ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Polygenic adaptation and negative selection across traits, years and environments in a long-lived plant species (*Pinus pinaster* Ait., Pinaceae)

Marina de Miguel[1,2] | Isabel Rodríguez-Quilón[3] | Myriam Heuertz[1] | Agathe Hurel[1] | Delphine Grivet[3] | Juan Pablo Jaramillo-Correa[4] | Giovanni G. Vendramin[5] | Christophe Plomion[1] | Juan Majada[6] | Ricardo Alía[3] | Andrew J. Eckert[7] | Santiago C. González-Martínez[1]

[1]INRAE, University Bordeaux, BIOGECO, Cestas, France

[2]EGFV, University Bordeaux, Bordeaux Sciences Agro, INRAE, Villenave d'Ornon, France

[3]Department of Forest Ecology and Genetics, Forest Research Centre, INIA, Madrid, Spain

[4]Department of Evolutionary Ecology, Institute of Ecology, Universidad Nacional Autónoma de México, México City, Mexico

[5]Institute of Biosciences and Bioresources, Division of Florence, National Research Council, Sesto Fiorentino, Italy

[6]Sección Forestal, SERIDA, Grado, Spain

[7]Department of Biology, Virginia Commonwealth University, Richmond, Virginia, USA

Correspondence
Santiago C. González-Martínez, UMR 1202 BIOGECO INRAE – University Bordeaux, 69 route d'Arcachon, F-33610 Cestas, France.
Email: santiago.gonzalez-martinez@inrae.fr

Abstract

A decade of genetic association studies in multiple organisms suggests that most complex traits are polygenic; that is, they have a genetic architecture determined by numerous loci, each with small effect-size. Thus, determining the degree of polygenicity and its variation across traits, environments and time is crucial to understand the genetic basis of phenotypic variation. We applied multilocus approaches to estimate the degree of polygenicity of fitness-related traits in a long-lived plant (*Pinus pinaster* Ait., maritime pine) and to analyse this variation across environments and years. We evaluated five categories of fitness-related traits (survival, height, phenology, functional, and biotic-stress response) in a clonal common-garden network planted in contrasted environments (over 20,500 trees). Most of the analysed traits showed evidence of local adaptation based on Q_{ST}–F_{ST} comparisons. We further observed a remarkably stable degree of polygenicity, averaging 6% (range of 0%–27%), across traits, environments and years. We detected evidence of negative selection, which could explain, at least partially, the high degree of polygenicity. Because polygenic adaptation can occur rapidly, our results suggest that current predictions on the capacity of natural forest tree populations to adapt to new environments should be revised, especially in the current context of climate change.

KEYWORDS
heritability, local adaptation, maritime pine, negative selection, polygenicity

1 | INTRODUCTION

Genetic adaptation of living organisms to the environment is a topic of foremost interest in the current context of rapid global change. Understanding how different organisms have adapted to past environments can improve knowledge on how species will adapt to future conditions. Population adaptive responses to environmental changes depend on the genetic architecture of fitness-related traits (Hayward & Sella, 2019). Thus, an important first step to improving our knowledge of adaptation is to study the heritability of complex traits. This is especially true considering that the genetic architecture underlying adaptive traits can readily be incorporated in predictive models aimed to estimate the capacity of species to adapt to new environments (Archambeau et al., 2020; Exposito-Alonso et al., 2019; Lu et al., 2019).

Studying the genetic architecture of adaptive traits has traditionally occurred following contrasting paradigms (Höllinger et al., 2019). Traditional population genetic approaches often emphasize on hard selective sweeps, where new beneficial mutations rapidly become fixed at a small number of loci (Smith & Haigh, 1974), hence many early genome-wide association studies (GWAS) approaches focused on detecting large effect (i.e., single locus) genetic polymorphisms associated with phenotypic variation (Yang et al., 2010). In contrast, quantitative genetic approaches view adaptation as resulting from changes in allele frequencies at an idealized infinite number of loci, each with infinitesimal effects on fitness (Fisher, 1918). Our current understanding of the genetic architecture of adaptation is that natural selection often acts through subtle allele frequency shifts on standing genetic variation at numerous loci distributed across the genome (Latta, 1998; Le Corre & Kremer, 2003, 2012; McKay & Latta, 2002; Orr & Coyne, 1992). Consequently, studying the genetic architecture of complex traits using single-locus approaches (e.g., early GWAS approaches) has only accounted for small fractions of trait heritability, causing the so-called "missing heritability" paradox (Maher, 2008).

To unify these conflicting views, Pritchard et al. (2010) proposed the "polygenic adaptation model." Under this model of adaptation, while some loci may harbour new mutations that have been fixed by natural selection, the most common pattern is the genome-wide increase of favored alleles without fixation. Thus, the expected genome-wide footprint resulting from natural selection would not be that of a classical hard sweep, but would rather involve a large number of causal variants, each with subtle allele frequency changes (Hermisson & Pennings, 2017; Pritchard et al., 2010; Pritchard & Rienzo, 2010). Recent theoretical and empirical studies have contributed to the convergence of quantitative and population genetics theories of adaptation by showing that signatures of selective sweeps do not preclude polygenic adaptation at other loci, because large- and small-effect alleles may have different temporal dynamics (Barghi et al., 2020). In addition, the "omnigenic model" has been proposed as a concrete explanation for the biological mechanisms underlying the infinitesimal model, which is the cornerstone of quantitative genetics theory (Boyle et al., 2017). This explanation

relies on the high degree of interconnection of gene regulatory networks. This implies that the vast majority of expressed genes probably influence the function of a small set of core genes directly linked to fitness-related traits. Consequently, the heritability of complex traits is often associated with loci that are widespread across the genome, also including loci without clear functional connections to the trait of interest.

Methodologically diverse experimental studies (e.gBerg & Coop, 2014; Field et al., 2016; Lloyd-Jones et al., 2019; Turchin et al., 2012) over the last decade have provided strong support for the polygenic adaptation model in a diversity of organisms, including humans (Berg et al., 2019; Gnecchi-Ruscone et al., 2018; Hancock et al., 2010), insects (Friedline et al., 2019), molluscs (Bernatchez et al., 2019), model plants (He et al., 2016), crops (Josephs et al., 2019; Wisser et al., 2019), and forest trees (De La Torre et al., 2019; Lind et al., 2017). In particular, joint gene analyses in pathways and modules have proven to be a promising strategy to identify polygenic adaptation in nonmodel species (Hämälä et al., 2020; Mayol et al., 2020), avoiding some common limitations related to the lack of common gardens. Such analyses provide also a direct connection with the physiological mechanisms underlying genetic adaptation. Despite numerous theoretical and methodological advances to the study of polygenic adaptation, there remain multiple open questions regarding the degree of polygenicity of adaptive traits, the distribution of effect sizes of causative loci, and how the genetic architecture of adaptation changes under varying selective forces, especially for nonmodel species (Lind et al., 2018).

Answering these open questions is crucial to understand the driving mechanisms of adaptive evolution. In this sense, whether positive or negative selection operates in a polygenic adaptation framework has been addressed both in empirical (Durvasula & Lohmueller, 2021; Hämälä et al., 2020; Mayol et al., 2020; Zeng et al., 2018) and theoretical studies (Barghi et al., 2020; O'Connor et al., 2019; Stephan, 2016). Landmark work on humans suggested negative selection as a pervasive mechanism determining the polygenic architecture of fitness-related traits (O'Connor et al., 2019; Zeng et al., 2018). In particular, negative selection has been proposed to favor polygenicity in complex traits by removing large-effect variants, because of their deleterious effects, while small-effect variants would remain unaffected; a process named "flattening," as the genetic signal is "flattened" relative to the expected distribution of effect-sizes (O'Connor et al., 2019).

So far, our knowledge about polygenic adaptation remains constrained by the specific life-history of model species, such as the annual life-cycle for *Arabidopsis thaliana*, or the difficulties to evaluate the same genotype in different environments or having exact replicates, that is, clones, or large sib families for humans. Maritime pine (*Pinus pinaster* Ait.) is an ideal model to investigate polygenic adaptation. It is a long-lived, ecologically and economically important species of tree inhabiting nearly undomesticated random mating populations with high levels of genetic diversity (González-Martínez et al., 2002; Jaramillo-Correa et al., 2015). It expanded from several isolated glacial refugia, and it is now distributed across the western

Mediterranean Basin and the European Atlantic front in scattered populations under contrasting environments (Bucci et al., 2007). In addition, an extensive artificial clonal propagation programme exists for this species, which allows the limitations previously reported for humans (i.e., evaluating the same genotype in different environments through clonal replicates) to be addressed. This allowed us to estimate precisely variance components for several fitness-related traits and to investigate selective forces driving trait evolution by evaluating the same genotypes in different environments, allowing the estimation of genotype-by-environment interaction effects, $G \times E$, on phenotypic variation. Specifically, we hypothesized (i) that most complex adaptive traits are polygenic in this long-lived plant, allowing a first estimate of the degree of polygenicity in a forest tree, and (ii) that their genetic architecture is driven by negative selection. We then described how these patterns change across time and environments. To do so, we selected two polygenic association methods that consider all single nucleotide polymorphisms (SNPs) simultaneously and that rely on different approaches, in order to test the robustness of our estimates: Bayesian model averaging and subset selection (VSR; Guan & Stephens, 2011), and Bayesian mixed linear modelling (MLM; Zeng et al., 2018). Finally, we used gene pathway analyses based on SNP effect-sizes from VSR polygenic models to provide additional information on underlying physiological mechanisms and selection at the pathway level (Daub et al., 2013).

2 | MATERIALS AND METHODS

2.1 | Clonal common garden network (CLONAPIN)

We studied phenotypic variation in a clonal common garden network (CLONAPIN). To establish the CLONAPIN network, open-pollinated seeds were collected in 36 natural stands sampled across the natural range of maritime pine and covering its six previously identified gene pools; that is, genetic groups reflecting the population genetic structure in this species (Jaramillo-Correa et al., 2015; Figure 1). Collected seeds were germinated in a nursery and one seedling per open-pollinated family was selected (i.e., selected

genotypes were unrelated) and vegetatively propagated by cuttings (following Majada et al., 2011). A total of 535 genotypes (clones) belonging to 35 populations were used to establish four clonal common gardens (three sites in Spain: Asturias, Cáceres and Madrid; and one in Portugal: Table 1), with eight ramets per clone set in a randomized complete block design ($n = 4272$ trees/common garden). A fifth common garden was established in Bordeaux, France, comprising 443 clones from all 36 populations ($n = 3434$ trees). The common gardens in Asturias, Portugal and Bordeaux are located in the Atlantic climatic region, which has high annual rainfall and mild temperatures. The common gardens in Cáceres and Madrid are in continental areas, under a Mediterranean influence, characterized by large seasonal temperature oscillations and a marked summer drought. In addition, clay soils in Cáceres hampered plant growth and diminished survival (Table 1).

2.2 | Phenotypic evaluation

Over 20,500 trees corresponding to the eight clonal replicates per genotype in each of the five common gardens (i.e., environments) were assessed for different complex traits (see Table S1 for further details on the total number of trees, clones, environments and tree ages evaluated for each trait), thus allowing for accurate estimations of genotype, environment and their interaction effects on phenotypic variation. A total of 28 phenotypic trait-environment combinations were evaluated. Assayed phenotypic traits were classified into five groups: survival, height and phenology, evaluated in several environments and different years; and functional and biotic-stress response, measured in a single environment and year (see Table S1 for an exhaustive list of the measured traits).

Tree survival and height were evaluated in the five common gardens (including different years in Bordeaux, with measures taken in 2013, 2015 and 2018). Phenology traits were evaluated in the Atlantic sites only (Asturias, Portugal and Bordeaux), including different years of evaluation in Bordeaux (2015 and 2017). In Asturias and Portugal, growth phenology was estimated using a Phenology Growth Index (1):

FIGURE 1 Sampled maritime pine populations (circles) and common garden sites (other symbols). Neutral gene pools (identified in Jaramillo-Correa et al., 2015) outline the species natural distribution range in different colours [Colour figure can be viewed at wileyonlinelibrary.com]
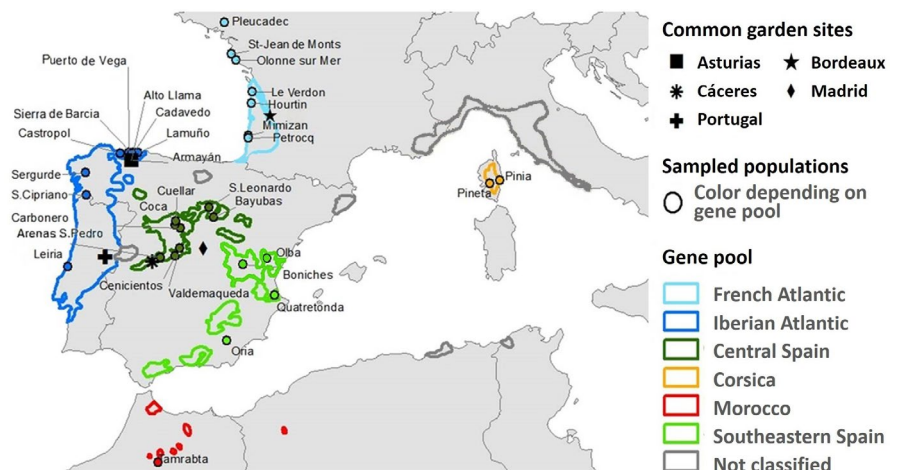
TABLE 1 CLONAPIN common garden network (443–535 clones planted in five sites, involving over 20,500 trees). Climatic data correspond to the mean of each parameter for the period 2005–2014, as obtained from the EuMedClim database (Fréjaville & Benito Garzón, 2018)

Site	Country	Coordinates	Environment	Plantation year	N trees (clones)	Annual precipitation (mm)	Summer precipitation (mm)	Annual mean temperature (°C)	Annual temperature range (°C)	Soil type
Asturias	Spain	43°25′17″N 06°32′38″W	Iberian Atlantic	2011	4272 (535)	890	126	12.9	24.0	Cambisol
Portugal	Portugal	40°06′38″N 07°28′58″W		2011	4272 (535)	1122	58	14.0	26.9	Cambisol
Bordeaux	France	44°44′42″N 00°47′04″W	French Atlantic	2011	3434 (443)	933	199	13.8	26.7	Arenosol
Madrid	Spain	40°30′47″N 03°18′44″W	Mediterranean	2010	4272 (535)	378	35	14.8	32.8	Arenosol
Cáceres	Spain	40°02′24″N 05°22′19″W		2011	4272 (535)	374	21	16.7	32.6	Fluvisol

$$\text{Phenology Growth Index} = \frac{\text{spring growth}}{\text{total growth}} = \frac{(\text{tree height } may_n - \text{tree height } dec_{n-1})}{(\text{tree height } dec_n - \text{tree height } dec_{n-1})}$$

(1)

where *may* and *dec* correspond to the months May and December of the year *n* and the year *n*−1, respectively. In these common gardens, the presence of polycyclism, that is, the ability of a plant to produce several flushes in the same growing season (Girard et al., 2011), was also assessed.

In Bordeaux, phenology of bud burst was estimated through a scale ranging from 0 to 5 (see Figure S1) (Hurel et al., 2019). The first Julian day at each stage (S1–S5) was scored for each tree. Julian days were converted into accumulated degree-days (with base temperature 0°C) from the first day of the year, to take into account the between-year variability in temperature. The number of degree-days between stages 1 and 4 defined the duration of bud burst. Daily mean temperatures to calculate accumulated degree-days were downloaded from the nearest climatic station (located just a few hundred metres from the common garden, station 33122004 of the INRAE Agroclim database: https://www6.paca.inrae.fr/agroclim/Les-outils).

Functional traits, including nitrogen and carbon content and isotopic composition (δ^{15}N and δ^{13}C, respectively), as well as specific leaf area (SLA, a measure of leaf area per unit of dry mass), were evaluated in the common garden located in Portugal (see Methods S1). SLA is an estimation of the compromise among light capture, CO_2 assimilation, and the restrictions imposed by water loss through transpiration (Sefton et al., 2002). Low SLA suggests high leaf construction cost, and thus higher stress tolerance (Díaz et al., 2016). Thus, this key leaf trait is also associated with fitness components, such as tree survival (Greenwood et al., 2017). Given that there is a positive relationship between δ^{13}C and water use efficiency (Farquhar & Richards, 1984), δ^{13}C has been widely used as a surrogate to study tree adaptation to water-limiting environments (e.g., Aranda et al., 2010; Walker et al., 2015). Similarly, δ^{15}N is an indirect index related to the nitrogen cycle (Craine et al., 2015).

Assessment of biotic-stress response in a high number of trees is logistically complex. Therefore, it was evaluated only for a subset of clones (Table S1) in the Bordeaux common garden (France). Biotic-stress response was evaluated based on susceptibility to two major pine pathogens, *Diplodia sapinea* and *Armillaria ostoyae*, as well as the incidence of the defoliator pest, *Thaumetopoea pityocampa* (pine processionary moth) (see Methods S1 and Hurel et al., 2019 for details).

2.3 | DNA extraction and SNP genotyping

All phenotypically evaluated clones were also sampled for SNP genotyping. Needles were collected from one ramet per clone in the Asturias common garden (*N* = 535). Genomic DNA was extracted using the Invisorb DNA Plant HTS 96 Kit/C (Invitek GmbH). An *Illumina Infinium* SNP array was used for genotyping (see Plomion et al., 2016 for further details). Genotyped SNPs covered all 12

putative chromosomes of *P. pinaster* according to linkage mapping (Plomion et al., 2016). For this study, 6100 SNPs were finally retained following standard filtering (GenTrain score >0.35, GenCall50 score >0.15 and Call frequency >0.85) and removal of SNPs with uncertain clustering patterns (visual inspection using GENOMESTUDIO v. 2.0). Individuals with more than 15% missing data were also removed. Finally, 5165 polymorphic SNPs genotyped in 523 clones were retained for subsequent analysis. Most of the SNPs were obtained from independent contigs of the maritime pine reference UniGene set (Canales et al., 2014; Fernández-Pozo et al., 2011), which resulted in overall low-levels of linkage disequilibrium (LD) (see below) and a good representation of maritime pine coding regions. Overall, minor allele frequency (MAF) ranged from 0.001% to 0.5%, with about 10% of the SNPs being at low frequency (MAF <5%). Pairwise LD (r^2) was calculated using the R package GENETICS. The resulting LD distribution was strongly right-skewed, with average r^2 of 0.003 ± 0.025 (median ± standard deviation), and only 78 pairwise comparisons (out of over 13 million tests), involving 49 different SNPs, presenting r^2 values higher than 0.2 (Figure S2).

2.4 | Quantitative genetics analysis

A first quantitative genetic model, described in Equation (2), was implemented for those phenotypic traits evaluated at multiple sites of the CLONAPIN common garden network (Table S1). To estimate the genetic control of the genotype-by-environment (G × E) interactions, a second model, described in Equation (3), was fitted for those traits measured at all sites of the CLONAPIN common garden network (i.e. height and survival).

$$y_{ijkl} = \mu + S_i + S(B)_{ij} + P_k + P(C)_{kl} + S_i * C_l + \varepsilon_{ijkl} \quad (2)$$

$$y_{ijkl} = \mu + S_i + S(B)_{ij} + P_k + P(C)_{kl} + S_i * P_k + S_i * C_l + \varepsilon_{ijkl} \quad (3)$$

where, for a given trait *y*, μ denotes the overall phenotypic mean, S_i refers to the fixed effect of site *i*, B_j represents the random effect of experimental block *j* nested within site *i*, P_k is the random effect of population *k*, *C* denotes the random effect of clone *l* nested within population *k*, and ε is the residual effect.

Simplified models with or without covariates represented by Equations (4) and (5) were implemented for phenotypic traits measured in just one site of the CLONAPIN common garden network (Table S1).

$$y_{ijk} = \mu + B_i + P_j + P(C)_{jk} + \varepsilon_{ijk} \quad (4)$$

$$y_{ijk} = \mu + B_i + \text{cov} + P_j + P(C)_{jk} + \varepsilon_{ijk} \quad (5)$$

where, for a given trait *y*, μ denotes the overall phenotypic mean, B_i represents the fixed effect of experimental block *i*, P_j is the random effect of population *j*, *C* denotes the random effect of clone *k* nested within population *j*, and ε is the residual effect. In (5), *cov* represents a

covariate implemented when modelling the presence of pine processionary moth nests (*cov* representing tree height) and necrosis caused by *A. ostoyae* (*cov* representing the level of humidity in the experimental jar).

All models were fitted using the R package MCMCGLMM v.2.32 (Hadfield, 2010). See Methods S1 and Table S2 for model specifications and parametrization.

Variance components estimated from the previous models were then used to compute broad-sense heritability (H^2) as (6):

$$H^2 = \frac{\sigma^2_{\text{clone}}}{\sigma^2_{\text{clone}} + \sigma^2_e} \quad (6)$$

where σ^2_{clone} is the variance among clones within populations obtained from the term $P(C)$, and σ^2_e the residual variance obtained from the term ε in Equations (2–5). To estimate broad-sense heritability for traits following a binomial distribution, we included an extra term in the denominator (+ $\pi^2/3$) to account for the implicit *logit* link function variance; similarly, we added one to the denominator to account for the *probit* link function (Nakagawa & Schielzeth, 2010).

The GLMMs described above were used to estimate genetic values using Best Linear Unbiased Predictors (BLUPs) (Henderson, 1973; Robinson, 1991). BLUPs were obtained as the posterior mode of each of the effects estimated in Equations (2–5). The genetic value of each clone was defined as the sum of population and clone BLUPs. BLUPs for G × E were obtained from Equation (3) and calculated following Equation (7) in Methods S1.

Parameter estimates from quantitative genetics analyses are presented as the mode of the posterior distribution; 95% credible intervals were computed as the highest density region of each posterior parameter distribution.

2.5 | Q_{ST}-F_{ST} comparison

Molecular population differentiation (F_{ST}) was estimated according to Weir and Cockerham (1984) using the 5165 SNPs from the *Illumina Infinium* SNP array and the DIVERSITY R package v.1.9.90 (Keenan et al., 2013). The 95% confidence interval of the global F_{ST} estimate was computed by bootstrapping across loci (1000 bootstrap iterations). Quantitative genetic differentiation among populations was calculated following Spitze (1993) using the variance components estimated from the models described in Equations (2–5):

$$Q_{st} = \frac{\sigma^2_{\text{pop}}}{\sigma^2_{\text{pop}} + 2\sigma^2_{\text{clone}}} \quad (8)$$

where σ^2_{pop} is the variance among populations, and σ^2_{clone} is the variance among clones within populations. Quantitative (Q_{ST}) and molecular (F_{ST}) genetic differentiation among populations were considered to be significantly different when Q_{ST} and F_{ST} posterior distributions had non-overlapping 95% confidence intervals.

2.6 | Polygenicity across traits, years and environments

Taking advantage of the clonal common-garden network, we used BLUPs as inputs for the polygenic association methods to exclude nongenetic effects on phenotypic variation. Additionally, we could estimate the genetic architecture of G × E using BLUPs obtained for the G × E term in Equations (3) and (7). Polygenicity was evaluated as the proportion of SNPs with nonzero effect-size on phenotypic traits using polygenic association methods that rely on different approaches, to assess the robustness of our estimates. First, we conducted posterior inferences via model averaging and subset selection (Bayesian variable selection regression [VSR]), as implemented in PIMASS software (Guan & Stephens, 2011). This method allows the identification of combinations of SNPs probably affecting a phenotype and to estimate the proportion of trait variance explained by the SNPs in the data set. Hereafter, we refer to this quantity as the genetic explained variance (GEV), which, in this study, represents the BLUP variance explained by SNP additive effects. Second, we used the Bayesian mixed linear model (MLM) framework developed by Zeng et al. (2018) as implemented in CGTB 2.0 software. This model simultaneously estimates: (i) SNP-based heritability (considering SNPs with nonzero effects on the trait), hereafter referred as GEV, analogously to VSR estimates; (ii) polygenicity (as defined above); and (iii) the relationship between SNP effect-size and minor allele frequency (S, a common indicator of negative selection). When negative selection is operating, S is expected to be negative, as most new mutations are deleterious and large effect SNPs are kept at low frequencies. Estimates with 95% credible intervals of parameter posterior distributions not overlapping zero were considered as significant. Prior to these analyses, neutral population genetic structure was accounted for by running linear models relating the genetic values for each trait (with site and block effects removed) to the admixture coefficients for each clone (Q-scores) obtained using a STRUCTURE run for $K = 6$ based on neutral markers (see Jaramillo-Correa et al., 2015 for further details). From this linear model, we extracted the normalized residuals for each trait, as recommended in piMASS manual. The population structure correction obtained with this method was verified with a PCA analysis on the residual BLUPs for the 28 measured phenotype-environment combinations (Figure S3). After the correction no apparent population structure remained for any trait.

Analyses were run separately for different traits, years, and environments (Table S1). VSR models were run for 2,000,000 MCMC iterations with a burnin of 100,000 iterations and a thinning interval of 100. After several preliminary runs, the maximum number of SNPs included in VSR models was fixed to 2000 (i.e., maximum allowed polygenicity of ~40%). MLM models were run for 500,100 iterations, including a burnin of 100 iterations, and a thinning interval of 10 iterations. Polygenicity using the CGTB method was estimated for an increasing number of input SNPs in order to evaluate the influence of SNP sample size on the polygenic association results (see Methods S1). Parameter estimates from both VSRs and MLMs were presented as the median of the posterior distribution, instead of the mode, for a better handling of bimodal distributions. The 95% credible intervals were computed as the highest density region of the posterior parameter distribution.

2.7 | Annotation and gene function enrichment at pathway level

The transcripts containing the 5165 polymorphic SNPs were downloaded from the SUSTAINPINE v.3.0 database (Canales et al., 2014). DNA sequences were translated with BIOEDIT v. 7.2.6 (Hall, 1999) and submitted to BlastKOALA (Kanehisa et al., 2016) for annotation and functional characterization using InterPro annotations, GO terms, and KEGG pathway identification. Annotations were compared with those available at SustainPine, and conflicting cases were examined individually by privileging similarity to genes correctly identified in other conifers or forest trees. Contigs with no clear annotations (e.g., hypothetical or unknown proteins, or unsolved conflicting annotations) were removed. For the retained contigs, the top-two KEGG terms were used for assignation to one or more specific metabolic pathways/modules based on KEGG orthology. Genes for which no hit with KEGG database was found, were assigned to metabolic pathways/modules based on the InterPro annotation. We privileged metabolic pathways/modules that could be unequivocally assigned to a given phenotypic response (e.g., circadian rhythm to bud phenology or pathogen interaction to biotic stress response) or linked to various stress responses (e.g., DNA recombination and repair, ubiquitin system or transcription factor machinery to survival and biotic stress response). In total, 17 pathways/modules were retained containing a total of 628 (19.7% out of 3194) genes, including 1233 polymorphic SNPs (Table S3).

For gene enrichment tests using POLYSEL (Daub et al., 2013), the 17 pathways/modules were defined as gene sets. First, we computed two statistics at the gene level (objStat) based on the per SNP estimates obtained from the VSR implemented in piMASS: the maximum, over all SNPs included in a gene, of the Rao-Backwellized posterior probability of inclusion (hereafter *postp*) and the maximum of the absolute value of Rao-Backwellized effect size (hereafter *beta*). The *postp* statistic provides information on the probability that a SNP is retained in the multilocus models of phenotypic variation while the *beta* statistic estimates the magnitude of its effect size. To account for a weak correlation of these statistics with the number of SNPs per gene, we used the AssignBins and RescaleBins functions in POLYSEL, which automatically assigns gene scores (objStat) into bins defined from the number of SNPs per gene. We then rescaled scores within bins and computed the sum(objStat) of each statistic over all genes per gene set. Since the sum(objStat) for random gene sets (sizes $n = 10, 50, 250$ genes) was not normally distributed, we built empirical null distributions by randomly sampling gene sets of the same size as the sets to be tested. Then, we performed one-sided tests evaluating whether the observed sum(objStat) was smaller than the fiftth or larger than the 95th percentile of the

sum(obStat) null distribution. Significant results in the upper tail for *postp* indicate gene sets enriched with higher overall probability of being selected during the VSR procedure implemented in piMASS. Significant results in the upper tail for *beta* identify gene sets enriched with higher overall SNP effect-sizes. In contrast, significant results in the lower tail for both statistics suggest gene sets containing genes with smaller overall probability of inclusion or SNP effect-size estimates. Significant results for *postp* and *beta* is interpreted as evidence of polygenic selection. We report *p*-values based on this comparison, as well as *q*-values from a false discovery rate (FDR) approach implemented in the R package QVALUE (R Core Team, 2019). The level of connection between gene sets was weak with only four genes associated with more than one gene set (633 gene—gene set combinations for 628 genes). For this reason, we did not assess enrichment for pruned gene sets (see Daub et al., 2013).

3 | RESULTS

3.1 | Broad-sense heritability and genetic differentiation among populations

All traits had low to moderate broad-sense heritability H^2 (maximum of 0.32 for bud burst measured in 2015, Table S1), with the exception of nitrogen and carbon amount that did not show any genetic variation. Consequently, these two phenotypic traits were excluded from further analyses. Interestingly, survival showed significant heritability only in the sites under the (harsher) Mediterranean climate, characterized by an intense drought period during summer. The highest H^2 estimates were obtained for phenology traits followed by tree height. H^2 for a given trait varied across environments (e.g., height, survival and phenology traits) but showed little variation across years (Table S1).

The global estimate of F_{ST} was 0.112 (95% confidence intervals: 0.090–0.141). All groups of phenotypic traits, except survival, had at least one trait with statistically higher Q_{ST} than F_{ST} (Table S1), suggesting that local adaptation is driven by these traits. The highest Q_{ST} was obtained for susceptibility to *D. sapinea* infection measured as necrosis length, followed by $\delta^{13}C$ and tree height, which also showed similar Q_{ST} values across environments and tree ages (Figure 2).

3.2 | Polygenicity of adaptive traits

Polygenicity estimates were consistent between the VSR and MLM methods (Table S4). In addition, estimates of polygenicity were consistent across increasing numbers of input SNPs (Figure S4). Both VSR and MLM methods showed substantial polygenic control for most of the phenotypic traits, with an average of 6% (0%–15%) of the genotyped SNPs having nonzero effects. Significant polygenicity was found in all five trait categories for at least one trait (Figures 3 and 4; Table S4). Polygenicity for height was stable across environments and years, when measured multiple times under the
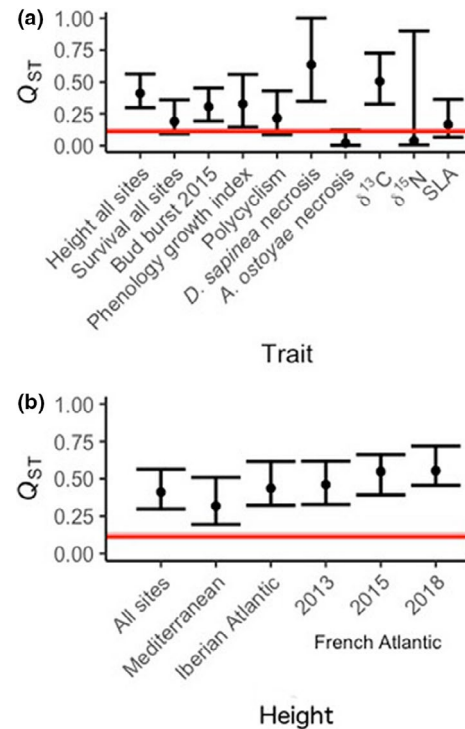

FIGURE 2 Comparison of Q_{ST} and F_{ST} estimates across traits, environments and years. (a) Q_{ST} for a selection of traits belonging to five categories: survival, height, phenology, functional and biotic-stress response traits (see Table S1 for all traits). (b) Q_{ST} for height estimated in three different environments: Mediterranean, Iberian Atlantic, and French Atlantic, and a global Q_{ST} for the three environments together. In the French Atlantic common garden, height was measured in three different years: 2013, 2015 and 2018. Global F_{ST} estimate is presented as a red line surrounded by the 95% confidence intervals computed by bootstrapping [Colour figure can be viewed at wileyonlinelibrary.com]

same environment (e.g., in the French Atlantic common garden at Bordeaux) (Figure 4). Along the same line, polygenicity for phenology traits and tree survival also remained stable across environments, although 95% credible intervals overlapped zero in one out of four cases and 2 out of 4 cases, respectively. The low polygenicity values observed for survival in the French Atlantic common garden are probably a consequence of low phenotypic variability in this site, with almost no mortality (97.12% of planted trees were alive at the evaluation time, Table S1). Polygenicity was heterogeneous for biotic stress response and functional traits (Figure 3). For instance, susceptibility to *D. sapinea* was more polygenic than susceptibility to *A. ostoyae* or than incidence of pine processionary moth. For functional traits, SLA and $\delta^{15}N$ showed the highest levels of polygenicity, while $\delta^{13}C$ showed a considerably lower proportion of SNPs with nonzero effect size.

Estimates of genetic explained variance, *GEV*, were consistent across methods, although VSR tended to give higher values (Table S4). On average *GEV* was 0.37 across traits (considering both VSR and MLM methods), with a minimum of 0.018 for survival in the French Atlantic environment in 2018, and a maximum of 0.99 for *D. sapinea* necrosis (both estimates obtained with the VSR method).
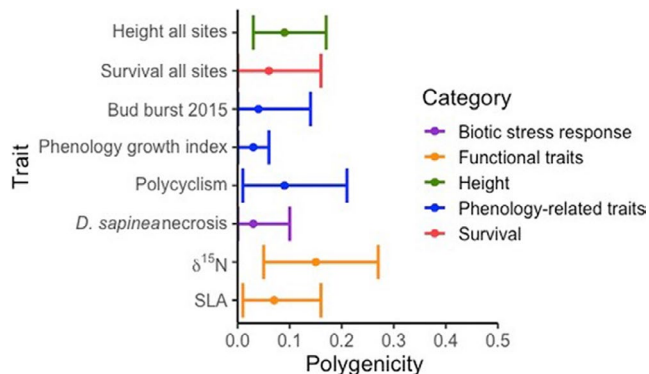
FIGURE 3 Polygenicity estimated from Bayesian mixed linear models (MLMs) for a selection of traits (see Table S4 for all traits). Polygenicity was estimated as the proportion of nonzero effect-size SNPs. Posterior median and 95% credible intervals are presented [Colour figure can be viewed at wileyonlinelibrary.com]

GEV estimated with the VSR method for the G × E component on tree height (considering Atlantic versus Mediterranean environments) was low but significant (median = 0.238, 95% credible interval = 0.043–0.409), indicating some SNPs with significant effects on growth plasticity. However, this result could not be confirmed with the MLM method. Furthermore, *GEV* for the G × E component on tree survival was not significant with any model.

Polygenicity and *GEV* were positively and consistently correlated for both VSR and MLM models (Figure 5). This positive correlation suggested that SNP-based heritability is mainly determined by genetic variants with similarly small effects, and that differences in polygenicity across traits are mostly accounting for differences in explained genetic variance, rather than the distribution of SNP effect-size (Figures S5 and S6).

3.3 | Evidence of negative selection

The correlation between SNP effect size and MAF, *S*, was used to identify the type and mode of natural selection acting upon phenotypic traits. Out of the 28 assayed trait-environment combinations, we were able to estimate *S* for 20 of them (the MCMC algorithm did not converge for the other eight traits). Estimates ranged from −1.68 (bud burst in 2017) to 0.55 (tree survival in French Atlantic environment), but only seven traits from four out of five trait categories (survival, height, phenology, and functional traits) were significant (Figure 6). No significant effect was observed for any trait belonging to the biotic-stress response category. Remarkably, all seven significant estimates of *S* were negative (ranging from −1.68 for bud burst in 2017 to −0.99 for survival in the Iberian Atlantic environment).

Estimates of *S* for tree height were consistent across years and environments. However, *S* estimated for tree survival was only significant in the Iberian Atlantic environment. For phenology traits, *S* was significant only for bud burst measured in 2017 (Figure 6). These results contrast with the consistent level of polygenicity for survival and phenology traits across years and environments.

3.4 | Gene function enrichment at pathway level

Tests for gene function enrichment at the pathway level were significant for survival in the Iberian Atlantic environment, phenology-related and biotic-stress response traits, and height in the French Atlantic and Mediterranean environments. Genes coding for transcription factors showed higher probability of being included in the VSR models (*postp* statistic) and higher estimated SNP effect-sizes
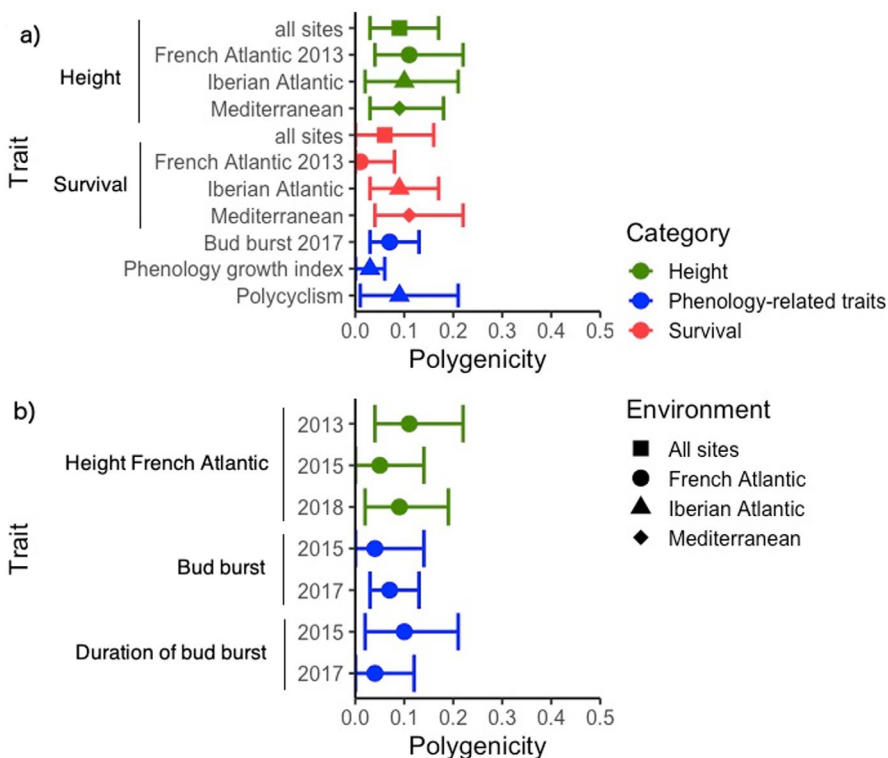

FIGURE 4 Polygenicity estimated from Bayesian mixed linear models (MLMs) across environments and years. (a) Variation of polygenicity across environments. (b) Temporal variation of polygenicity. Polygenicity was estimated as the proportion of nonzero effect-size SNPs. Posterior median and 95% credible intervals are presented [Colour figure can be viewed at wileyonlinelibrary.com]
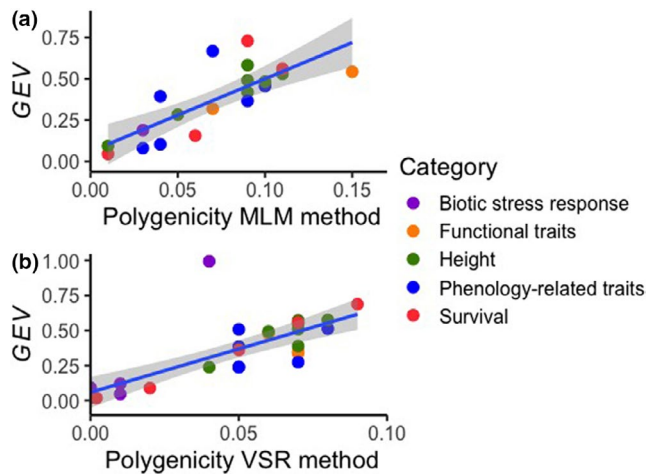
FIGURE 5 (a) MLM method implemented in CGTB software. (b) VSR method implemented in PIMASS software. Each point represents the posterior median [Colour figure can be viewed at wileyonlinelibrary.com]

(*beta* statistic) for survival in the Iberian Atlantic environment (Table 2). Two gene sets associated to bud burst in 2015 showed signals of polygenic selection: monolignol biosynthesis, which had high overall values of both *postp* and *beta*, and glycan metabolism, which showed low overall *beta* estimates (Table 2). Furthermore, the phenology growth index was associated with enrichment for genes related to cell growth and death, DNA recombination and repair and UV response, which mostly have low *beta* values (Table 2). *D. sapinea* susceptibility was associated with high overall *beta* and *postp* in genes from the ubiquitin system for necrosis length, and in genes from the signal transduction and flavonoid biosynthesis for needle discoloration (Table 2). Interestingly, tree height was enriched for genes from different pathways when measured in contrasting environments. For instance, in the French Atlantic environment genes coding for transcription factors showed high *beta* and *postp,* while

genes within the cytoskeleton pathway showed overall low beta values in the Mediterranean environment.

4 | DISCUSSION

Unraveling the genetic architecture of adaptive traits is challenging because of the difficulty to identify variants with small effect sizes using GWAS. Here, we addressed this challenge by maximizing the power to detect significant associations through the estimation of the genetic effects on phenotypic variation in a clonal common garden network for an extensive number of fitness-related traits combined with the implementation of polygenic methods that simultaneously evaluate the effect of multiple SNPs. Specifically, we tested if a large proportion of the genetic variance of fitness-related traits in a long-lived forest tree, maritime pine, can be explained by a large number of small effect size variants, in line with the polygenic adaptation model. We also tested whether negative selection is pervasive for such polygenic traits. Our results showed patterns of local adaptation for most phenotypic traits, highlighting their relationship with fitness, and also revealed a high and remarkably stable degree of polygenicity, across traits, years, and environments. Moreover, using two complementary multilocus approaches, we were able to account for a considerable proportion of the heritability, and identified negative selection as a relevant driver of local adaptation in maritime pine.

4.1 | Evidence of local adaptation in maritime pine

All phenotypic trait categories presented significant within-population genetic variation (i.e., broad-sense heritability, H^2); thus, significant responses to natural selection are expected in maritime pine (Visscher et al., 2008). Estimates of heritability were consistent

FIGURE 6 Correlation between SNP effect-size and minor allele frequency (MAF). The coefficient of correlation between SNP effect-size and MAF (S) was estimated through the MLM method. The posterior distribution of S (median and 95% credible intervals) are presented. Trait names on the left side indicate traits that have been measured in different common gardens (i.e., environments) or years [Colour figure can be viewed at wileyonlinelibrary.com]
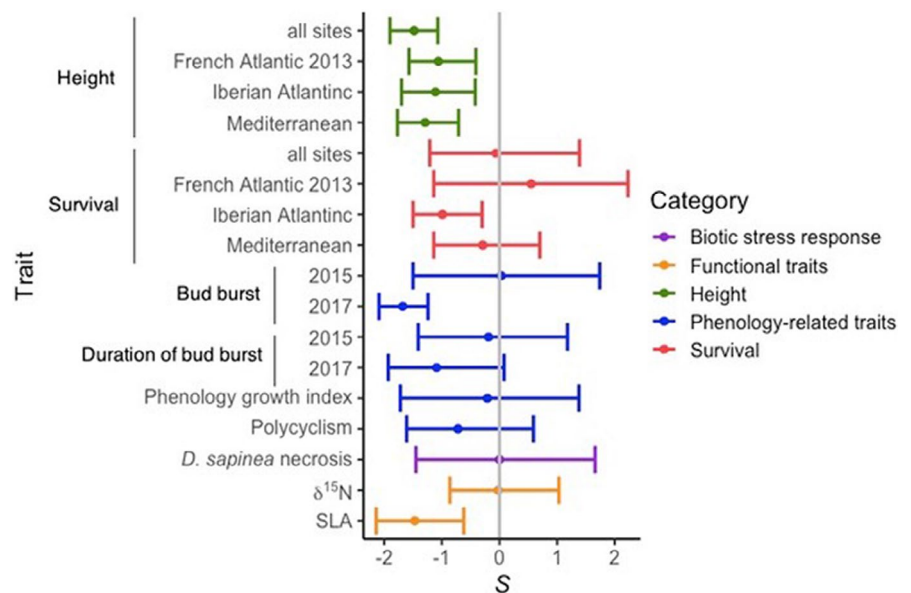
TABLE 2 Gene sets with gene function enrichment at pathway/module level. Two statistics obtained from the VSR method were tested: the maximum of any SNP per gene of the Rao-Backwellized posterior probability of inclusion (*postp*) and the maximum of any SNP per gene of the absolute value of the Rao-Backwellized effect-size (*beta*). Sign of enrichment refers to two-tailed null hypothesis testing

Trait	Environment	Gene set	Statistic tested	Sign of enrichment	p-value	q-value (<0.10)
Height	French Atlantic	Transcription factor	*beta*	Higher	.003	0.05
			postp	Higher	.004	0.07
	Mediterranean	Cytoskeleton	*beta*	Lower	.003	0.06
Survival	Iberian Atlantic	Transcription factor	*beta*	Higher	.001	0.01
			postp	Higher	<.001	0.005
Bud burst 2015	French Atlantic	Monolignol biosynthesis	*beta*	Higher	.003	0.05
		Monolignol biosynthesis	*postp*	Higher	.005	0.08
		Glycan metabolism	*beta*	Lower	.040	0.09
Phenology growth index	Iberian Atlantic	Cell growth and death	*beta*	Lower	.010	0.03
		DNA recomb and repair	*beta*	Lower	.008	0.03
		UV response	*beta*	Lower	.005	0.03
D. sapinea necrosis	French Atlantic	Ubiquitin system	*beta*	Higher	.002	0.04
			postp	Higher	.003	0.06
D. sapinea discoloration	French Atlantic	Signal transduction	*beta*	Higher	.004	0.08
			postp	Higher	.003	0.06
		Flavonoid biosynthesis	*postp*	Higher	.007	0.07

with studies in forest trees (reviewed by Lind et al., 2018). In addition, our results were consistent with adaptive differentiation ($Q_{ST} > F_{ST}$) for 11 out of 26 analysed trait-environment combinations, involving four out of the five trait categories. There was no evidence, however, of adaptive differentiation for survival traits. These results are in accordance with reports of pervasive local adaptation in forest trees (Alberto et al., 2013; Lind et al., 2018; Savolainen et al., 2007, 2013). Considering that we found negative selection to be a relevant driver of population adaptation in maritime pine (see below), this means that differential purifying selection across environments may underlie the observed pattern of widespread local adaptation. This pattern could be the result of different environmental constraints across populations or to the fact that different gene pathways could be affected by negative selection as a consequence of genetic redundancy (Barghi et al., 2019).

The stability of Q_{ST} estimates for height across environments and years highlights the strength of directional selection for this trait in maritime pine; a trait that can thus be used for the delimitation of conservation and management units (Rodríguez-Quilón et al., 2016). Contrarily, phenology traits showed contrasting estimates of Q_{ST} depending on the environment and year of measurement (i.e., we cannot discard that genetic differentiation between populations for phenology was produced by drift alone for some years of measurement). This result highlights that the evolutionary forces driving population genetic differences in some phenological traits are environmentally and temporally dependent, which can slow down attaining local phenotypic optima under rapidly changing climates. Polygenic adaptation could be specially relevant for these traits because it can produce rapid phenotypic changes, as it would only

require small adjustments in allele frequencies in the contributing loci rather than selective sweeps on new mutations (Dayan et al., 2019; Hermisson & Pennings, 2017; Jain & Stephan, 2017; Wisser et al., 2019).

Unexpectedly, survival, a trait directly related with a component of fitness (i.e., viability), did not show evidence of local adaptation in maritime pine. The low levels of phenotypic variability observed for survival in this study may explain these results. Future studies should focus on quantitative evaluations of survival (e.g., adding a time frame, such as time until death or order of dead trees) to better capture the complexity of this trait and better discern genetic differences among populations. The strong selective pressure in the Mediterranean region exacerbated genetic differences in survival among clones and resulted in slightly higher estimates of heritability (similarly to Gaspar et al., 2013). Additionally, we observed significant phenotypic plasticity for height and survival, the two traits measured in all five experimental sites. While our results were consistent with a heritable component for plasticity, judging by a high number of SNPs with nonzero effect sizes, this question requires further investigation for a more detailed characterization of phenotypic plasticity in the adaptive response of maritime pine to changing environmental conditions (Alía et al., 2014; Vizcaíno-Palomar et al., 2019).

Two traits in particular had remarkably high levels of adaptive genetic differentiation among populations, $\delta^{13}C$ and *D. sapinea necrosis* (Figure 2), but their genetic variation within populations was low, compromising their adaptive potential. These traits deserve special attention because of the implication of water-use efficiency in drought resistance (reviewed by Plomion, Bartholomé,

Bouffier et al., 2016) and the new pathogenic outbreaks of *D. sapinea* expected on maritime pine plantations fostered by climate change (Brodde et al., 2019; Fabre et al., 2011). In contrast to our findings, a lack of adaptive genetic differentiation for $\delta^{13}C$ was previously reported for maritime pine by Lamy et al. (2011), as well as for broad-leaved trees (Torres-Ruiz et al., 2019). Although this disagreement may be influenced by the much larger number of populations we analysed (see Whitlock & Guillaume, 2009) as compared to Lamy et al. (2011), we cannot rule out discrepancies due to the estimation of total genetic variance in our study (i.e., based on clones), instead of additive genetic variance (i.e., based on half-sib families). Nevertheless, nonadditive genetic effects in maritime pine traits related to drought resistance have been reported to be of little importance (Gaspar et al., 2013), and they probably did not affect our estimates much.

4.2 | Polygenicity of adaptive traits

The implementation of polygenic adaptation studies outside of humans is slowly emerging (Barghi et al., 2019; Csilléry et al., 2014; Friedline et al., 2019; He et al., 2016; Lind et al., 2017; Wisser et al., 2019), providing increased evidence that polygenic adaptation in complex traits may be pervasive (Barghi et al., 2020; Sella & Barton, 2019). In our study, most traits had a considerable degree of polygenicity, with an average of 6% across traits, environments and years, which is on the same order of magnitude as for humans (Zeng et al., 2018). Polygenicity was relatively similar across all analysed traits and therefore did not depend on the level of genetic control, as estimated by heritability through quantitative genetic analysis. Mei et al. (2018) predicted different genetic architectures as a function of genome size. Surprisingly, although the maritime pine genome is more than seven times larger than that of humans (De La Torre et al., 2014), we found similar estimates of polygenicity as in humans. In maritime pine, the distribution of SNP effect sizes showed that hundreds of SNPs with near-zero effect size contributed to phenotypic differences among clones. In addition, we did not detect any large effect size SNPs. This highly polygenic architecture could be explained by genetic redundancy (Barghi et al., 2019, 2020). Nevertheless, we cannot disregard the explanation that our study failed to detect large effect size SNPs because of partial genome coverage.

According to genetic redundancy, different individuals of the same population would have used different combination of alleles to attain the same phenotypic value leading to the observed pattern of subtle allele frequency shifts in a high number of genes. Genetic redundancy can speed up the evolution of phenotypic optima through multiple genetic pathways leading to similar phenotypes (Barghi et al., 2019; Höllinger et al., 2019). Unraveling the role of genetic redundancy in conifers, whose genomes are characterized by a high number of paralogs (Diaz-Sala et al., 2013), may shed new light about how rapidly these taxa can adapt to environmental changes. Moreover, the influence of genome size in the genetic architecture

of fitness-related traits, as well as the relationship between heritability and polygenicity, deserve further investigation including a better coverage of conifer genomes, as well as improved knowledge of non-coding regions (Mackay et al., 2012).

Recent studies of human height have suggested that detecting polygenicity may be affected by subtle biases in GWAS caused by population structure (Berg et al., 2019; Sohail et al., 2019). In our study, the clonal common garden network allowed separating the genetic and the environmental effects on phenotypes, so as to better identify which traits are contributing to adaptation. In addition, we corrected the BLUPs estimates for the effect of neutral population genetic structure (see Section 2 and Figure S3). In this sense, our work highlights the potential of combining precise estimation of the genetic effect on phenotypes using clones with multilocus genotype-phenotype association models, so as to elucidate the mechanisms that allow the maintenance of genetic variation in adaptive traits, especially in species with complex demographic histories. Undoubtedly, the next steps to decipher polygenic adaptation in species with varied life-history traits should implement upcoming polygenic association methods that directly correct for population stratification (e.g., Josephs et al., 2019).

4.3 | Performance of polygenic adaptation approaches (VSR and MLM)

We evaluated the performance of polygenic approaches (VSR and MLM) through the comparison of SNP-based genetic variance estimates, *GEV*. Despite some slight differences, notably for biotic-stress response traits that were limited by low sample sizes and, in some cases, low heritability, both methods were robust and provided consistent estimates. The large proportion of the genetic variance explained by SNP-based models, usually higher than 50%, suggests that, by adopting a polygenic analytical model, we were able to account for a significant part of the heritability inferred through pedigree-based analysis, even when using a modest number of SNPs. It is worth noting that the performance of polygenic models did not depend on the estimated degree of heritability, as evidenced by the absence of correlation between *GEV* and H^2 ($\rho = 0.04$ for VSR, $\rho = -0.05$ for MLM, $p > .05$ in both cases).

Insights provided by SNP-based estimations of *GEV* should be interpreted with caution. First, because the genome size of maritime pine is huge (around 28 Gbp; Grotkopp et al., 2004; Zonneveld, 2012) and linkage disequilibrium decays rapidly (Neale & Savolainen, 2004), a larger number of genotyped SNPs will be needed to obtain complete genomic coverage. Second, rare variants are usually difficult to incorporate in genotyping platforms, such as the one used in our study (although some rare variants were captured in our SNP array), and such rare variants may account for an important proportion of the heritability in complex traits (Young & Flint, 2019). Even though further investigation is needed to draw stronger conclusions, the robust and consistent estimates of polygenicity across methods obtained in our study was probably fostered by a precise

phenotypic evaluation of a large number of clonally-replicated individuals ($N > 20{,}500$ trees).

4.4 | Stability of polygenicity estimates across environments and years

The temporal and spatial heterogeneity of selection can impact the evolution of the genetic architecture underlying adaptation (Sella & Barton, 2019). Monitoring the patterns of genetic architecture not only across environments but also across years is an important issue in long-lived forest trees that may experience changing selection pressures throughout their lifetimes. Our study is not only a validation of the polygenic adaptation model in a new organism, but a contribution to improving our understanding of adaptation. Surprisingly, the estimated degree of polygenicity remained stable across environments for all trait categories, especially tree height. Additionally, we observed highly stable genetic architectures for height, phenology, and survival across years. For the case of tree height, polygenicity was highly stable across three time points taken throughout a time span of 6 years, comprising seedling and juvenile stages, during which trees are more vulnerable and selection pressures are more pronounced (Leck et al., 2008). However, analysis of gene function enrichment (see below) suggests that different genetic pathways could be underlying phenotypic variation in contrasting environments. Moreover, differences in gene expression may also underlie adaptation in different environments and years (Hämälä et al., 2020; Mähler et al., 2017).

4.5 | The role of negative selection in polygenic adaptation

All significant correlations between SNP effect sizes and MAF were negative (for tree height, bud burst and SLA), suggesting a genetic architecture modeled, at least partially, by the action of negative selection (O'Connor et al., 2019). Interestingly, our results suggest a stable polygenic architecture, but an environment- and year-dependent impact of negative selection for some fitness-related traits. The MLM method did not allow elucidating whether negative estimates of S were the consequence of an enrichment of trait increasing or trait decreasing alleles (Zeng et al., 2018), but its results clearly were consistent with these traits being under some form of negative selection. Our estimates of negative selection may have been limited by the low coverage of the maritime pine genome that did not account for many of the rare variants, which considerably affect S estimates (Zeng et al., 2018). Nevertheless, the analysed SNPs included both species-wide and gene-pool specific alleles, with about 10% of the SNPs having frequencies lower than 5%. This suggests that S was estimated over an extensive range of allele frequencies, including also some low-frequency variants.

Evidence of the pervasive effects of purifying selection is abundant in model plant genomes (Wright & Andolfatto, 2008), and is also emerging in forest trees (De La Torre et al., 2017; Eckert et al., 2013; Grivet et al., 2017; Krutovsky & Neale, 2005; Palmé et al., 2009). Indeed, negative selection, and its variation across populations and through time, has been pointed out as a main cause for maintaining polygenicity (O'Connor et al., 2019; Zeng et al., 2018). Nevertheless, strikingly, the negative selection patterns observed across environments and years did not mimic the trend observed for polygenicity. That is, negative selection was consistently inferred for height, but its strength changed across environments and years for survival and phenology traits. This result suggests that the expression of survival and phenology traits in different environments and years may be affected by different selective forces, which is in accordance with the environment- and time-dependent selection observed in the analyses at the pathway level (see below).

We detected signals of gene enrichment for 10 pathways that had higher values than expected of the maximum SNP effect size or posterior probability of being included in the polygenic models. Assuming that evolution of these pathways is driven by negative selection, as suggested above, these patterns could be explained by the accumulation of slightly deleterious alleles in highly redundant and large conifer genomes (Krakauer & Nowak, 1999; Nowak et al., 1997). Otherwise, if we were to assume a higher impact of positive rather than negative selection, the observed patterns would imply an accumulation of beneficial mutations in these pathways, which is a hypothesis worth exploring in future studies. Conversely, another five pathways were enriched with lower than expected effect size alleles. The involved pathways (see Section 3) perform general functions. In this case, the observed patterns suggest higher genetic constraints on these functionally important genes, for which negative selection should be highly efficient (Wright & Andolfatto, 2008). Interestingly, our results suggest that even for stable estimates of polygenicity, different gene pathways could underlie polygenic adaptation for height in contrasting environments. Finally, although our gene enrichment analysis revealed some pathways with stronger evidence for polygenic adaptation, we cannot discard the influence of other nonstudied gene pathways, as pointed by the "omnigenic" theory (Boyle et al., 2017).

5 | CONCLUSIONS

The study of the genetics of adaptation is currently facing new challenges. The advancement of GWAS relies on the development of methods able to detect causal variants of small effect size and/or at low allele frequencies. Our study, which tested the fit of a polygenic adaptation model using well-characterized maritime pine clones planted in contrasted environments, contributes to a better understanding of the heritability of complex adaptive traits in long-lived organisms, including the underlying genetic architecture of these traits. Our results were consistent with the hypothesis that most complex and adaptive traits are polygenic, with some of them also showing signatures of negative selection. The degree of polygenicity was similar for traits spanning different functional categories

and this genetic architecture was considerably stable over time and across environments. Current models for predicting population trajectories in forest trees under climate change are based on identification of outlier SNPs with relatively large effects on phenotypes and/or strong correlation with climate variables (Jaramillo-Correa et al., 2015; Lu et al., 2019; Rellstab et al., 2016). Because polygenic adaptation can take place rapidly (see, for example, Jain & Stephan, 2017), current prediction models are probably underestimating the capacity of natural forest tree populations to adapt to new environments. Thus, adopting a polygenic adaptation perspective could significantly improve prediction accuracy, and provide new scenarios to inform forest conservation and reforestation programmes (Fady et al., 2016; Valladares et al., 2014). A better understanding of the genetic architecture of economically valuable complex traits can also improve genomic-assisted breeding, thus allowing construction of better genomic selection models (Grattapaglia et al., 2018).

AUTHOR CONTRIBUTION

Marina de Miguel, collected field data, carried out the statistical analyses and drafted the manuscript. Juan Majada, Ricardo Alía and Christophe Plomion designed and established the common gardens, and helped with field data collection. Isabel Rodríguez-Quilón, Delphine Grivet, Christophe Plomion, Giovanni G. Vendramin and Santiago C. González-Martínez contributed to the SNP assay design and molecular laboratory work. Andrew J. Eckert, Ricardo Alía and Santiago C. González-Martínez conceived and designed the study. Isabel Rodríguez-Quilón and Agathe Hurel collected field data and helped with the statistical analyses. Juan-Pablo Jaramillo-Correa identified gene pathways and defined gene-sets. Myriam Heuertz, Santiago C. González-Martínez, Delphine Grivet, Juan-Pablo Jaramillo-Correa and Giovanni G. Vendramin contributed to the statistical analysis of genomic data. Santiago C. González-Martínez coordinated the study. All authors contributed to manuscript discussion and review, and gave final approval for publication.

BENEFIT-SHARING STATEMENT

A research collaboration was developed with scientists from the countries providing genetic samples and maintaining the common gardens, all collaborators are included as coauthors and the results have been shared broadly, including outside the scientific community. In addition, benefits from this research accrue from the sharing of our data and results on public databases as described above. Finally, authors are committed to international scientific partnerships, as well as institutional capacity building.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

OPEN RESEARCH BADGES

This article has earned an Open Data Badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. All the data necessary for confirming the conclusions presented in this article is given fully in Data INRAE. SNP genotypes: https://doi.org/10.15454/UBSQUK. Raw phenotypes and BLUPs: https://doi.org/10.15454/L3JM22.

DATA AVAILABILITY STATEMENT

All the data necessary for confirming the conclusions presented in this article is given fully in Data INRAE public repository. SNP genotypes: https://doi.org/10.15454/UBSQUK. Raw phenotypes and BLUPs: https://doi.org/10.15454/L3JM22.

ORCID

Marina de Miguel https://orcid.org/0000-0001-6398-2660
Myriam Heuertz https://orcid.org/0000-0002-6322-3645
Delphine Grivet https://orcid.org/0000-0001-8168-4456
Christophe Plomion https://orcid.org/0000-0002-3176-2767
Juan Majada https://orcid.org/0000-0003-0009-4847
Ricardo Alía https://orcid.org/0000-0002-9426-0967
Andrew J. Eckert https://orcid.org/0000-0002-6522-2646
Santiago C. González-Martínez https://orcid.org/0000-0002-4534-3766

REFERENCES

Alberto, F. J., Aitken, S. N., Alía, R., González-Martínez, S. C., Hänninen, H., Kremer, A., Lefèvre, F., Lenormand, T., Yeaman, S., Whetten, R., & Savolainen, O. (2013). Potential for evolutionary responses to climate change evidence from tree populations. *Global Change Biology*, 19(6), 1645–1661. https://doi.org/10.1111/gcb.12181

Alía, R., Chambel, R., Notivol, E., Climent, J., & González-Martínez, S. C. (2014). Environment-dependent microevolution in a Mediterranean pine (*Pinus pinaster* Aiton). *BMC Evolutionary Biology*, 14(1), 200. https://doi.org/10.1186/s12862-014-0200-5

Aranda, I., Alía, R., Ortega, U., Dantas, Â. K., & Majada, J. (2010). Intra-specific variability in biomass partitioning and carbon isotopic discrimination under moderate drought stress in seedlings from four *Pinus pinaster* populations. *Tree Genetics & Genomes*, 6(2), 169–178. https://doi.org/10.1007/s11295-009-0238-5

Archambeau, J., Garzón, M. B., Barraquand, F., de Miguel Vega, M., Plomion, C., & González-Martínez, S. C. (2020). Combining climatic and genomic data improves range-wide tree height growth prediction in a forest tree. *bioRxiv*. https://doi.org/10.1101/2020.11.13.382515

Barghi, N., Hermisson, J., & Schlötterer, C. (2020). Polygenic adaptation: A unifying framework to understand positive selection. *Nature Reviews Genetics*, 21, 1–13. https://doi.org/10.1038/s41576-020-0250-z

Barghi, N., Tobler, R., Nolte, V., Jakšić, A. M., Mallard, F., Otte, K. A., Dolezal, M., Taus, T., Kofler, R., & Schlötterer, C. (2019). Genetic redundancy fuels polygenic adaptation in *Drosophila*. *PLoS Biology*, *17*(2), e3000128. https://doi.org/10.1371/journal.pbio.3000128

Berg, J. J., & Coop, G. (2014). A population genetic signal of polygenic adaptation. *PLoS Genetics*, *10*(8), e1004412. https://doi.org/10.1371/journal.pgen.1004412

Berg, J. J., Harpak, A., Sinnott-Armstrong, N., Joergensen, A. M., Mostafavi, H., Field, Y., Boyle, E. A., Zhang, X., Racimo, F., Pritchard, J. K., & Coop, G. (2019). Reduced signal for polygenic adaptation of height in UK Biobank. *eLife*, *8*, e39725. https://doi.org/10.7554/eLife.39725

Bernatchez, S., Xuereb, A., Laporte, M., Benestan, L., Steeves, R., Laflamme, M., Bernatchez, L., & Mallet, M. A. (2019). Seascape genomics of eastern oyster (*Crassostrea virginica*) along the Atlantic coast of Canada. *Evolutionary Applications*, *12*(3), 587–609. https://doi.org/10.1111/eva.12741

Boyle, E. A., Li, Y. I., & Pritchard, J. K. (2017). An expanded view of complex traits: From polygenic to omnigenic. *Cell*, *169*(7), 1177–1186. https://doi.org/10.1016/j.cell.2017.05.038

Brodde, L., Adamson, K., Julio Camarero, J., Castaño, C., Drenkhan, R., Lehtijärvi, A., Luchi, N., Migliorini, D., Sánchez-Miranda, Á., Stenlid, J., Özdağ, Ş., & Oliva, J. (2019). Diplodia Tip blight on its way to the north: Drivers of disease emergence in Northern Europe. *Frontiers in Plant Science*, *9*, 1818. https://doi.org/10.3389/fpls.2018.01818

Bucci, G., González-Martínez, S. C., Provost, G. L., Plomion, C., Ribeiro, M. M., Sebastiani, F., Alía, R., & Vendramin, G. G. (2007). Range-wide phylogeography and gene zones in *Pinus pinaster* Ait. revealed by chloroplast microsatellite markers. *Molecular Ecology*, *16*(10), 2137–2153. https://doi.org/10.1111/j.1365-294X.2007.03275.x

Canales, J., Bautista, R., Label, P., Gómez-Maldonado, J., Lesur, I., Fernández-Pozo, N., Rueda-López, M., Guerrero-Fernández, D., Castro-Rodríguez, V., Benzekri, H., Cañas, R., Guevara, M.-A., Rodrigues, A., Seoane, P., Teyssier, C., Morel, A., Ehrenmann, F., Le Provost, G., Lalanne, C., … Cánovas, F. M. (2014). De novo assembly of maritime pine transcriptome: Implications for forest breeding and biotechnology. *Plant Biotechnology Journal*, *12*(3), 286–299. https://doi.org/10.1111/pbi.12136

Craine, J. M., Brookshire, E. N. J., Cramer, M. D., Hasselquist, N. J., Koba, K., Marin-Spiotta, E., & Wang, L. (2015). Ecological interpretations of nitrogen isotope ratios of terrestrial plants and soils. *Plant and Soil*, *396*(1), 1–26. https://doi.org/10.1007/s11104-015-2542-1

Csilléry, K., Lalagüe, H., Vendramin, G. G., González-Martínez, S. C., Fady, B., & Oddou-Muratorio, S. (2014). Detecting short spatial scale local adaptation and epistatic selection in climate-related candidate genes in European beech (*Fagus sylvatica*) populations. *Molecular Ecology*, *23*(19), 4696–4708. https://doi.org/10.1111/mec.12902

Daub, J. T., Hofer, T., Cutivet, E., Dupanloup, I., Quintana-Murci, L., Robinson-Rechavi, M., & Excoffier, L. (2013). Evidence for polygenic adaptation to pathogens in the human genome. *Molecular Biology and Evolution*, *30*(7), 1544–1558. https://doi.org/10.1093/molbev/mst080

Dayan, D. I., Du, X., Baris, T. Z., Wagner, D. N., Crawford, D. L., & Oleksiak, M. F. (2019). Population genomics of rapid evolution in natural populations: Polygenic selection in response to power station thermal effluents. *BMC Evolutionary Biology*, *19*, 61. https://doi.org/10.1186/s12862-019-1392-5

De La Torre, A. R., Birol, I., Bousquet, J., Ingvarsson, P. K., Jansson, S., Jones, S. M., Keeling, C. I., MacKay, J., Nilsson, O., Ritland, K., Street, N., Yanchuk, A., Zerbe, P., & Bohlmann, J. (2014). Insights into conifer giga-genomes. *Plant Physiology*, *166*(4), 1724–1732. https://doi.org/10.1104/pp.114.248708

De La Torre, A. R., Li, Z., Van de Peer, Y., & Ingvarsson, P. K. (2017). Contrasting rates of molecular evolution and patterns of selection

among Gymnosperms and flowering plants. *Molecular Biology and Evolution*, *34*(6), 1363–1377. https://doi.org/10.1093/molbev/msx069

De La Torre, A. R., Wilhite, B., & Neale, D. B. (2019). Environmental genome-wide association reveals climate adaptation is shaped by subtle to moderate allele frequency shifts in Loblolly Pine. *Genome Biology and Evolution*, *11*(10), 2976–2989. https://doi.org/10.1093/gbe/evz220

Díaz, S., Kattge, J., Cornelissen, J. H. C., Wright, I. J., Lavorel, S., Dray, S., Reu, B., Kleyer, M., Wirth, C., Colin Prentice, I., Garnier, E., Bönisch, G., Westoby, M., Poorter, H., Reich, P. B., Moles, A. T., Dickie, J., Gillison, A. N., Zanne, A. E., … Gorné, L. D. (2016). The global spectrum of plant form and function. *Nature*, *529*(7585), 167–171. https://doi.org/10.1038/nature16489

Diaz-Sala, C., Cabezas, J. A., Fernández de Simón, B., Abarca, D., Guevara, M. Á., de Miguel, M., Cadahía, E., Aranda, I., & Cervera, M.-T. (2013). The uniqueness of conifers. In P. Poltronieri, N. Burbulis, & C. Fogher (Eds.), *From plant genomics to plant biotechnology* (1st ed., pp. 67–96). Woodhead Publishing.

Durvasula, A., & Lohmueller, K. E. (2021). Negative selection on complex traits limits phenotype prediction accuracy between populations. *The American Journal of Human Genetics*, *108*(4), 620–631. https://doi.org/10.1016/j.ajhg.2021.02.013

Eckert, A. J., Bower, A. D., Jermstad, K. D., Wegrzyn, J. L., Knaus, B. J., Syring, J. V., & Neale, D. B. (2013). Multilocus analyses reveal little evidence for lineage-wide adaptive evolution within major clades of soft pines (*Pinus* subgenus *Strobus*). *Molecular Ecology*, *22*(22), 5635–5650. https://doi.org/10.1111/mec.12514

Exposito-Alonso, M., Burbano, H. A., Bossdorf, O., Nielsen, R., & Weigel, D. (2019). A map of climate change-driven natural selection in *Arabidopsis thaliana*. *BioRxiv*, https://doi.org/10.1101/321133

Fabre, B., Piou, D., Desprez-Loustau, M.-L., & Marçais, B. (2011). Can the emergence of pine Diplodia shoot blight in France be explained by changes in pathogen pressure linked to climate change? *Global Change Biology*, *17*(10), 3218–3227. https://doi.org/10.1111/j.1365-2486.2011.02428.x

Fady, B., Cottrell, J., Ackzell, L., Alía, R., Muys, B., Prada, A., & González-Martínez, S. C. (2016). Forests and global change: What can genetics contribute to the major forest management and policy challenges of the twenty-first century? *Regional Environmental Change*, *16*(4), 927–939. https://doi.org/10.1007/s10113-015-0843-9

Farquhar, G. D., & Richards, R. A. (1984). Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. *Functional Plant Biology*, *11*(6), 539–552. https://doi.org/10.1071/pp9840539

Fernández-Pozo, N., Canales, J., Guerrero-Fernández, D., Villalobos, D. P., Díaz-Moreno, S. M., Bautista, R., Flores-Monterroso, A., Guevara, M. Á., Perdiguero, P., Collada, C., Cervera, M. T., Soto, Á., Ordás, R., Cantón, F. R., Avila, C., Cánovas, F. M., & Claros, M. G. (2011). EuroPineDB: A high-coverage web database for maritime pine transcriptome. *BMC Genomics*, *12*(1), 366. https://doi.org/10.1186/1471-2164-12-366

Field, Y., Boyle, E., Telis, N., Gao, Z., Gaulton, K. J., Golan, D., Yengo, L., Rocheleau, C., Froguel, P., McCarthy, M. I., & Pritchard, J. K. (2016). Detection of human adaptation during the past 2000 years. *Science*, *354*(6313), 760–764. https://doi.org/10.1126/science.aah5114

Fisher, R. A. (1918). The correlation between relatives on the supposition of Mendelian inheritance. *Transaction Royal Society Edinburgh*, *52*, 399–433.

Fréjaville, T., & Benito Garzón, M. (2018). The EuMedClim database: Yearly climate data (1901–2014) of 1 km resolution grids for Europe and the Mediterranean Basin. *Frontiers in Ecology and Evolution*, *6*, 31. https://doi.org/10.3389/fevo.2018.00031

Friedline, C. J., Faske, T. M., Lind, B. M., Hobson, E. M., Parry, D., Dyer, R. J., Johnson, D. M., Thompson, L. M., Grayson, K. L., & Eckert, A. J. (2019). Evolutionary genomics of gypsy moth populations sampled

along a latitudinal gradient. *Molecular Ecology*, 28(9), 2206–2223. https://doi.org/10.1111/mec.15069

Gaspar, M. J., Velasco, T., Feito, I., Alía, R., & Majada, J. (2013). Genetic variation of drought tolerance in *Pinus pinaster* at three hierarchical levels: A comparison of induced osmotic stress and field testing. *PLoS One*, 8(11), e79094. https://doi.org/10.1371/journal.pone.0079094

Girard, F., Vennetier, M., Ouarmim, S., Caraglio, Y., & Misson, L. (2011). Polycyclism, a fundamental tree growth process, decline with recent climate change: The example of *Pinus halepensis* Mill. in Mediterranean France. *Trees*, 25(2), 311–322. https://doi.org/10.1007/s00468-010-0507-9

Gnecchi-Ruscone, G. A., Abondio, P., De Fanti, S., Sarno, S., Sherpa, M. G., Sherpa, P. T., Marinelli, G., Natali, L., Di Marcello, M., Peluzzi, D., Luiselli, D., Pettener, D., & Sazzini, M. (2018). Evidence of polygenic adaptation to high altitude from Tibetan and Sherpa genomes. *Genome Biology and Evolution*, 10(11), 2919–2930. https://doi.org/10.1093/gbe/evy233

González-Martínez, S. C., Alía, R., & Gil, L. (2002). Population genetic structure in a Mediterranean pine (*Pinus pinaster* Ait.): A comparison of allozyme markers and quantitative traits. *Heredity*, 89(3), 199–206. https://doi.org/10.1038/sj.hdy.6800114

Grattapaglia, D., Silva-Junior, O. B., Resende, R. T., Cappa, E. P., Müller, B. S. F., Tan, B., Isik, F., Ratcliffe, B., & El-Kassaby, Y. A. (2018). Quantitative genetics and genomics converge to accelerate forest tree breeding. *Frontiers in Plant Science*, 9, 1693. https://doi.org/10.3389/fpls.2018.01693

Greenwood, S., Ruiz-Benito, P., Martínez-Vilalta, J., Lloret, F., Kitzberger, T., Allen, C. D., Fensham, R., Laughlin, D. C., Kattge, J., Bönisch, G., Kraft, N. J. B., & Jump, A. S. (2017). Tree mortality across biomes is promoted by drought intensity, lower wood density and higher specific leaf area. *Ecology Letters*, 20(4), 539–553. https://doi.org/10.1111/ele.12748

Grivet, D., Avia, K., Vaattovaara, A., Eckert, A. J., Neale, D. B., Savolainen, O., & González-Martínez, S. C. (2017). High rate of adaptive evolution in two widespread European pines. *Molecular Ecology*, 26(24), 6857–6870. https://doi.org/10.1111/mec.14402

Grotkopp, E., Rejmánek, M., Sanderson, M. J., & Rost, T. L. (2004). Evolution of genome size in Pines (*Pinus*) and its life-history correlates: Supertree analyses. *Evolution*, 58(8), 1705. https://doi.org/10.1554/03-545

Guan, Y., & Stephens, M. (2011). Bayesian variable selection regression for genome-wide association studies and other large-scale problems. *The Annals of Applied Statistics*, 5(3), 1780–1815. https://doi.org/10.1214/11-AOAS455

Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: The MCMCglmm *R* package. *Journal of Statistical Software*, 33(2), 1–22. https://doi.org/10.18637/jss.v033.i02

Hall, T. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Window 95/98/NT. *Nucleic Acids Symposium Series*, 41, 95–98.

Hämälä, T., Guiltinan, M. J., Marden, J. H., Maximova, S. N., dePamphilis, C. W., & Tiffin, P. (2020). Gene expression modularity reveals footprints of polygenic adaptation in *Theobroma cacao*. *Molecular Biology and Evolution*, 37(1), 110–123. https://doi.org/10.1093/molbev/msz206

Hancock, A. M., Witonsky, D. B., Ehler, E., Alkorta-Aranburu, G., Beall, C., Gebremedhin, A., Sukernik, R., Utermann, G., Pritchard, J., Coop, G., & Di Rienzo, A. (2010). Human adaptations to diet, subsistence, and ecoregion are due to subtle shifts in allele frequency. *Proceedings of the National Academy of Sciences of the United States of America*, 107(Suppl 2), 8924–8930. https://doi.org/10.1073/pnas.0914625107

Hayward, L. K., & Sella, G. (2019). Polygenic adaptation after a sudden change in environment. *BioRxiv*, https://doi.org/10.1101/792952

He, F., Arce, A. L., Schmitz, G., Koornneef, M., Novikova, P., Beyer, A., & de Meaux, J. (2016). The footprint of polygenic adaptation on stress-responsive cis-regulatory divergence in the *Arabidopsis* genus. *Molecular Biology and Evolution*, 33(8), 2088–2101. https://doi.org/10.1093/molbev/msw096

Henderson, C. R. (1973). Sire evaluation and genetic trends. *Journal of Animal Science*, 1973(Issue Symposium), 10-41. https://doi.org/10.1093/ansci/1973.Symposium.10

Hermisson, J., & Pennings, P. S. (2017). Soft sweeps and beyond: Understanding the patterns and probabilities of selection footprints under rapid adaptation. *Methods in Ecology and Evolution*, 8(6), 700–716. https://doi.org/10.1111/2041-210X.12808

Höllinger, I., Pennings, P. S., & Hermisson, J. (2019). Polygenic adaptation: From sweeps to subtle frequency shifts. *PLoS Genetics*, 15, e1008035. https://doi.org/10.1371/journal.pgen.1008035

Hurel, A., de Miguel, M., Dutech, C., Desprez-Loustau, M.-L., Plomion, C., Rodríguez-Quilón, I., Cyrille, A., Guzman, T., Alía, R., González-Martínez, S. C., & Budde, K. B. (2019). Genetic basis of susceptibility to *Diplodia sapinea* and *Armillaria ostoyae* in maritime pineGenetic basis of growth, spring phenology, and susceptibility to biotic stressors in maritime pine. *Evolutionary Applications*, 14, 2750–2772. https://doi.org/10.1111/eva.13309

Jain, K., & Stephan, W. (2017). Rapid adaptation of a polygenic trait after a sudden environmental shift. *Genetics*, 206(1), 389–406. https://doi.org/10.1534/genetics.116.196972

Jaramillo-Correa, J.-P., Rodríguez-Quilón, I., Grivet, D., Lepoittevin, C., Sebastiani, F., Heuertz, M., Garnier-Géré, P. H., Alía, R., Plomion, C., Vendramin, G. G., & González-Martínez, S. C. (2015). Molecular proxies for climate maladaptation in a long-lived tree (*Pinus pinaster* Aiton, Pinaceae). *Genetics*, 199(3), 793–807. https://doi.org/10.1534/genetics.114.173252

Josephs, E. B., Berg, J. J., Ross-Ibarra, J., & Coop, G. (2019). Detecting adaptive differentiation in structured populations with genomic data and common gardens. *Genetics*, 211, 989–1004. https://doi.org/10.1101/368506

Kanehisa, M., Sato, Y., & Morishima, K. (2016). BlastKOALA and GhostKOALA: KEGG tools for functional characterization of genome and metagenome sequences. *Journal of Molecular Biology*, 428(4), 726–731. https://doi.org/10.1016/j.jmb.2015.11.006

Keenan, K., McGinnity, P., Cross, T. F., Crozier, W. W., & Prodöhl, P. A. (2013). diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors. *Methods in Ecology and Evolution*, 4(8), 782–788. https://doi.org/10.1111/2041-210X.12067

Krakauer, D. C., & Nowak, M. A. (1999). Evolutionary preservation of redundant duplicated genes. *Seminars in Cell & Developmental Biology*, 10(5), 555–559. https://doi.org/10.1006/scdb.1999.0337

Krutovsky, K. V., & Neale, D. B. (2005). Nucleotide diversity and linkage disequilibrium in cold-hardiness- and wood quality-related candidate genes in Douglas-fir. *Genetics*, 171(4), 2029–2041. https://doi.org/10.1534/genetics.105.044420

Lamy, J.-B., Bouffier, L., Burlett, R., Plomion, C., Cochard, H., & Delzon, S. (2011). Uniform selection as a primary force reducing population genetic differentiation of cavitation resistance across a species range. *PLoS One*, 6(8), e23476. https://doi.org/10.1371/journal.pone.0023476

Latta, R. G. (1998). Differentiation of allelic frequencies at quantitative trait loci affecting locally adaptive traits. *The American Naturalist*, 151(3), 283–292. https://doi.org/10.1086/286119

Le Corre, V., & Kremer, A. (2003). Genetic variability at neutral markers, quantitative trait loci and trait in a subdivided population under selection. *Genetics*, 164(3), 1205–1219. https://doi.org/10.1093/genetics/164.3.1205

Le Corre, V., & Kremer, A. (2012). The genetic differentiation at quantitative trait loci under local adaptation. *Molecular Ecology*, 21(7), 1548–1566. https://doi.org/10.1111/j.1365-294X.2012.05479.x

Leck, M. A., Parker, V. T., & Simpson, R. L. (2008). *Seedling ecology and evolution*. Cambridge University Press.

Lind, B. M., Friedline, C. J., Wegrzyn, J. L., Maloney, P. E., Vogler, D. R., Neale, D. B., & Eckert, A. J. (2017). Water availability drives signatures of local adaptation in whitebark pine (*Pinus albicaulis* Engelm.) across fine spatial scales of the Lake Tahoe Basin, USA. *Molecular Ecology*, 26(12), 3168–3185. https://doi.org/10.1111/mec.14106

Lind, B. M., Menon, M., Bolte, C. E., Faske, T. M., & Eckert, A. J. (2018). The genomics of local adaptation in trees: Are we out of the woods yet? *Tree Genetics & Genomes*, 14(2), 29. https://doi.org/10.1007/s11295-017-1224-y

Lloyd-Jones, L. R., Zeng, J., Sidorenko, J., Yengo, L., Moser, G., Kemper, K. E., Wang, H., Zheng, Z., Magi, R., Esko, T., Metspalu, A., Wray, N. R., Goddard, M. E., Yang, J., & Visscher, P. M. (2019). Improved polygenic prediction by Bayesian multiple regression on summary statistics. *Nature Communications*, 10(1), 1–11. https://doi.org/10.1038/s41467-019-12653-0

Lu, M., Krutovsky, K. V., & Loopstra, C. A. (2019). Predicting adaptive genetic variation of loblolly pine (*Pinus taeda* L.) populations under projected future climates based on multivariate models. *Journal of Heredity*, 110, 857–865. https://doi.org/10.1093/jhered/esz065

Mackay, J., Dean, J. F. D., Plomion, C., Peterson, D. G., Cánovas, F. M., Pavy, N., Ingvarsson, P. K., Savolainen, O., Guevara, M. Á., Fluch, S., Vinceti, B., Abarca, D., Díaz-Sala, C., & Cervera, M.-T. (2012). Towards decoding the conifer giga-genome. *Plant Molecular Biology*, 80(6), 555–569. https://doi.org/10.1007/s11103-012-9961-7

Maher, B. (2008). Personal genomes: The case of the missing heritability. *Nature*, 456, 18–21. https://doi.org/10.1038/456018a

Mähler, N., Wang, J., Terebieniec, B. K., Ingvarsson, P. K., Street, N. R., & Hvidsten, T. R. (2017). Gene co-expression network connectivity is an important determinant of selective constraint. *PLoS Genetics*, 13(4), e1006402. https://doi.org/10.1371/journal.pgen.1006402

Majada, J., Martínez-Alonso, C., Feito, I., Kidelman, A., Aranda, I., & Alía, R. (2011). Mini-cuttings: An effective technique for the propagation of *Pinus pinaster* Ait. *New Forests*, 41(3), 399–412. https://doi.org/10.1007/s11056-010-9232-x

Mayol, M., Riba, M., Cavers, S., Grivet, D., Vincenot, L., Cattonaro, F., Vendramin, G. G., & González-Martínez, S. C. (2020). A multiscale approach to detect selection in non-model tree species: Widespread adaptation despite population decline in *Taxus baccata* L. *Evolutionary Applications*, 13, 143–160. https://doi.org/10.1111/eva.12838

McKay, J. K., & Latta, R. G. (2002). Adaptive population divergence: Markers, QTL and traits. *Trends in Ecology & Evolution*, 17(6), 285–291. https://doi.org/10.1016/S0169-5347(02)02478-3

Mei, W., Stetter, M. G., Gates, D. J., Stitzer, M. C., & Ross-Ibarra, J. (2018). Adaptation in plant genomes: Bigger is different. *American Journal of Botany*, 105(1), 16–19. https://doi.org/10.1002/ajb2.1002

Nakagawa, S., & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: A practical guide for biologists. *Biological Reviews*, 85(4), 935–956. https://doi.org/10.1111/j.1469-185X.2010.00141.x

Neale, D. B., & Savolainen, O. (2004). Association genetics of complex traits in conifers. *Trends in Plant Science*, 9(7), 325–330. https://doi.org/10.1016/j.tplants.2004.05.006

Nowak, M. A., Boerlijst, M. C., Cooke, J., & Smith, J. M. (1997). Evolution of genetic redundancy. *Nature*, 388(6638), 167–171. https://doi.org/10.1038/40618

O'Connor, L. J., Schoech, A. P., Hormozdiari, F., Gazal, S., Patterson, N., & Price, A. L. (2019). Extreme polygenicity of complex traits is explained by negative selection. *The American Journal of Human Genetics*, 105(3), 456–476. https://doi.org/10.1016/j.ajhg.2019.07.003

Orr, H. A., & Coyne, J. A. (1992). The genetics of adaptation: A reassessment. *The American Naturalist*, 140(5), 725–742. https://doi.org/10.1086/285437

Palmé, A. E., Pyhäjärvi, T., Wachowiak, W., & Savolainen, O. (2009). Selection on nuclear genes in a *Pinus* phylogeny. *Molecular Biology and Evolution*, 26(4), 893–905. https://doi.org/10.1093/molbev/msp010

Plomion, C., Bartholomé, J., Bouffier, L., Brendel, O., Cochard, H., De Miguel, M., Delzon, S., Gion, J. M., González-Martínez, S. C., Guehl, J. M., Lagraulet, H., Le Provost, G., Marguerit, E., & Porté, A. (2016). Understanding the genetic bases of adaptation to soil water deficit in trees through the examination of water use efficiency and cavitation resistance: Maritime pine as a case study. *Journal of Plant Hydraulics*, 3, e008. https://doi.org/10.20870/jph.2016.e008

Plomion, C., Bartholomé, J., Lesur, I., Boury, C., Rodríguez-Quilón, I., Lagraulet, H., Ehrenmann, F., Bouffier, L., Gion, J. M., Grivet, D., de Miguel, M., de María, N., Cervera, M. T., Bagnoli, F., Isik, F., Vendramin, G. G., & González-Martínez, S. C. (2016). High-density SNP assay development for genetic analysis in maritime pine (*Pinus pinaster*). *Molecular Ecology Resources*, 16(2), 574–587. https://doi.org/10.1111/1755-0998.12464

Pritchard, J. K., Pickrell, J. K., & Coop, G. (2010). The genetics of human adaptation: Hard sweeps, soft sweeps, and polygenic adaptation. *Current Biology*, 20(4), 208–215. https://doi.org/10.1016/j.cub.2009.11.055

Pritchard, J. K., & Rienzo, A. D. (2010). Adaptation – Not by sweeps alone. *Nature Reviews Genetics*, 11(10), 665–667. https://doi.org/10.1038/nrg2880

R Core Team (2019). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Rellstab, C., Zoller, S., Walthert, L., Lesur, I., Pluess, A. R., Graf, R., Bodénès, C., Sperisen, C., Kremer, A., & Gugerli, F. (2016). Signatures of local adaptation in candidate genes of oaks (*Quercus* spp.) with respect to present and future climatic conditions. *Molecular Ecology*, 25(23), 5907–5924. https://doi.org/10.1111/mec.13889

Robinson, G. K. (1991). That BLUP is a good thing: The estimation of random effects. *Statistical Science*, 6(1), 15–32. https://doi.org/10.1214/ss/1177011926

Rodríguez-Quilón, I., Santos-del-Blanco, L., Serra-Varela, M. J., Koskela, J., González-Martínez, S. C., & Alía, R. (2016). Capturing neutral and adaptive genetic diversity for conservation in a highly structured tree species. *Ecological Applications*, 26(7), 2254–2266. https://doi.org/10.1002/eap.1361

Savolainen, O., Lascoux, M., & Merilä, J. (2013). Ecological genomics of local adaptation. *Nature Reviews Genetics*, 14(11), 807–820. https://doi.org/10.1038/nrg3522

Savolainen, O., Pyhäjärvi, T., & Knürr, T. (2007). Gene flow and local adaptation in trees. *Annual Review of Ecology, Evolution, and Systematics*, 38(1), 595–619. https://doi.org/10.1146/annurev.ecolsys.38.091206.095646

Sefton, C. A., Montagu, K., Atwell, B. J., & Conroy, J. P. (2002). Anatomical variation in juvenile eucalypt leaves accounts for differences in specific leaf area and CO_2 assimilation rates. *Australian Journal of Botany*, 50(3), 301–310. https://doi.org/10.1071/bt01059

Sella, G., & Barton, N. H. (2019). Thinking about the evolution of complex traits in the era of genome-wide association studies. *Annual Review of Genomics and Human Genetics*, 20(1), 461–493. https://doi.org/10.1146/annurev-genom-083115-022316

Smith, J. M., & Haigh, J. (1974). The hitch-hiking effect of a favourable gene. *Genetics Research*, 23(1), 23–35. https://doi.org/10.1017/S0016672300014634

Sohail, M., Maier, R. M., Ganna, A., Bloemendal, A., Martin, A. R., Turchin, M. C., Chiang, C. W., Hirschhorn, J., Daly, M. J., Patterson, N., Neale, B., Mathieson, I., Reich, D., & Sunyaev, S. R. (2019). Polygenic adaptation on height is overestimated due to uncorrected stratification in genome-wide association studies. *eLife*, 8, e39702. https://doi.org/10.7554/eLife.39702

Spitze, K. (1993). Population structure in *Daphnia obtusa*: Quantitative genetic and allozymic variation. *Genetics*, *135*, 367–374. https://doi.org/10.1093/genetics/135.2.367

Stephan, W. (2016). Signatures of positive selection: From selective sweeps at individual loci to subtle allele frequency changes in polygenic adaptation. *Molecular Ecology*, *25*(1), 79–88. https://doi.org/10.1111/mec.13288

Torres-Ruiz, J. M., Kremer, A., Carins-Murphy, M. R., Brodribb, T. J., Lamarque, L. J., Truffaut, L., Bonne, F., Ducousso, A., & Delzon, S. (2019). Genetic differentiation in functional traits among European sessile oak populations. *Tree Physiology*, *39*, 1736–1749. https://doi.org/10.1093/treephys/tpz090

Turchin, M. C., Chiang, C. W., Palmer, C. D., Sankararaman, S., Reich, D., & Hirschhorn, J. N. (2012). Evidence of widespread selection on standing variation in Europe at height-associated SNPs. *Nature Genetics*, *44*(9), 1015–1019. https://doi.org/10.1038/ng.2368

Valladares, F., Matesanz, S., Guilhaumon, F., Araújo, M. B., Balaguer, L., Benito-Garzón, M., Cornwell, W., Gianoli, E., van Kleunen, M., Naya, D. E., Nicotra, A. B., Poorter, H., & Zavala, M. A. (2014). The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecology Letters*, *17*(11), 1351–1364. https://doi.org/10.1111/ele.12348

Visscher, P. M., Hill, W. G., & Wray, N. R. (2008). Heritability in the genomics era—Concepts and misconceptions. *Nature Reviews Genetics*, *9*(4), 255–266. https://doi.org/10.1038/nrg2322

Vizcaíno-Palomar, N., Fady, B., Alía, R., Raffin, A., Mutke, S., & Garzón, M. B. (2019). Patterns of phenotypic plasticity among populations of three Mediterranean pine species and implications for evolutionary responses to climate change. *Science of the Total Environment*, *749*, 141454. https://doi.org/10.1101/716084

Walker, X. J., Mack, M. C., & Johnstone, J. F. (2015). Stable carbon isotope analysis reveals widespread drought stress in boreal black spruce forests. *Global Change Biology*, *21*(8), 3102–3113. https://doi.org/10.1111/gcb.12893

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution*, *38*(6), 1358–1370. https://doi.org/10.1111/j.1558-5646.1984.tb05657.x

Whitlock, M. C., & Guillaume, F. (2009). Testing for spatially divergent selection: Comparing Q_{ST} to F_{ST}. *Genetics*, *183*(3), 1055–1063. https://doi.org/10.1534/genetics.108.099812

Wisser, R. J., Fang, Z., Holland, J. B., Teixeira, J. E. C., Dougherty, J., Weldekidan, T., de Leon, N., Flint-Garcia, S., Lauter, N., Murray, S. C., Xu, W., & Hallauer, A. (2019). The genomic basis for short-term evolution of environmental adaptation in maize. *Genetics*, *213*(4), 1479–1494. https://doi.org/10.1534/genetics.119.302780

Wright, S. I., & Andolfatto, P. (2008). The impact of natural selection on the genome: Emerging patterns in *Drosophila* and *Arabidopsis*. *Annual Review of Ecology, Evolution, and Systematics*, *39*(1), 193–213. https://doi.org/10.1146/annurev.ecolsys.39.110707.173342

Yang, J., Benyamin, B., McEvoy, B. P., Gordon, S., Henders, A. K., Nyholt, D. R., Madden, P. A., Heath, A. C., Martin, N. G., Montgomery, G. W., Goddard, M. E., & Visscher, P. M. (2010). Common SNPs explain a large proportion of the heritability for human height. *Nature Genetics*, *42*(7), 565–569. https://doi.org/10.1038/ng.608

Young, A. I., & Flint, J. (2019). Solving the missing heritability problem. *PLoS Genetics*, *15*(6), e1008222. https://doi.org/10.1371/journal.pgen.1008222

Zeng, J., de Vlaming, R., Wu, Y., Robinson, M. R., Lloyd-Jones, L. R., Yengo, L., Yap, C. X., Xue, A., Sidorenko, J., McRae, A. F., Powell, J. E., Montgomery, G. W., Metspalu, A., Esko, T., Gibson, G., Wray, N. R., Visscher, P. M., & Yang, J. (2018). Signatures of negative selection in the genetic architecture of human complex traits. *Nature Genetics*, *50*(5), 746–753. https://doi.org/10.1038/s41588-018-0101-4

Zonneveld, B. J. M. (2012). Conifer genome sizes of 172 species, covering 64 of 67 genera, range from 8 to 72 picogram. *Nordic Journal of Botany*, *30*(4), 490–502. https://doi.org/10.1111/j.1756-1051.2012.01516.x

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.