results). For each species, 29-86 populations spanning most of their ranges were sampled, with total
281 sample sizes per species ranging from 490 to 1,729 mature trees (except *for F. excelsior* where young
282 trees were sampled; see Supplementary Files 1 and 2). Particular attention was paid to sample
283 populations with different degrees of genetic differentiation, including both core and range-edge
284 populations.

285 Genomic data for 490 trees representing 29 populations of *T. baccata* were obtained from a previous
286 study[17]. This dataset comprised single nucleotide polymorphisms (SNPs) generated by combining
287 gene-capture and single-primer enrichment technology (SPET) data.

288 Genomic data for 461 trees from 31 natural populations of *F. excelsior* were compiled from one
289 previous data paper and one study: 286 trees sequenced using SPET[75] and 175 using low-coverage

290 whole genome sequencing[76], following Doonan *et al*.[77]. SNPs were called against the same reference
291 genome, FRAX_0001_PL[78].

292 Genomic data for 1,224 trees from 51 populations of *F. sylvatica* were generated by combining two
293 previous datasets: 370 trees from a data paper[75] and 854 trees from a published dataset[79], both of
294 which produced using SPET. SNP quality filtering and the removal of outliers and duplicates were
295 performed on the merged dataset following the procedure described in Pinosio *et al*.[75].

296 Genomic data for the three pine species (*P. pinea*: 952 trees from 48 populations; *P. pinaster*: 1,579
297 trees from 86 populations; *P. sylvestris*: 1,729 trees from 81 populations) were retrieved: from a
298 previous study[80] for *P. pinaster* and from a data paper[81] for *P. pinea* and *P. sylvestris*. SNP genotypes
299 were generated using the multispecies 4TREE (*P. pinea*, *P. pinaster*) and the PiSy50k (*P. sylvestris*)
300 Axiom arrays (Thermo Fisher Scientific, USA) at IGA Technology Services (Udine, Italy). Following
301 Olsson *et al*.[81], we applied the quality thresholds for markers CR ≥ 85, FLD ≥ 3.2, HetSO ≥ -0.3 and
302 samples DQC ≥ 0.4 with QC call rate ≥ 85, in order to maximise SNP retention while ensuring high
303 data quality.

304 Overall, genomic datasets included 6,435 trees from 326 populations, mainly in Europe and
305 bordering regions in North Africa and Asia (see details in Supplementary Files 1 and 2). For each
306 species, we performed population genetic structure analyses to ensure that merged datasets were
307 biologically consistent and appropriate for downstream analyses (Figs. S6, S7). Two of the 326
308 populations (COM and FUEbis of *P. pinaster*) were excluded because they were duplicated or had too
309 few samples. For each species, two datasets were then generated after removing monomorphic SNPs
310 and variants that failed additional filtering thresholds (coverage depth < 7 or > 150, and allele
311 balance at heterozygous sites < 0.2 or > 0.8). The first dataset (*dataset 1*) retained SNPs and
312 individuals with less than 15% missing data and SNPs with a minor allele count of at least three
313 (except for *T. baccata*, see Francisco *et al*.[17]). This dataset was used for population genetic structure
314 (PCA, STRUCTURE) and differentiation (population-level F_{ST}) analyses, as well as for calculating

genetic diversity (*Hs*), as these analyses are sensitive to high rates of missing data and low-frequency SNPs[82]. *Dataset 1* comprised between 3,563 (*P. pinea*) and 34,760 SNPs (*P. sylvestris*), depending on the species, with a total of 6,262 trees genotyped across the 324 populations (Table S4). The second dataset (*dataset 2*), used for computing climate adaptation and genetic load statistics, was filtered out for missing data > 30% (both for SNPs and individuals) and for minor allele counts lower than twice the number of individuals in the smallest population before filtering (Table S4). This latter filter was applied to remove low-frequency alleles that could otherwise increase false-positive rates, while retaining potential climate-associated SNPs or deleterious mutations that are fixed in small populations. *Dataset 2* included between 3,790 (*P. pinea*) and 34,848 SNPs (*P. sylvestris*), depending on the species, with a total of 6,326 trees genotyped across the 324 populations (Table S4). From these datasets, SNPs from coding regions that could be polarised and annotated using the *Pinus tabuliformis* or *Taxus chinensis* reference genomes[83,84] were used to estimate genetic load (see below). This yielded 4,700; 1,195; 2,219; and 12,808 SNPs for *T. baccata*, *P. pinea*, *P. pinaster*, and *P. sylvestris*, respectively. Finally, for climate adaptation analyses, missing data in *dataset 2* were imputed using the most common genotype within each gene pool.

Climatic data

Climatic data were extracted at high spatial resolution (30 arc-seconds) from Climate Downscaling Tool (ClimateDT[85]). The 1901-1950 period was defined as the reference climate to which the populations are currently adapted, except for *F. excelsior*, for which the 1991-2020 period was used instead. This more recent time interval may better reflect the climate to which populations are adapted in this species, given the relatively young age of the sampled trees and the species' known capacity for rapid adaptation[86]. For each species, the retained climatic variables (Table S5) were selected following the approach described in Francisco *et al.*[17]: (i) a pre-selection step based on species life-history traits and the available literature, (ii) forward selection analysis based on genomic importance, and (iii) removal of correlated variables (Pearson's $|r| > 0.75$) to ensure that

340 multicollinearity remained low and the variance inflation factor below 10 across variables (see
341 Supplementary Material).

342 *Population genetic structure and differentiation*

343 For each species, principal component analysis (PCA) was used to assess population genetic
344 structure, as implemented in the VEGAN R package[87] (Fig. S6). Genetic clusters were also examined
345 based on previously[17,88] or newly run Bayesian clustering analyses, as implemented in STRUCTURE
346 v2.3.4[89] (Fig. S7). For newly run STRUCTURE analyses, ten independent MCMC runs were performed
347 for each number of potential clusters (K = 1-10), with 100,000 iterations per run and a burn-in of
348 10,000 iterations. The most probable number of genetic clusters was determined using the ΔK
349 method[90].

350 Population-level genetic differentiation (F_{ST}), used here as a proxy for population-specific
351 demographic history, encompassing historical population size fluctuation, gene flow and connectivity
352 (e.g.,[91,92]), was calculated using BayeScan v2.1[93]. In this approach, F_{ST} represents the genetic
353 differentiation of each population relative to a predicted ancestral gene pool, making it less
354 influenced by local outliers and more reflective of broad historical patterns of divergence. Given the
355 comprehensive sampling effort, including both core and range-edge populations for each species,
356 populations with high F_{ST} can be considered more historically isolated than the species average.

357 *Genetic metrics of vulnerability to climate change*

358 Genetic differences among individuals of the same species can produce diverse phenotypes within
359 populations, some of which may confer advantages in shifting climate. The extent of this genetic
360 diversity can determine the population's evolutionary potential[12]. Genetic variation within
361 populations was quantified using Nei's genetic diversity (*H*s), as implemented in the HIERFSTAT R
362 package[94]. Due to the differences in experimental design to obtain genomic data (e.g., gene capture,
363 SPET designs and different genotyping arrays), genetic diversity values cannot be compared across
364 species.

365 Genetic load was estimated only for the conifer species in this study (*T. baccata*, *P. pinea*, *P. pinaster*,
366 and *P. sylvestris*), as the accumulation of deleterious mutations in these species has been shown to
367 be greater than in broadleaves and linked to fitness-related traits (e.g.,[95]; see Introduction). First,
368 SNPs were mapped onto the *Pinus tabuliformis* reference genome[83] (*P. pinea*, *P. pinaster* and *P.*
369 *sylvestris*; see Supplementary Material) or onto the *Taxus chinensis* reference genome[84] (*T. baccata*).
370 SnpEff software (version 5.1) was then used to identify SNPs causing amino acid changes in protein-
371 coding sequences[96]. Finally, the functional impact of mutations was predicted using PROVEAN
372 (version 1.1.5), a software that assesses the impact of mutations by evaluating sequence
373 conservation and alignment scores across homologous proteins[97]. For each SNP, PROVEAN predicted
374 its impact on the biological function of the corresponding protein and assigned a 'deleteriousness'
375 score. Mutations were considered deleterious when the PROVEAN score was lower than -2.5[97].
376 Realised genetic load was then computed following González-Martínez *et al.*[27], as the ratio of the
377 expected number of deleterious mutations in homozygosis to the number of expected neutral
378 mutations. As most deleterious alleles are thought to be recessive, the realised genetic load is
379 therefore expected to have a stronger immediate impact on fitness than the absolute number of
380 deleterious mutations[27,65].

381 Population climate adaptation was quantified using a two-step procedure. First, we investigated the
382 presence of genomic markers potentially involved in climate adaptation by carrying out genotype-
383 environment association (GEA) analyses. For each species, six GEA methods (correcting or not for
384 population genetic structure) were applied to identify candidate loci: redundancy analysis (RDA),
385 partial redundancy analysis (pRDA), latent factor mixed models (LFMM), BayPass, gradient forest (GF)
386 uncorrected for population genetic structure (GF-raw) and GF corrected for population genetic
387 structure (GF-corrected; see Table S6 and Supplementary Material for details). We then defined an
388 *all-outlier* SNP set comprising all candidate SNPs identified across the GEA analyses. Following
389 Archambeau *et al.*[98], an *outlier* SNP set was further defined, including only unlinked candidate SNPs
390 identified by at least two GEA methods. For this, we used a linkage disequilibrium threshold of R^2 <

391 |0.7| within contigs or 2,000 bp, depending on the available information for each species, and
392 filtered the datasets for linked loci using the GENETICS R package[99]. For each species, a *random* SNP set
393 was also generated by randomly selecting SNPs not identified as candidates by any GEA method,
394 matching both the number and allele frequency distribution of the *outlier* set (see Supplementary
395 Material).

396 Second, climate adaptation was quantified for each population within species by computing the
397 genomic discrepancy index (GDI[17]) using the RDA method. GDI computes the Euclidean distances
398 between the observed and predicted population scores along the retained RDA axes. Higher
399 population GDI indicates a greater deviation from the established GEA relationship relative to other
400 populations. For most species, the *all-outlier* SNP set was used to build the reference GEA model for
401 GDI estimates. However, for *T. baccata* and *P. pinaster*, we applied SNP sets (*random* and *outlier*,
402 respectively) previously identified as most suitable through model evaluation with fitness proxies[17,98].

403 For all species, GDI estimates based on alternative SNP sets were highly correlated with those
404 obtained from the selected sets (Pearson's correlation > 0.64; Table S2).

405 *Linking demographic history to vulnerability to climate change*

406 To assess the effects of demographic history on population vulnerability to climate change, we
407 examined, for each species, the relationship between population-level F_{ST} and genetic diversity,
408 genetic load and GDI (see Supplementary File 3). Associations were evaluated using linear models
409 with *F*-test *p*-values, as implemented in the STATS R package[100]. To avoid interpreting associations
410 driven solely by extreme values, linear models were investigated with and without populations
411 exhibiting atypically high F_{ST}, defined as those falling outside 2.4 times the interquartile range of
412 population-level F_{ST} within each species.

413

Author Contributions

Designed research: TF, AP, SCG-M; acquired and pretreated genomic data: SO, MK, MW, SP, GGV, KBB, LRN, JMD, DG, EV; analysed data: TF, IL-K, SCG-M; wrote the paper: TF, SCG-M; obtained funding: MW, GGV, DG, AP, SCG-M, LRN; all authors participated in discussions and revised multiple versions of the manuscript, including the final version.

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Data Archiving Statement

Genomic data for *Taxus baccata* from Francisco *et al*.[17] is available at Data INRAE (doi: https://doi.org/10.57745/JJ0ZEI); for *Pinus pinaster* from Olsson *et al*.[80] at Zenodo (doi: 10.5281/zenodo.14950394); for *Pinus pinea* and *Pinus sylvestris* from Olsson *et al*.[81] at Zenodo (doi: 10.5281/zenodo.17455887 and 10.5281/zenodo.17462035, respectively). Newly combined genomic

data for *Fraxinus excelsior* and *Fagus sylvatica* are openly available at Data INRAE (doi: 10.57745/OG2FBY).

Code availability

Code used for data processing and analysis will be made publicly available upon publication at a GitHub public repository.

Table and Figures

Table 1. **Association between population genetic differentiation (F_{ST}) and genetic diversity (*Hs*)**. Linear model coefficient signs (β), explained variance (R^2), and *F*-test *p*-values, summarising the direction and strength of the relationship between population-level F_{ST}, used here as a proxy for demographic history, and genetic diversity (*Hs*). Analyses were run including and excluding populations exhibiting atypically high F_{ST} values and accounting or not for geographical variation in *Hs* (longitude 'Lon' and latitude 'Lat').

Species	***Excluding atypical populations***		***Including atypical populations***	
	***Hs* ~ F_{ST}**	***Hs* ~ F_{ST} | Lon + Lat**	***Hs* ~ F_{ST}**	***Hs* ~ F_{ST} | Lon + Lat**
T. baccata	(-) 0.59***	(-) 0.51***	(-) 0.59***	(-) 0.51***
P. pinea	(-) 0.87***	(-) 0.70***	(-) 0.87***	(-) 0.70***
P. pinaster	(-) 0.46***	(-) 0.24***	(-) 0.52***	(-) 0.28***
F. excelsior	(-) 0.13^{m}	(-) 0.12^{m}	(-) 0.43***	(-) 0.33***
F. sylvatica	(-) 0.56***	(-) 0.50***	(-) 0.88***	(-) 0.71***
P. sylvestris	(-) 0.33***	(-) 0.31***	(-) 0.48***	(-) 0.40***

$^{m:}$ $0.10 < p < 0.05$; ***: $p < 0.001$

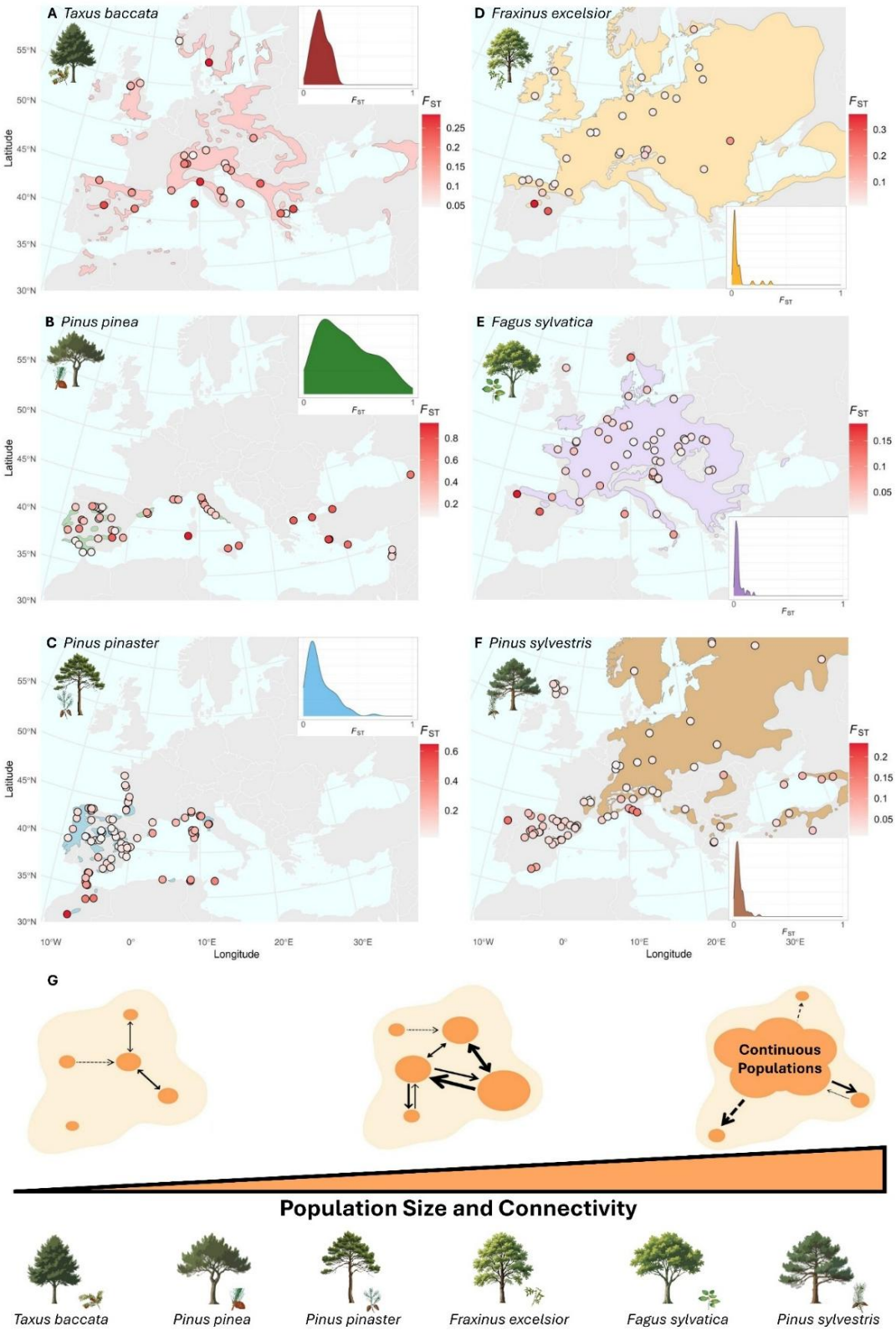

Figure 1. **Description of the six tree species**. **A-F** Geographical range and population-level genetic differentiation (F_{ST}). Each circle represents a population, with colour intensity indicating the magnitude of F_{ST}. Inserts show the density distribution of population-level F_{ST} values, represented on

a common scale to facilitate visual comparison across species. Shaded areas depict the approximate species range[101] for the geographic extent of latitude 30°N-60°N and longitude 10°W-37°E. Sampled populations of *P. sylvestris* occurring outside this range are not shown but can be found in Fig. S1. Notice that species such as *T. baccata*, despite having a large geographical range, have a scattered distribution while others (e.g., *P. sylvestris*) constitute large, stand-forming populations. **G** Conceptual illustration of the gradient in population size and connectivity represented by the six species under study[30,40-43,46,50], which spans from species with predominantly small and isolated populations to species with large and well-connected populations. The schematic is inspired by[43].

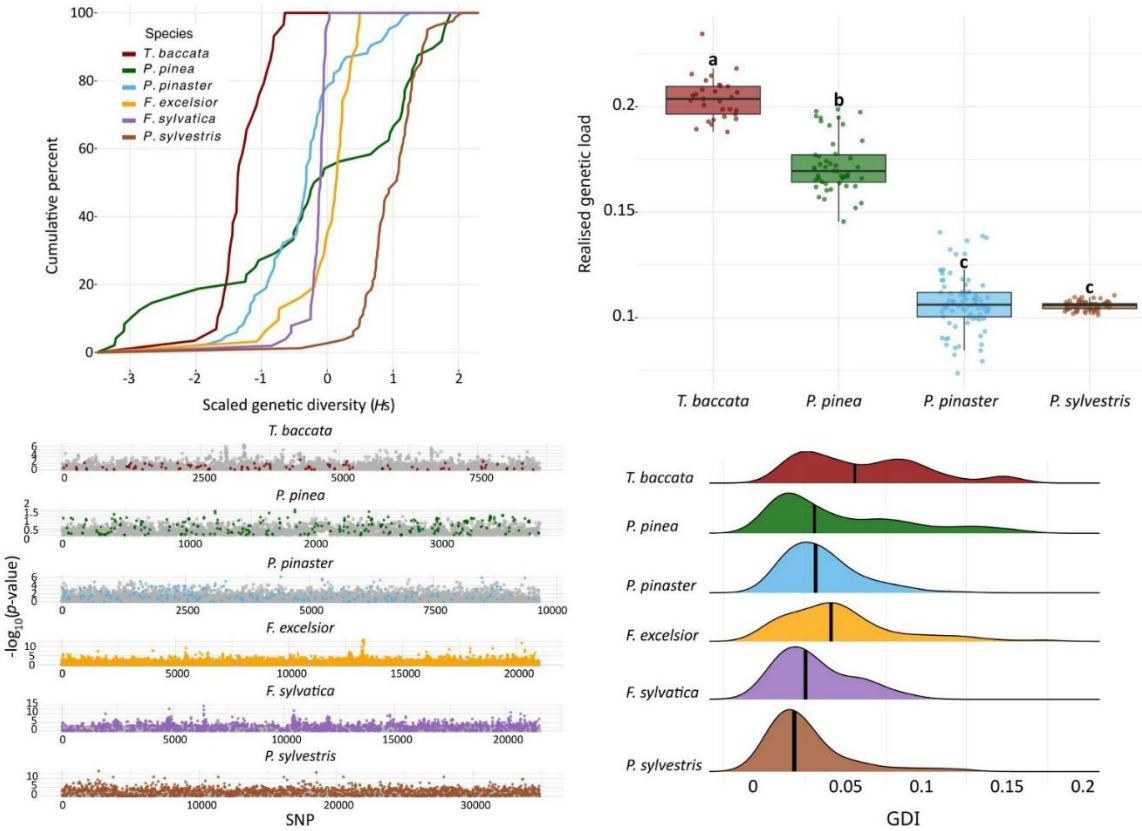

Figure 2. **Genetic metrics of population vulnerability to climate change across the studied species**. **A** Cumulative distribution of scaled genetic diversity (*Hs*; calculated by subtracting the mean and dividing by the standard deviation) across populations. A stepped curve indicates low variability in *Hs* across populations (e.g., *F. sylvatica*), whereas a flatter curve indicates high variability (e.g., *P. pinea*). **B** Comparison of realised genetic load across the four conifers species, with Tukey post-hoc groups indicated by lowercase letters. **C** Manhattan plots showing the retained candidate SNPs (coloured) used for GDI calculation, plotted against the total number of SNP, which varied across species (Table S2). *P*-values were obtained from the RDA GEA models. **D** GDI density plot across populations for each species. Black line represents the median GDI values. Note that values are not directly comparable among species.

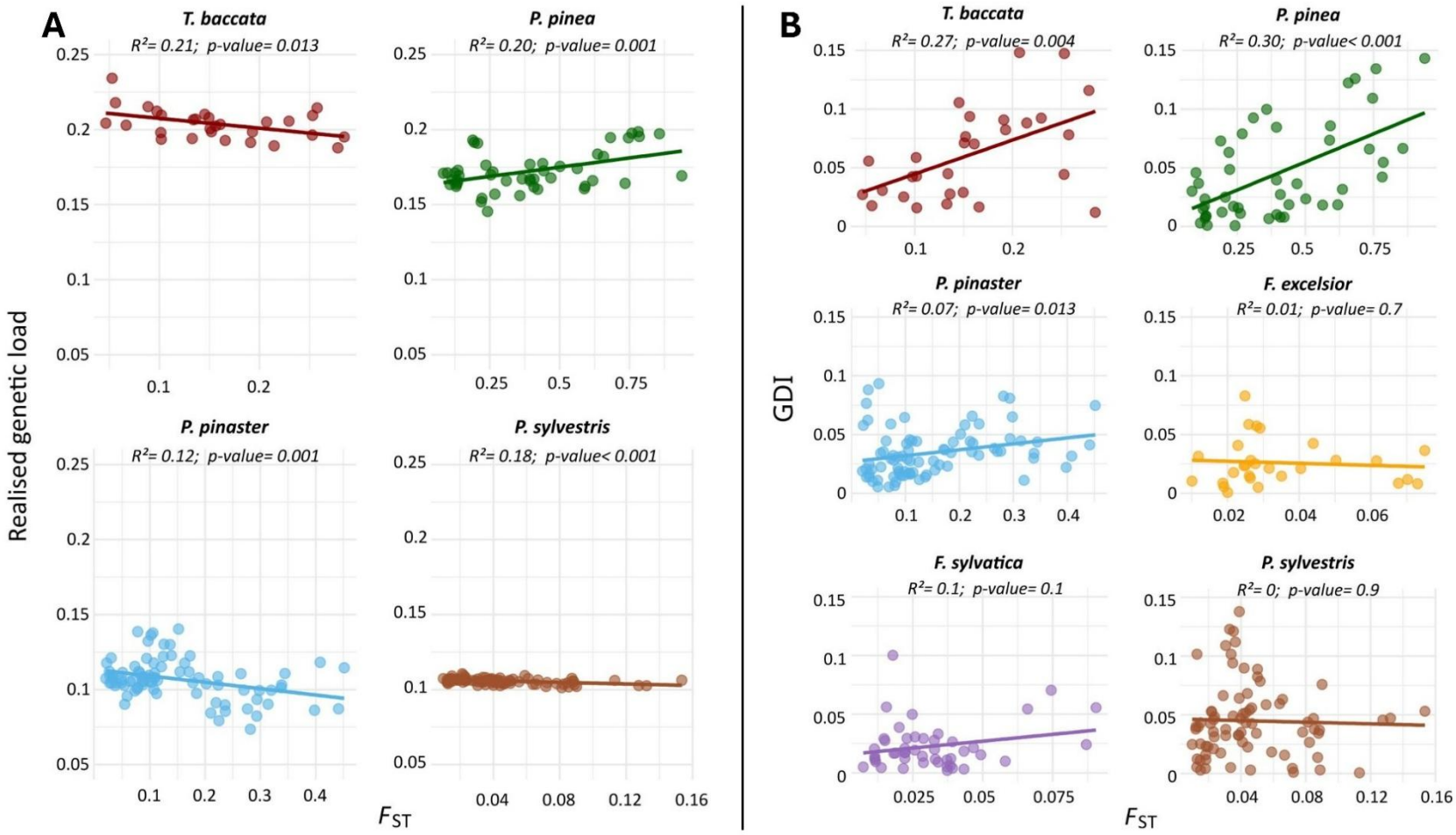

Figure 3. **Population-level genetic differentiation (F_{ST}) as a key factor explaining vulnerability to climate change.** Linear relationships between (**A**) realised genetic load (only conifers) or (**B**) GDI, and population-level F_{ST}, after excluding populations with atypically high F_{ST} values (see Methods and Fig. S5 for relationships including atypical populations). The coefficient of determination (R^2) and *F*-test *p*-values are shown for each model.

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