Genetic basis of susceptibility to *Diplodia sapinea* and *Armillaria ostoyae* in maritime pine

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Summary

- Forest ecosystems are increasingly challenged by extreme events, e.g. pest and pathogen outbreaks, causing severe ecological and economical losses. Understanding the genetic basis of adaptive traits in tree species is of key importance to preserve forest ecosystems
- Adaptive phenotypes, including susceptibility to two fungal pathogens (*Diplodia sapinea* and *Armillaria ostoyae*) and an insect pest (*Thaumetopoea pityocampa*), height and needle phenology were assessed in a range-wide common garden of maritime pine (*Pinus pinaster* Aiton), a widespread conifer in the western Mediterranean Basin and parts of the Atlantic coast.
- Broad-sense heritability was significant for height (0.497), needle phenology (0.231-0.468) and pathogen symptoms (0.413 for *D. sapinea* and 0.066 for *A. ostoyae*) measured after inoculation under controlled conditions, but not for pine processionary moth incidence assessed in the common garden. Genetic correlations between traits revealed contrasting trends for pathogen susceptibility to *D. sapinea* and *A. ostoyae*. Maritime pine populations from areas with high summer temperatures and frequent droughts were less susceptible to *D. sapinea* but more susceptible to *A. ostoyae*. An association study using 4,227 genome-wide SNPs revealed several loci significantly associated to each trait.
- This study provides important insights to develop genetic conservation and breeding strategies integrating tree responses to pathogens.

Key words: *Armillaria ostoyae*, association genetics, *Diplodia sapinea*, genetic correlations, height, heritability, needle phenology, pathogen susceptibility, *Pinus pinaster*

Introduction

Forest ecosystems are challenged worldwide by changing environmental conditions (Turner, 2010). Warmer and drier climates are expected to increase the risks of fire, droughts and insect outbreaks while warmer and wetter climates will probably increase storm and pathogen incidence on forests (Petr *et al.*, 2017), leading to episodes of high tree mortality (Castro *et al.*, 2009) and in consequence severe economic losses (Hanewinkel *et al.*, 2013). Changing environmental conditions can also cause range shifts in previously locally restricted pests and pathogens or shifts to increased pathogenicity (Desprez-Loustau *et al.*, 2006). Thus, understanding the variability in disease response and the genetic basis of adaptive traits related to biotic and abiotic factors in tree species is crucial to develop informed restoration, conservation and management strategies. Additionally, knowledge about genes underlying adaptive traits can also serve tree breeding and increase forest productivity, e.g. targeting resistance to drought or against pests and pathogens in forest plantations (Neale & Kremer, 2011).

Forest trees are long-lived organisms characterized by mainly outcrossing mating systems, high standing genetic variation, large effective population sizes, and the production of vast numbers of seeds and seedlings exposed to strong selection (Petit *et al.*, 2004; Petit & Hampe, 2006). High genetic and phenotypic differentiation has been observed in tree species along environmental gradients (e.g. Savolainen *et al.*, 2007, 2013) or between contrasting habitats, indicating local adaptation (e.g. Lind *et al*., 2017). Common garden experiments (i.e. experiments evaluating trees from a wide range of populations under the same environmental conditions) provide valuable insights in the phenotypic and genotypic variation of tree species (Morgenstern, 2011). They have revealed genetic differentiation for adaptive traits (such as flushing, senescence or growth) along latitudinal and altitudinal gradients (Mimura & Aitken, 2007; Delzon *et al.*, 2009). Geographical variation can also be found for disease resistance against certain pests (Menéndez-Gutiérrez *et al.*, 2017) and pathogens (e.g. Hamilton *et al*., 2013; Freeman *et al*., 2019). Phenological traits, such as flowering or leaf flushing time and autumn leaf senescence are sometimes genetically correlated with disease resistance in forest trees and can give hints about resistance or avoidance mechanisms (Elzinga *et al.*, 2007).

Disease resistance is generally thought to be the result of selective pressures exerted by the pathogen, in areas where host and pathogen have co-existed during considerable periods of time, under the co-evolution hypothesis (e.g. Burdon and Thrall, 2000; Ennos, 2015). In this line, geographical variation in disease resistance has been interpreted in some cases as a result of past heterogeneous pathogen pressures within the range of a host species (Ennos, 2015; Perry *et al.*, 2016). However, the past distribution of pathogen species is often unknown (Desprez-Loustau *et al.*, 2016), therefore, other processes than co-evolution, such as “ecological fitting” or “exaptation” should not be excluded (Agosta & Klemens, 2008). These biological processes have been suggested when, for example, variability in disease resistance has been observed in tree species with no co-evolutionary history with

a pathogen (Leimu & Koricheva, 2006; Freeman *et al.*, 2019). Such resistance may have evolved in response to other pathogens but shows broad-range efficacy, even to a novel pathogen. Generic mechanisms of resistance in conifers include the production of large amounts of non-volatile compounds (resin acids) that can act as mechanical barriers to infections (Shain, 1967; Phillips & Croteau, 1999) and volatile compounds (such as monoterpenes or phenols) that can be toxic to fungi (Cobb *et al.*, 1968; Rishbeth, 2006). The composition of secondary metabolites can show marked differences between trees with distinct geographic origins (Meijón *et al.*, 2016). The evolution of plant defences against biotic stressors can also be shaped by differences in resource availability and environmental constraints throughout the host's species distribution. Depending on resource availability plants have evolved distinct strategies by investing either more in growth, to increase competition ability, or more in chemical and structural defences, to better respond to herbivores and pathogens (Herms & Mattson, 2004). Typically, faster growing trees invest more in inducible defences while slow growing trees invest more in constitutive defences (Moreira *et al.*, 2014).

Many quantitative traits in forest species, including disease resistance, show significant heritability and often stronger differentiation (Q_{ST}) between populations than neutral genetic markers (F_{ST}) (Hamilton *et al.*, 2013; Lind *et al.*, 2018). Major resistance genes against certain forest pathogens have been identified (e.g. Kuhlman *et al.*, 2002; Sniezko, 2010) but most adaptive traits have a highly polygenic basis of quantitative inheritance, typically involving many loci with rather small effects (Goldfarb *et al.*, 2013; de la Torre *et al.*, 2019). The identification of genes underlying adaptive traits in forest trees is becoming more feasible, with the increasing availability of genetic and genomic markers. Many association genetic studies in forest tree species focused on wood property and growth traits to assist tree breeding (e.g. Pot *et al.*, 2005; Neale *et al.*, 2006; Beaulieu *et al.*, 2011). Also, loci associated to other ecologically important traits, such as cold hardiness (e.g. Eckert *et al.*, 2009; Holliday *et al.*, 2010), drought tolerance (reviewed in Moran *et al.*, 2017) or disease resistance (e.g. Liu *et al.*, 2014; Resende *et al.*, 2017) have been suggested based on this approach. However, association studies addressing biotic interaction traits, including responses to pests and pathogens, are still scarce.

Our study focused on maritime pine (*Pinus pinaster* Aiton), a long-lived conifer with a highly fragmented natural range in the western Mediterranean Basin, the Atlantic coasts of southern France and of the Iberian Peninsula. The species has a wide ecological amplitude and grows from sea level to 2000 m altitude. Genetic diversity in natural populations of maritime pine is high (Salvador *et al.*, 2000; Bucci *et al.*, 2007) and strongly structured (Petit *et al.*, 1995; Jaramillo-Correa *et al.*, 2015). In addition, traits, such as stem form, height (González-Martínez *et al.*, 2002), metabolite content (Meijón *et al.*, 2016), drought (Aranda *et al.*, 2010; Gaspar *et al.*, 2013) and disease resistance (Desprez-Loustau & Baradat, 1991; Burban *et al.*, 1999; Elvira-Recuenco *et al.*, 2014), are highly variable in maritime pine and often strongly differentiated between geographic provenances. Maritime pine has

also been widely planted and is currently exploited for timber and paper, covering ~0.8 million ha in the Landes region in southwestern France, one of the largest plantation forests in Europe (Labbé *et al.*, 2015). Despite the ecological and economical importance of maritime pine, only a few genetic association studies have been developed in this species. Lepoittevin *et al.*, (2012) identified two loci associated to growth and wood cellulose content, respectively, Cabezas *et al.*, (2015) revealed four SNPs in *korrigan* (gene ortholog to an Arabidopsis degrading enzyme cellulase) also as significantly associated to growth traits (total height and polycyclism) and Bartholomé *et al.*, (2016) reported four loci for stem straightness and three loci for height growth. Budde *et al.*, (2014) were able to predict 29% of the phenotypic variation in a fire adaptive trait (proportion of serotinous cones) in eastern Spain based on 17 significantly associated loci. However, none of these studies targeted biotic interaction traits, such as disease resistance.

In our study, we assessed susceptibility to pests/pathogens, height and needle phenology (bud burst and duration of bud burst) in a clonal common garden (CLONAPIN, planted in Cestas, southwestern France), which allowed us to explore variation in disease response and genetic correlations with other traits in range-wide populations of maritime pine. Considering disease and growth traits together is relevant from an evolutionary and ecological perspective, and can also have important implications in terms of management, especially in breeding programs. We selected three important disease agents: two fungal pathogens, *Diplodia sapinea* (Botryosphaeriaceae) and *Armillaria ostoyae* (Physalacriaceae), as well as the pine processionary moth, *Thaumetopoea pityocampa* (Thaumetopoeidae), a main defoliator of pine forests.

Diplodia sapinea is the causal agent of several diseases, such as tip-blight, canker or root collar necrosis in needles, shoots, stems and roots of conifers, eventually leading to mortality in case of severe attacks (Piou *et al.*, 1991; Luchi *et al.*, 2014). The pathogenicity of *D. sapinea* is associated to environmental conditions. It can remain in an endophytic form, i.e. without causing any symptoms, until stressful environmental conditions, such as drought (Stanosz *et al.*, 2002; Desprez-Loustau *et al.*, 2006), hail storms (Zwolinski *et al.*, 1990), or changes in the nitrogen concentration of the soil (Piou *et al.*, 1991; Stanosz *et al.*, 2004) weaken the host and trigger *D. sapinea* pathogenicity. Trees from all ages are affected (Chou, 1978; Georgieva & Hlebarska, 2017), though seedlings and old trees show increased susceptibility (Swart & Wingfield, 1991). The fungus can be found in many conifers, especially in the genus *Pinus* and *P. pinaster* was classified as moderately susceptible by Iturritxa *et al.*, (2013). The species was first described in Europe in 1823 (synonym *Sphaeria sapinea*; Piou *et al.*, 1991) and recent surveys showed that *D. sapinea* is currently very broadly distributed in all pine forests throughout the world, though its origin is unknown (Burgess *et al.*, 2004; Brodde *et al.*, 2019). Serious damage associated with *D. sapinea* in Europe has only been reported in the last decades but it may become a serious threat to pine forests, as climate change will certainly favor pathogen activity by increasing temperature and the frequency and intensity of drought events (Woolhouse *et al.*, 2005;

Desprez-Loustau *et al.*, 2006; Boutte, 2018). Recent outbreaks associated with *D. sapinea* in northern Europe suggest an ongoing northward expansion (Brodde *et al.*, 2019).

Armillaria ostoyae is a root pathogen that causes white rot and butt rot disease in conifers, leading to growth deprivation, high mortality and major losses in timber wood, hence, its economic importance (Lung-Escarmant & Guyon, 2004; Heinzelmann *et al.*, 2018). The species can be traced back to six millions years ago, both in Eurasia and North-America (Tsykun *et al.*, 2013; Koch *et al.*, 2017). *Armillaria ostoyae* has been reported in all the coniferous forests of the Northern Hemisphere but it is replaced by *A. mellea* (Marxmüller & Guillaumin, 2005) in the Mediterranean as its distribution is limited by high temperatures and drought. It is likely that *A. ostoyae* would have co-existed for a long time with maritime pine in Europe (Labbé *et al.*, 2017a) and consequently has been affected by the same extinction-recolonization events associated to past climatic changes. It is one of the most common fungal species in maritime pine forests, and it is particularly dangerous, as it can act as a parasite and saprophyte (Cruickshank *et al.*, 1997; Labbé *et al.*, 2017b), i.e. the death of its host does not prevent its spread. In maritime pine, the severity of the symptoms is related with the age of its host, with higher mortality in young trees (Lung-Escarmant *et al.*, 2002; Lung-Escarmant & Guyon, 2004). Climate change is predicted to increase the impact of *A. ostoyae* on conifer forests in the coming years (Kubiak *et al.*, 2017).

Thaumetopoea pityocampa, the pine processionary, is considered the most severe defoliator insect in pine forests in southern Europe and northern Africa (Jactel *et al.*, 2015) and can lead to severe growth loss (Jacquet *et al.*, 2013). The species typically reproduces in summer followed by larval development during autumn and winter. Caterpillars and moths of *T. pityocampa* are sensitive to climatic and environmental conditions and the pine processionary moth is expected to expand its range following events of climate warming (Battisti *et al.*, 2006; Toïgo *et al.*, 2017).

The specific objectives of our study are to 1) estimate genetic variability and heritability within and among range-wide populations of maritime pine for pathogen/pest-related traits, height and needle phenology, 2) test for adaptive divergence across the maritime pine range for these traits (i.e. Q_{ST} vs. F_{ST} approach); 3) analyze the genetic correlations between these traits that could be useful for conservation and breeding programs; and 4) identify loci associated to disease-related, growth and phenology traits by a genotype-phenotype association approach.

Material and Methods

Plant material and common garden measurements

A clonal common garden (CLONAPIN) was planted in 2011 in Cestas, southwestern France (for details see Rodríguez-Quilón, 2017). It includes trees from 35 populations of maritime pine covering

the whole species distribution (see Table **S1.1**, Supporting Information for number of individuals, genotypes, and population coordinates of 33 populations included in this study), representing all differentiated gene pools (French Atlantic, Iberian Atlantic, Corsica, Central Spain, Southeastern Spain, and Morocco; see Jaramillo-Correa *et al.*, 2015). The common garden design consisted of eight randomized complete blocks, with one clonal copy (ramet) of each genotype replicated in each block.

Height, bud burst, duration of bud burst, and incidence of processionary moth were measured in all individuals from 5-8 blocks, depending on the trait (sample size of 1,440-3,330 trees, see Table **S1.1**, Supporting Information). Tree height was measured in 2015, four years after the establishment of the trial. Bud burst stage was evaluated using a phenological scale ranging from 0 (bud without elongation during winter) to 5 (total elongation of the needles; see Fig. **S2.1**, Supporting Information). The Julian day of entry in each stage was scored for each tree. Julian days were converted into accumulated degree-days (0°C basis) 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 defines the duration of bud burst. Both needle phenology phenotypes, bud burst and duration of bud burst, were assessed in 2015 and 2017. The presence or absence of pine processionary moth nests in the tree crowns was assessed in March 2018.

Experimental evaluation of susceptibility to Diplodia sapinea

For *D. sapinea* inoculations, we used the pathogen strain Pier4, isolated from *P. nigra* cones in Pierroton, France (May 2017) and maintained on malt-agar medium. The identity of this strain as *D. sapinea*, was confirmed by its ITS sequence (ITS1-F and ITS4 see Gardes and Bruns 1993), and blasting against the NCBI nucleotide data base (Benoît Laurent, personal communication).
We sampled a total of 453 branches, from 151 genotypes (i.e. one branch from each of three replicate trees per genotype) in the CLONAPIN common garden representing all differentiated maritime pine gene pools. On several days between June 12th and July 31st 2018, one lateral branch per tree was cut from 30 randomly selected trees and taken to the laboratory for inoculation. Inoculations were carried out on the current year leader shoot (phenological stage 3 to 5, see Supporting Information S2.1) of excised branches (for a detailed laboratory protocol see Supporting Information S3.1).
For the inoculation, we removed a needle fascicle in the middle of each shoot with a scalpel and placed an infected malt-agar plug (5 mm diameter) on the wound and wrapped it in cellophane. For control shoots, plugs of sterile rather than colonized malt-agar were used. The shoots were put in water and kept in a climatic chamber set at 20°C with a daily cycle of 12h of light and 12h of dark (Blodgett & Bonello, 2003; Iturritxa *et al.*, 2013). Six days after the inoculation, we removed the cellophane and measured the lesion length around the inoculation point with a caliper. Needle discoloration was assessed using a scale from 0: no discoloration to 3: all needles along the necrosis showed discoloration (see Fig. **S3.1**, Supporting Information).

Experimental evaluation of susceptibility to Armillaria ostoyae

For the inoculation with *A. ostoyae*, we used the pathogen strain A4, collected from a dying maritime pine tree in La Teste (Gironde, France) in 2010 (Labbé *et al.*, 2017b). For the experiment, mycelium culture was prepared in 180 ml plastic jars filled with a mixture of industrial vegetable soup, malted water and hazelnut wood chips and two plugs of 5 mm diameter of malt agar with *A. ostoyae* mycelium on top (Heinzelmann & Rigling, 2016; for a detailed laboratory protocol see Supporting Information S3.2). The lid was closed loosely enough to allow some oxygen flow. The jars were placed in a heat chamber set at 23°C and 80% humidity during three month before inoculation.

We randomly sampled 10 maritime pine genotypes for each of the six differentiated gene pools represented in the CLONAPIN common garden. Fully elongated current year shoots were selected (bud stage 4 and 5) with a minimum diameter of 250 mm and a minimum length of 10 cm. A total of 180 branches from 60 genotypes (i.e. one branch from each of three replicate trees per genotype) were measured, cut and taken to the laboratory to be inoculated, on October 3rd-4th 2018.

The basal part of the shoots (ca. 8 cm) was placed in the center of the jars with mycelial culture in the heat chamber, maintaining the same temperature and humidity settings as for the mycelium growth, but adding an additional 12h cycle of light/dark. After 3 weeks, inoculation success was evaluated visually by confirming the presence of mycelium under the bark. The length of the colonizing mycelium and length of the lesion in the sapwood (i.e. wood browning, hereafter referred to as necrosis) were measured. In the jar, we visually evaluated the level of humidity (dry, medium and very humid) and *A. ostoyae* growth. Controls were prepared in the exact same manner, but with plugs of sterile malt-agar as opposed to those colonized by *A. ostoyae*.

Climatic Data

Summary climate data for the years 1950–2000 were retrieved for 32 variables from Worldclim (Hijmans *et al.*, 2005) and a regional climatic model (Gonzalo, 2007) for the 11 non-Spanish and the 22 Spanish populations, respectively. Climate variables included monthly mean, highest, and lowest temperatures and mean monthly precipitation. Gonzalo's (2007) model was favored for climate data in Spain because it considers a much denser network of meteorological stations than Worldclim, which is known to underperform in this region (see Jaramillo-Correa *et al.*, 2015).

DNA extraction and SNP genotyping

Needles were collected from one replicate per genotype (N=416, including all genotypes used for pathogen susceptibility assays) and desiccated using silica gel. Genomic DNA was extracted using the Invisorb® DNA Plant HTS 96 Kit/C kit (Invitek GmbH, Berlin, Germany). An Illumina Infinium SNP array developed by Plomion *et al.* (2016), comprising potentially neutral and adaptive genetic polymorphisms, was used for genotyping. After standard filtering followed by removal of SNPs with

uncertain clustering patterns (visual inspection using *GenomeStudio v. 2.0*), we kept 5,176 polymorphic SNPs, including 4,227 SNPs with a minor allele frequency (MAF) above 0.1.

Quantitative genetic analyses

To estimate the genetic variance components of the analyzed traits, we fitted the following mixed-effect model:

$$y_{ijk} = \mu + block_i + cov + pop_j + pop(genotype)_{jk} + \varepsilon_{ijk} \quad (1)$$

where for any trait y, μ denotes de overall phenotypic mean, $block_i$ represents the fixed effect of experimental block i, pop_j is the random effect of population j, $pop(genotype)_{jk}$ denotes the random effect of genotype k nested within population j, ε is the residual effect and *cov* represents the covariates that were only implemented when modeling the presence of pine processionary moth nests (i.e. tree height in 2015) and necrosis caused by *A. ostoyae* (i.e. a categorical evaluation of jar humidity).

All models were fitted in a Bayesian framework using Markov chain Monte Carlo (MCMC) methods implemented in the R package *MCMCglmm* (Hadfield, 2010; see Supporting Information S4.1 and Table **S4.1** for further details on model specifications).

Variance components were then used to compute broad-sense heritability either including the population random effect (H_p^2) or not (H^2) :

$$H^2 = \frac{\sigma^2_{genotype}}{(\sigma^2_{genotype}+\sigma^2_{pop}+\sigma^2_e)} \quad (2)$$

$$H_p^2 = \frac{\sigma^2_{genotype}+\sigma^2_{pop}}{(\sigma^2_{genotype}+\sigma^2_{pop}+\sigma^2_e)} \quad (3)$$

where $\sigma^2_{genotype}$ is the variance among genotypes within populations, σ^2_{pop} is the variance between populations and σ^2_e the residual variance. When appropriate, we included an extra term in the denominator to account for implicit *logit* and *probit* link function variance ($\pi^2/3$ and +1, respectively; Nakagawa and Schielzeth 2010). We also estimated the evolvability, defined as the genotype plus population variances to phenotypic mean ratio for each trait, which represents the ability of a population/genetic group to respond to selection on a certain trait (Houle, 1992). Genetic differentiation among populations for the analyzed traits was calculated as (Spitze, 1993).

$$Q_{ST} = \frac{\sigma^2_{pop}}{\sigma^2_{pop}+2\sigma^2_{genotype}} \quad (4)$$

Additionally, we estimated the global F_{ST} using all available SNP genotypes in SPAGeDi 1.5 (Hardy & Vekemans, 2002). The difference between global F_{ST} and Q_{ST} values for each adaptive trait was considered significant when the 95% confidence intervals (CI) did not overlap. Genetic correlations between traits were calculated with the Pearson's coefficient of correlation using the Best Linear

Unbiased Predictors (BLUPs) of the combined population and genotype effects (Henderson, 1973; Robinson, 1991) for each trait. Finally, climate and environmental correlations were performed at the population level (using population BLUPs).

Genetic association of SNPs with growth, needle phenology and susceptibility to pathogens

We used a mixed linear regression approach (MLM, Yu *et al.*, 2006) implemented in Tassel v. 5.0 (Bradbury *et al.*, 2007) to identify single SNPs associated to each of the phenotypes (combining population and genotype BLUPs). Ancestry proportions of each sample were computed using STRUCTURE 2.3 (Pritchard *et al*., 2000) for K=6 representing the six maritime pine gene pools (see Jaramillo-Correa *et al*., 2015). These ancestry proportions were included as covariates in the MLM. A covariance matrix accounting for relatedness between all sample pairs was estimated using Loiselle's kinship coefficient (Loiselle *et al.*, 1995) in SPAGeDi 1.5 and was included as random effect. Negative kinship values were set to zero following Yu *et al.* (2006). Only loci with a P-value below 0.005 in the Tassel analyses and with a minimum allele frequency above 0.1 were used for further analyses. We used a Bayesian mixed-effect association approach (Bayesian Association with Missing Data, BAMD; Quesada *et al.*, 2010; Li *et al.*, 2012) in R v.3.4.1 (R Core Team, 2017) to estimate single-locus allelic effects under three genetic models accounting for additive, over-dominance and dominance effects (as in Budde *et al.*, 2014). The STRUCTURE ancestry proportions were used as covariates and the relatedness matrix as random factor. Mean allelic effects (γ) and 95% confidence intervals were obtained from the distribution of the last 20,000 iterations (50,000 in total). Only those SNPs with confidence intervals not overlapping zero were considered to have a significant (non-zero) effect on the trait. Functional annotations, SNP motives, linkage groups and blast results were retrieved from Plomion *et al.*, (2016).

Results

Phenotypic variability, heritability and genetic differentiation

Most traits showed strong differences among populations, whereas the intra-population variation (genotype effect) was smaller, as indicated by lower broad-sense heritability of the genotype variance (Table **1**). Thus, we will base all results and interpretations on the BLUPs that combine the population and genotype effects if not otherwise indicated. Heritability was strongest for height (H^2_p: 0.497, CI [0.398-0.576]) (Table **1**). The highest trees were found in populations from the Atlantic French, Atlantic Iberian and Corsican gene pools, whereas the smallest trees originated from southeastern Spain and Morocco (Fig. **S5.1**, Supporting Information). Heritability of susceptibility to *D. sapinea*, assessed as the necrosis length, was not significant at the genotype level (H^2: 0.096, CI [0.000-0.186]), but was higher and significant when the population effect was taken into account (H^2_p: 0.413 [0.248-

0.675]). The trees from northern Africa and southern Spain showed shorter necrosis length than trees from Atlantic populations (Fig. **1a**). Heritability of needle discoloration caused by *D. sapinea* was lower, but still significant (H^2_p: 0.175 [0.040-0.345]). Necrosis length caused by *A. ostoyae* was also significantly heritable (H^2_p: 0.066 [0.018-0.203]) and indicated more damage in southern populations, especially in Morocco and southern Spain and less damage in northern populations, especially in populations from the French Atlantic gene pool (Fig. **1b**). The importance of the population effect in several traits was also highlighted by high Q_{ST} values indicating strong population differentiation (Table **1**). Global F_{ST} calculated using the available SNPs was 0.109 ([0.0129; 0.3247], p-value < 0.001) which is significantly lower than the Q_{ST} estimates obtained for height and necrosis length caused by *D. sapinea* (Table **1**). The evolvability was highest for height and lowest for the necrosis length caused by *A. ostoyae*.

Correlations between traits and with environmental variables

The genetic correlation (including the population and genotype effect) between necrosis length caused by each of the two fungal pathogens was negative (-0.692, p-value<0.001; Table **2**, Fig. **2**). We also observed significant genetic correlations with height, negative for necrosis length caused by *A. ostoyae* (-0,653, p-value<0.001) and positive for necrosis length caused by *D. sapinea* (0.679, p-value<0.001). However, genetic correlations for height and necrosis length of the two pathogens at the genotype level were not significant (see Table **S5.1**, Supporting Information). Furthermore, susceptibility to *D. sapinea* indicated by necrosis length was positively correlated with precipitation in winter (0.741, p-value=0.028 for precipitation in January) and negatively with mean and maximum temperatures in the summer months (-0.827, p-value=0.008 for mean temperature in July and -0.780, p-value= 0.0165 for maximum temperature in July) in the population of origin (Table **3**, Fig. **3**). A similar effect was found for needle discoloration although the correlations were less strong.

Genotype-phenotype associations

Between three and 26 SNPs were significantly associated with each of the phenotypic traits evaluated under different genotype effect models (see Table **S6.1**, Supporting Information). Here we only report the SNPs that were significant under the additive genetic model, this model being built on three genotypic classes and therefore considered the most robust. Based on this model, seven SNPs were associated to height, 37 SNPs were associated with needle phenology (considering altogether the different phenology traits and measurement years), and eight with pathogen susceptibility (Table **4**). In total, four significantly associated SNPs showed non-synonymous changes. Two non-synonymous SNPs were significantly associated to bud burst in 2017 (Fig. **S6.1**, Supporting Information) and one non-synonymous SNP was associated to each needle discoloration caused by *D. sapinea* and duration of bud burst in 2015 (Table **5**, Fig. **4** and Fig. **S6.2**, Supporting Information). All the remaining SNPs associated under the additive model were either non-coding or the effect of the substitution was

unknown (Table **S6.1**, Supporting Information). The allele frequency distribution of the associated SNPs was quite variable and did not reflect the population genetic structure of the species (Fig. **5**).

Discussion

In the current context of climate change, understanding the genetic basis of adaptive traits in tree species is key for an informed forest management. In this study, we assessed genetic variation in maritime pine for response to two pathogenic fungi, *D. sapinea* and *A. ostoyae*, at the range-wide scale, using trees grown in a clonal common garden and a novel inoculation protocol based on excised branches. Broad-sense heritability of pine susceptibility (necrosis length), both across and within populations, was estimated for the first time for the two diseases. We found a strong population effect for height, needle phenology and infection-related traits. Variation of susceptibility between geographical provenances, as well as height, followed a latitudinal gradient, corresponding to a climatic gradient, but in opposite direction for the two pathogens. Genetic associations revealed several loci significantly associated to pathogen susceptibility, height and phenological traits in maritime pine. The presence of pine processionary moth nests evaluated in the common garden was not heritable but future studies should consider the level of infestation or damage quantitatively.

Genetic and climate related correlations

Genetic correlations between susceptibility to *D. sapinea* and *A. ostoyae*, height and needle phenology possibly indicated similar climate factors and environmental clines driving differentiation at these traits. Especially, maximum temperatures during summer months and precipitation at the end of the summer or in winter showed significant correlations with genetic variability of phenotypic traits across maritime pine populations. Trees from populations with low winter precipitation and high maximum summer temperatures were less susceptible to *D. sapinea*. This result can be interpreted in different ways: 1) If we assume that *D. sapinea* is native in Europe, the pathogen pressure can be expected to be stronger in southern regions, with a climate more favourable to *D. sapinea* pathogenic outbreaks, triggered by stress in the host plant, especially by droughts (Luchi *et al.*, 2014). Maritime pine populations growing in regions, such as Morocco and southern Spain, would then be more likely to have evolved resistance to the disease. On the contrary, trees from populations where severe drought periods have most likely not been common so far, e.g. Atlantic populations from Iberia and France, would be more susceptible. 2) In case, maritime pine and *D. sapinea* did not have sufficient time to co-evolve or pathogen pressure was not strong enough, differences in susceptibility among maritime pine populations might be due to exaptation or ecological fitting, i.e. traits selected for other functions (Agosta & Klemens, 2008). Populations of maritime pine strongly vary geographically in many traits related to growth and response to drought, along the gradient from North Africa to Atlantic regions of Iberia and France (Correia *et al.*, 2008; Aranda *et al.*, 2010; Corcuera *et al.*, 2012; Gaspar *et al.*, 2013;

de la Mata *et al.*, 2014). Some of these traits may indirectly influence their susceptibility to pathogens, as observed here for *D. sapinea*. For example, faster growing maritime pine trees from northern populations are known to invest more in inducible defences while slow growing trees from southern populations invest more in constitutive defences (López-Goldar *et al.*, 2018). The positive genetic correlation between height and necrosis length caused by *D. sapinea* might indicate that constitutive defences confer better resistance to this pathogen in the southern populations. Also, Meijón *et al.* (2016) showed that the metabolomes in needles of maritime pine trees from populations with distinct geographic origin (notably Atlantic versus Mediterranean provenances) were quite differentiated and flavonoids showed a significant correlation with the water regime of the population of origin. However, the expression of metabolites is organ specific (de Miguel *et al.*, 2016) and knowledge about secondary metabolites involved in resistance to *D. sapinea* is still lacking.

A study on the invasive pathogen *Fusarium circinatum*, which did certainly not co-evolve with maritime pine, also revealed a geographic cline in susceptibility, with Atlantic maritime pine populations showing less susceptibility than Moroccan populations (Elvira-Recuenco *et al.*, 2014). A similar pattern was observed for *A. ostoyae* in our study. Heritability for necrosis length caused by *A. ostoyae* was low but significant, at both population and genotype level. Intra-population variability of susceptibility to *A. ostoyae* was higher than for *D. sapinea* where no significant variability at intra-population level was found. Our results indicated that maritime pine from southwestern France, where *A. ostoyae* outbreaks have been reported frequently (Labbé *et al.*, 2015), may have developed some resistance or might show exapted resistance to the disease. Considering the absence of reports of *A. ostoyae* from the south of the Iberian Peninsula (Marxmüller & Guillaumin, 2005), which is in line with the species' preference for humid forest sites (Cruickshank *et al.*, 1997; Heinzelmann *et al.*, 2018), trees in Morocco and southern Spain have most likely never co-evolved with this pathogen. However, a study by Guillaumin *et al.*, (2005) on the mortality of potted maritime pine plants revealed an opposite pattern with the Landes population in Atlantic France being the most susceptible and the Moroccan population the least susceptible to *A. ostoyae*. Also, Zas *et al.*, (2007) found moderate narrow-sense heritability for mortality due to *A. ostoyae* at the family level (h^2_f=0.35) in an infested progeny trial of maritime pine seedlings, which is much higher than broad-sense heritability of necrosis length in our study. *Armillaria ostoyae* is a root pathogen and a critical point during natural infestation is the penetration of the root that might be key for resistance mechanisms (Prospero *et al.*, 2004; Solla *et al.*, 2011; Labbé *et al.*, 2017b), as the pathogen grows faster once it enters the organism and reaches the cambium (Solla *et al.*, 2002). This step was bypassed in our inoculation protocol on excised branches. In the future, it would therefore be interesting to carry out inoculations on potted seedlings or young trees from range-wide maritime pine populations to evaluate susceptibility.

Suitable strategies to evaluate susceptibility to *D. sapinea* and *A. ostoyae* will become increasingly important as climate change increases pathogen pressure. Droughts are expected to become more

frequent throughout Europe (IPCC, 2014) which will most likely trigger *D. sapinea* outbreaks also in regions where the pathogen has not caused severe disease symptoms so far. Recently, a northward expansion of *D. sapinea* outbreaks in Europe, probably driven by higher spring temperatures, has been recorded and is causing severe damage on *P. sylvestris* in Sweden and eastern Baltic countries (Adamson *et al.*, 2015; Brodde *et al.*, 2019). Our results suggested that an increase of drought events e.g. in the Landes region in France will most likely cause severe damage in these vast maritime pine forests due to high susceptibility of this population of maritime pine. In the case of *A. ostoyae*, the main threat resides in the condition of the host. As mentioned before, a weaker host will be more susceptible to the fungus, and future extreme weather events are bound to weaken trees, also increasing the pathogenic power of *A. ostoyae* (Kubiak *et al.*, 2017). This is in line with a mathematical model that predicted a drastic northward shift of *A. ostoyae* in the Northwestern United States for the years 2061-2080, leading to increased mortality of stressed and maladapted trees (Hanna *et al.*, 2016). In this study, trees maladapted to new temperatures are also expected to be more susceptible to biotic stress.

A shift in temperatures will not only affect pathogen susceptibility, but also other traits, notably growth and spring phenology (Badeck *et al.*, 2004; Lindner *et al.*, 2010). Height is a crucial, frequently studied trait in forest trees (e.g. Kremer & Lascoux, 1988; Cornelius, 1994) and showed a moderate-high broad-sense heritability of 0.497, the highest of all traits in our study. This is well in line with estimates in other conifer species e.g. ranging from 0.21 in *Pinus taeda* to 0.78 in *Picea abies* (reviewed in Lind *et al.*, 2018) and from 0.148 to 0.282 in maritime pine saplings depending on the common garden site and the provenance (Rodríguez-Quilón *et al.*, 2016). Height is known to be a highly integrative trait closely related e.g. to abiotic factors (Alía *et al.*, 2014; Jaramillo-Correa *et al.*, 2015) and has been used in combination with genetic markers to identify relevant conservation units in maritime pine (Rodríguez-Quilón *et al.*, 2016). Our study showed that not only climate factors but also biotic interaction effects, such as pathogen susceptibility, were genetically correlated with height (positively for *D. sapinea* and negatively for *A. ostoyae*). Neutral genetic differentiation, i.e. F_{ST}, was moderate (F_{ST} = 0.109 [0.0129; 0.3247], p-value < 0.001) and significantly lower than Q_{ST} estimates obtained for height and necrosis length caused by *D. sapinea* indicating that divergent selection is promoting local adaptation in these traits (Whitlock & Guillaume, 2009; Lamy *et al.*, 2011).

Bud burst related phenological traits showed low to moderate broad-sense heritability depending on the year. Differentiation (Q_{ST}) for bud burst reached from 0.191 to 0.275 which is comparable to a mean of 0.249 for bud flush averaged over several forest tree species (reviewed in Alberto *et al.*, 2013). In our study, trees originating from northern populations flushed later than trees from southern populations. Similar clines have been observed for other conifers (reviewed in Alberto *et al.*, 2013), which is not surprising, as spring phenology, such as flushing time, is known to be correlated with climatic factors (e.g. Zohner & Renner, 2014). Spring phenology can also play a role in resistance to

or avoidance of forest tree pathogens (e.g. Swedjemark *et al.*, 1998; Ghelardini & Santini, 2009; Nielsen *et al.*, 2017). In line with this, we found a positive genetic correlation between needle discoloration and necrosis length caused by *D. sapinea* with needle phenology indicating that earlier flushing trees with faster developing needles showed less severe disease symptoms. Krokene *et al.*, (2012) showed that the concentrations of starch and total sugars (glucose, fructose and sucrose) in twigs of *Picea abies* change during shoot development, which affects pathogen-related symptoms. In our study, inoculations were carried out on twigs with elongated shoots, however, the chemical composition of twigs might differ with time since bud burst.

Genotype-phenotype associations

We revealed significantly associated loci for all heritable traits. However, genotype effects were small, pointing to a highly polygenic nature of studied traits, as often reported for adaptive traits in forest trees. In addition, for susceptibility to *D. sapinea* and *A. ostoyae*, no resistance alleles with major effects were detected. We retrieved annotations from Plomion *et al.*, (2016) and found four non-synonymous SNPs significantly associated to duration of bud burst in 2015 (one locus), bud burst in 2017 (two loci) and needle discoloration caused by *D. sapinea* (one locus), see Table **5**. The potential function of these genes has to be interpreted with caution as this information usually derives from studies in distantly related model species. Nevertheless, the locus (BX679001_1418), which was significantly associated to needle discoloration caused by *D. sapinea*, possibly codes for a translation initiation factor eIF-5 that has previously been reported to be involved in pathogen-induced cell death and development of disease symptoms in *Arabidopsis thaliana* (Hopkins *et al.*, 2008). Furthermore, the locus AL749768_562, significantly associated to bud burst, matched a putative 60S ribosomal protein L9 with higher expression in active buds compared to dormant buds in *Cunninghamia lanceolata* (Xu *et al.*, 2016). These two genes deserve further attention in future studies addressing the genetic control of adaptive traits in conifers.

Based on a well-replicated clonal common garden and state-of-the-art genotyping technology, we were able to study key adaptive traits in maritime pine and found evidence for non-synonymous mutations underlying genetic variation for these traits. Association studies for highly polygenic traits are still challenging. Lind *et al.*, (2017) reported an average of 236 SNPs associated to each of four fitness-related traits in *Pinus albicaulis* by detecting signals of significantly higher covariance of allele frequencies than would be expected to arise by chance alone. In the near future, multilocus association methods should be used to reveal genome wide loci with non-zero effects for polygenic traits in forest trees (Goldfarb *et al.*, 2013; de la Torre *et al.*, 2019).

Conclusions

In our study, we have shown that several adaptive traits in maritime pine were genetically correlated and also significantly correlated to climate factors. The evolution of suits of functional traits along

environmental clines is a common pattern (e.g. Chapin *et al.*, 1993; Reich *et al.*, 1996) and populations are typically best adapted to their environment of origin (Kawecki & Ebert, 2004). Currently, locally adapted populations are challenged by changing climate conditions and emergent pests and pathogens expanding their range (Petr *et al.*, 2017). Susceptibility to *D. sapinea* was highest in the northern maritime pine populations where it is expected to cause severe outbreaks due to increased incidence of drought events in the future (Brodde *et al.*, 2019). Opposing trends in pathogen susceptibility among maritime pine populations e.g. for *D. sapinea* and *A. ostoyae* (this study), and for the invasive pathogen *F. circinatum* (Elvira-Recuenco *et al.*, 2014) challenge forest tree breeding and natural forest resilience. An improved understanding of integrated phenotypes, including responses to known pests and pathogens, and their underlying genetic architecture is fundamental to assist new-generation tree breeding and the conservation of valuable genotypes. Coupled with early detection methods (see e.g. Kenis *et al.*, 2018), knowledge on genetic responses to emerging pests and pathogens will help to ensure the health of forests in the future.

Data availability

SNP genotypes and phenotypic BLUPs will be made available at the DRYAD repository as soon as the manuscript has been accepted.

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Author contributions

SCGM, RA and CP conceived the idea of the study, supervised its development, and obtained the funding; AH, AC and TG carried out the pathogen inoculation experiments supervised by CD and

MLDL, who also contributed to protocol development; IRQ contributed to phenotyping and carried out the SNP genotyping; AH, MDM and KBB carried out data analyses; AH and KBB wrote the manuscript and all co-authors critically read and contributed to the final version of the manuscript.

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Table captions

Table 1 Heritability of adaptive traits in *Pinus pinaster*. Variability refers to the standard deviation of the raw phenotypic data. H^2, broad-sense heritability of the genotype effect; H^2_p, broad-sense heritability of the combined genotype and population effect; Q_{ST}, population differentiation; bb, bud burst; dbb, duration of bud burst; disc., needle discoloration; Processionary, presence/absence of processionary moth nests; dd, degree-days; NA: not applicable. Heritability for incidence of the processionary moth was computed using height as a covariate. Values in bold are significant. Values in squared brackets indicate the 95% confidence intervals.

Table 2 Pearson's correlation coefficients for genetic correlations of the best linear unbiased predictors (BLUPs) of the combined population and genotype effects between adaptive traits in *Pinus pinaster*. bb, bud burst; dbb, duration of bud burst; disc., needle discoloration. Significance levels after false discovery rate (FDR) correction: *<0.05; **<0.01; ***0.001.

Table 3 Pearson's correlation coefficients between population effect BLUPs for adaptive traits, and climatic and environmental data in *Pinus pinaster*. disc., needle discoloration; meanT^a, mean temperature; T^amax, maximum temperature; prec, precipitation; Jan, January; Feb, February; Aug, August; Sep, September; *N*, number of genotypes available for the trait. Significance levels after false discovery rate (FDR) correction: *<0.05; **<0.01; ***0.001.

Table 4 Single nucleotide polymorphisms (SNPs) significantly associated to height, needle phenology and pathogen susceptibility traits in *Pinus pinaster* under the additive genetic model as identified by a two-step approach based on mixed-effects linear models (MLMs) implemented in Tassel and the Bayesian framework in BAMD (BMLMs). Bayesian mean SNP effects and 95% confidence intervals (CIs) were obtained from the distribution of the last 20 000 iterations in BAMD. Marker codes and linkage groups as reported in Plomion *et al.*, (2016). disc., needle discoloration.

Table 5 Annotation for SNPs significantly associated under the additive model and coding for a non-synonymous amino acid change, as retrieved from Plomion *et al.*, (2016) disc., needle discoloration

Figure captions

Figure 1 Stripchart of the best linear unbiased predictors (BLUPs, including both genotype and population effect) of necrosis length caused by *D. sapinea* (A) and *A. ostoyae* (B) for each of the *Pinus pinaster* populations included in each experiment. Populations were assigned to one of six gene pools (see Jaramillo-Correa *et al.*, 2015) which correspond to the six colours and ordered by latitude (north to south) within each gene pool. Black lines indicate the average necrosis length in each population.

Figure 2 Genetic correlation of necrosis length caused by *Diplodia sapinea* and *Armillaria ostoyae* based on best linear unbiased predictors (BLUPs, including both genotype and population effect). A linear trend line is shown.

Figure 3 Stripchart of necrosis length caused by *Diplodia sapinea* (BLUPs, including both genotype and population effect) plotted against the maximum temperature in July in each *Pinus pinaster* population of origin. Black lines indicate the average necrosis length in each population.

Figure 4 Genotypic effects (box plots) for two exemplary single nucleotide polymorphisms (SNPs) showing significant association with needle discoloration caused by *Diplodia sapinea* (A) and duration of bud burst in 2015 (B) in *Pinus pinaster*.

Figure 5 Allele frequency distribution of SNP BX679001_1418 in natural populations of *Pinus pinaster*. This locus was significantly associated to needle discoloration caused by *Diplodia sapinea* (see main text and Fig. **4**).

Supporting Information

Table S1.1 Number of genotypes from the CLONAPIN clonal common garden used to study adaptive traits in *Pinus pinaster.*

Figure S2.1 Phenological stages of bud burst.

Protocol S3.1 Laboratory protocol for *Diplodia sapinea* inoculations.

Figure S3.1 Pictures of the four scales of needle discoloration found along the necrosis caused by artificial *Diplodia sapinea* inoculations on excised branches of maritime pine.

Protocol S3.2 Laboratory protocol for *Armillaria ostoyae* inoculations.

S4.1 Model parametrization of fixed-effect models

Table S4.1 MCMCglmm Bayesian model parametrization.

Figure S5.1 Box plots of the best linear unbiased predictors (BLUP) of each phenotypic trait in each provenance (corresponding to the six gene pools) of *Pinus pinaster*.

Table S5.2 Pearson's correlation coefficients for genetic correlations of the best linear unbiased predictors (BLUPs) of the genotype values between adaptive traits in *Pinus pinaster*.

Table S6.1 (see separate pdf-file: TableS6.1.pdf) All significant allele effects (including additive, dominance and overdominance effects) of single nucleotide polymorphisms (SNPs, minor allele frequency (MAF) > 0.1) on height, needle phenology and pathogen susceptibility traits in *Pinus pinaster* identified by a two-step approach based on mixed-effects linear models (MLMs) implemented in Tassel and the Bayesian framework in BAMD (BMLMs). Bayesian mean SNP effects and 95% confidence intervals (CIs) were obtained from the distribution of the last 20 000 iterations in BAMD. Marker names and linkage groups (LG) as reported in Plomion *et al*., (2016). Site annotations: nc, non-coding (untranslated regions or introns); non-syn, non-synonymous; syn, synonymous; unk, unknown. *N*, number of phenotypic observations included in the analyses.

Figure S6.1 Density plots of the effect sizes based on 20,000 BAMD simulations and genotypic effects for three single nucleotide polymorphisms showing significant association with bud burst in 2017 and coding for a non-synonymous change in *Pinus pinaster*.

Figure S6.2 Density plots of the effect sizes based on 20,000 BAMD simulations and genotypic effects for two single nucleotide polymorphisms showing significant association with needle discoloration caused by *D. sapinea* and bud burst in 2015 and coding for a non-synonymous change in *Pinus pinaster*.

Tables

Table 1

	Phenotypic mean	Variability	H^2	H^2_p	Q_{ST}	Evolvability
height (cm)	170.647	± 48.228	**0.156[0.111, 0.185]**	**0.497[0.398, 0.576]**	**0.549[0.392, 0.662]**	6.223
bb2015 (dd)	1311.950	± 82.411	**0.241[0.194, 0.29]**	**0.465[0.389, 0.538]**	**0.275[0.186, 0.443]**	2.091
dbb2015 (dd)	814.713	± 116.708	**0.194[0.161, 0.238]**	**0.308[0.247, 0.364]**	**0.191[0.100, 0.332]**	4.551
bb2017 (dd)	1286.245	± 79.853	**0.134[0.085, 0.192]**	**0.231[0.165, 0.293]**	**0.191[0.106, 0,404]**	1.031
dbb2017 (dd)	901.149	± 78.922	**0.178[0.132, 0.226]**	**0.468[0.383, 0.557]**	**0.463[0.293, 0.579]**	3.043
A. ostoyae necrosis (mm)	48.533	± 29.625	**0.021[0.004, 0.121]**	**0.066[0.018, 0.203]**	**0.217[0.041, 0.787]**	0.713
D. sapinea necrosis (mm)	43.348	± 17.931	0.096 [0.000, 0.186]	**0.413[0.248, 0.675]**	**0.636[0.349, 1.000]**	2.380
D. sapinea disc.	0 - no disc.: 183 1 - low: 123 2 - medium: 141 3 - high: 9		0.106 [0.000, 0.221]	**0.175 [0.040, 0.345]**	0.093 [0.000, 0.752]	NA
Processionary	1 - presence: 48 0 - absence: 3282		0.001 [0.000, 0.206]	0.031 [0.000, 0.246]	0.006 [0.000, 0.985]	NA

Table 2

	bb2015	dbb2015	bb2017	dbb2017	*D. sapinea* necrosis	D. *sapinea* disc.	*A. ostoyae* necrosis
height	0.194***	0.117*	0.021 n.s.	0.800***	0.679***	0.414***	-0.653***
bb2015		0.846***	0.593***	0.147**	0.564***	0.470***	-0.321*
dbb2015			0.547***	0.079 n.s.	0.511***	0.492***	-0.284*
bb2017				0.018 n.s.	0.309***	0.337***	-0.056 n.s.
dbb2017					0.639***	0.392***	-0.53***
D. sapinea necrosis						0.727***	-0.692***
D. sapinea disc.							-0.367**

Table 3

Trait	*N*	Latitude	Longitude	Altitude	meanTªJuly	maxTªJuly	maxTªAug	precJan	precFeb	precAug	precSep
height	31	-0.014	0.719***	-0.864***	-0.635***	-0.857***	-0.84***	0.581*	0.55*	0.744***	0.767***
bb2015	31	-0.614***	0.457*	-0.558**	-0.653***	-0.606***	-0.589**	0.597**	0.566**	0.532**	0.62**
dbb2015	31	-0.593**	0.26	-0.428*	-0.452*	-0.49*	-0.467*	0.576**	0.533**	0.356	0.483*
bb2017	31	-0.569**	0.336	-0.268	-0.536**	-0.377	-0.379	0.386	0.371	0.467*	0.477*
dbb2017	31	-0.174	0.654***	-0.804***	-0.714***	-0.845***	-0.84***	0.654***	0.659***	0.713***	0.754***
D. sapinea necrosis	10	-0.483	0.618	-0.762*	-0.827**	-0.78*	-0.769*	0.741*	0.671	0.546	0.569
D. sapinea disc.	10	-0.485	0.327	-0.536	-0.738*	-0.691*	-0.69*	0.733*	0.733*	0.265	0.302
A. ostoyae necrosis	6	-0.011	-0.895*	0.832	0.594	0.782	0.746	-0.373	-0.129	-0.678	-0.712

Table 4

Trait	SNP name	SNP motif	Site annotation	LG	MAF	MLMs			BMLMs		
						F	*P*	R^2	Mean	(95% CIs)	
height	AL750825_659	[A/G]	unk	8	0.146	6.560	0.0016	0.025	5.0943	1.8647	8.2793
	BX249583-420	[A/G]	unk	1	0.266	6.950	0.0011	0.026	-3.8429	-6.5598	-1.1059
	CT2714-442	[T/A]	unk		0.381	5.719	0.0036	0.021	2.6072	0.2398	4.9727
	CT575717-1382	[T/C]	nc		0.206	5.891	0.0030	0.022	4.2584	1.3104	7.2411
	F51TW9001AZG2W-933	[C/G]	unk	4	0.438	10.138	0.0001	0.038	-3.2096	-5.8672	-0.6074
	FN694775-756	[A/G]	nc		0.132	6.116	0.0024	0.023	4.8086	1.3510	8.2424
	sp_v3.0_unigene17345-1191	[T/G]	nc	9	0.344	6.270	0.0021	0.023	3.4580	0.8379	6.0616
bb2015	BX249218-322	[A/C]	nc		0.315	6.502	0.0017	0.030	7.3080	2.2536	12.3645
	BX249671_307	[T/C]	unk	7	0.397	6.712	0.0014	0.030	6.0802	0.7338	11.4138
	BX253890-151	[T/G]	nc	12	0.157	6.296	0.0020	0.028	11.0578	4.5013	17.5928
	CL2033CT1302CN1398-513	[A/G]	nc	1	0.408	5.386	0.0049	0.024	6.9646	1.8207	12.0634
	CL544Contig1_03.Pipn-84	[T/G]	unk		0.135	8.981	0.0002	0.040	10.3545	3.0149	17.7447
	FN692276-550	[A/G]	unk		0.402	7.851	0.0005	0.035	5.5271	0.5534	10.4575
	i13066s710	[A/C]	nc		0.242	6.033	0.0026	0.028	8.3046	2.1705	14.3741
	i16267s380	[A/G]	unk	2	0.411	7.525	0.0006	0.034	-10.4358	-15.8176	-5.1493
	LP3-3-298	[T/C]	unk		0.143	5.567	0.0041	0.025	8.5705	1.8966	15.3310
dbb2015	F51TW9001BWV4H-219	[T/C]	non-syn		0.462	5.929	0.0029	0.028	7.1947	0.5013	13.7846
	F51TW9002FPGRE-170	[A/G]	nc		0.346	5.512	0.0043	0.026	8.7035	1.6592	15.7458
bb2017	0_12730_01_contig1-159	[A/C]	unk	12	0.379	10.119	0.0001	0.048	4.5331	2.0115	7.0269
	AL749768_562	[A/T]	non-syn	1	0.126	5.513	0.0044	0.026	3.9365	0.0920	7.7265
	AL750545-695	[T/A]	non-syn	1	0.487	5.417	0.0048	0.025	3.4631	0.7855	6.1680
	AL750755_1441	[A/C]	unk	2	0.432	5.964	0.0028	0.028	-3.6781	-6.4034	-0.9512
	AL750773_910	[T/A]	unk	3	0.499	5.936	0.0029	0.029	-3.4815	-6.2165	-0.7566
	BX252045-412	[A/G]	unk	12	0.164	5.688	0.0037	0.027	3.7144	0.3232	7.1098
	BX676789-1926	[A/T]	nc	12	0.273	5.548	0.0042	0.026	-5.6823	-8.6306	-2.7130
	CL2640CT2248CN2410-1340	[A/C]	unk	6	0.477	6.109	0.0024	0.029	-4.0448	-6.7826	-1.3158

	CT576106-142	[C/G]	unk	10	0.180	6.712	0.0014	0.032	4.5886	1.1391	8.0506
	F7JJN6E01B7BCW-157	[A/G]	syn	5	0.117	6.164	0.0023	0.029	6.7047	2.8720	10.6170
	FM945796-840	[T/G]	unk		0.214	6.754	0.0013	0.032	-4.4837	-7.6293	-1.2690
	i10996s1211	[T/C]	unk		0.301	7.769	0.0005	0.036	3.3180	0.3730	6.2603
dbb2017	AL749850_679	[A/G]	unk		0.402	5.451	0.0046	0.021	-4.6602	-9.0130	-0.2439
	CT582680-451	[A/C]	unk		0.201	7.048	0.0010	0.027	-9.9823	-15.1060	-4.8771
	F51TW9001BAW7V-405	[A/G]	unk	12	0.163	8.936	0.0002	0.035	7.6805	3.0770	12.3826
	i17647s350pg	[G/C]	unk		0.157	5.664	0.0037	0.022	6.3654	1.4410	11.2070
A. ostoyae necrosis.	F51TW9001AI9YZ-1847	[T/C]	unk	7	0.273	5.928	0.0048	0.081	-0.7349	-1.3448	-0.1294
	F51TW9001CXU1D-1264	[T/C]	unk	6	0.364	6.594	0.0028	0.090	-0.9972	-1.7872	-0.2250
D. sapinea necrosis	BX250531-554	[A/G]	unk		0.214	5.842	0.0036	0.032	-1.3467	-2.0488	-0.6471
	CT578935-1350	[T/C]	unk	2	0.391	5.793	0.0038	0.032	0.7483	0.1585	1.3385
	F51TW9001B2RB8-159	[A/C]	unk	1	0.326	6.028	0.0031	0.033	0.8452	0.2912	1.4063
	F51TW9002FT2ZF-1060	[T/G]	unk	12	0.485	8.241	0.0004	0.045	-0.9744	-1.6630	-0.2956
	PFK-39	[T/C]	unk	12	0.155	8.504	0.0002	0.040	0.9951	0.1908	1.8024
D. sapinea disc.	BX679001-1418	[T/C]	non-syn	7	0.192	5.551	0.0048	0.049	-0.0561	-0.1064	-0.0064

nc: non-coding (untranslated regions or introns), syn: synonymous, non-syn: non-synonymous, unk: unknown

Table 5

Trait	SNP name	Motif	Protein change	Putative protein function
dbb2015	F51TW9001BWV4H-219	[T/C]	Asparagine - Serine	LANC-like domain containing protein
bb2017	AL749768_562	[A/T]	Stop codon - Leucine	Putative 60S ribosomal protein L9
bb2017	AL750545-695	[A/T]	Glutamate/Glutamine - Valine	Catalase
D. sapinea disc.	BX679001-1418	[T/C]	Isoleucine - Valine	Translation initiation factor eIF-5

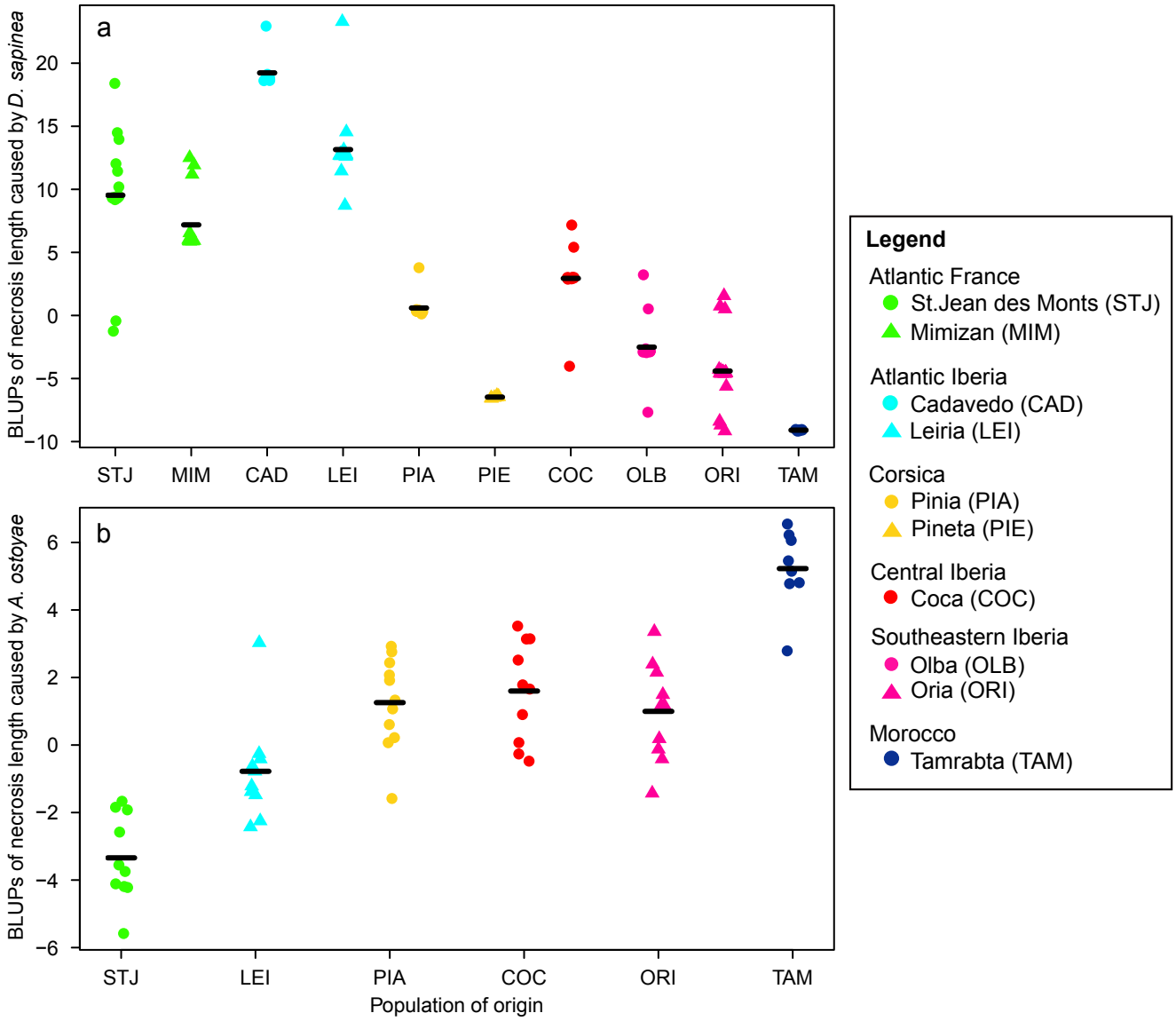
a
BLUPs of necrosis length caused by D. sapinea
20
15
10
5
0
−5
−10
STJ
MIM
CAD
LEI
PIA
PIE
COC
OLB
ORI
TAM
b
BLUPs of necrosis length caused by A. ostoyae
6
4
2
0
−2
−4
−6
STJ
LEI
PIA
COC
ORI
TAM
Population of origin
Legend
Atlantic France
St.Jean des Monts (STJ)
Mimizan (MIM)
Atlantic Iberia
Cadavedo (CAD)
Leiria (LEI)
Corsica
Pinia (PIA)
Pineta (PIE)
Central Iberia
Coca (COC)
Southeastern Iberia
Olba (OLB)
Oria (ORI)
Morocco
Tamrabta (TAM)

Figure 1

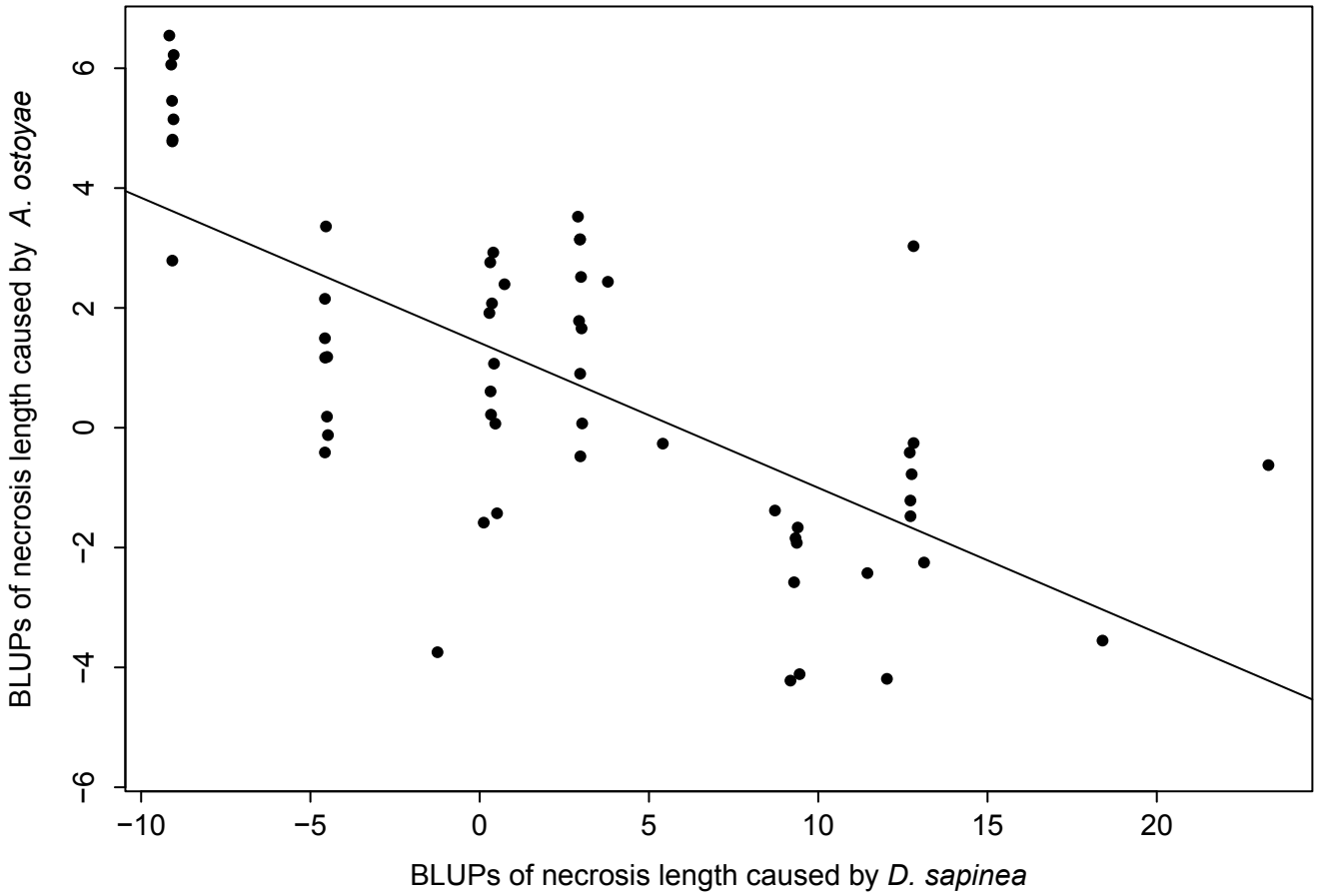
BLUPs of necrosis length caused by A. ostoyae
BLUPs of necrosis length caused by D. sapinea
−6
−4
−2
0
2
4
6
−10
−5
0
5
10
15
20

Figure 2

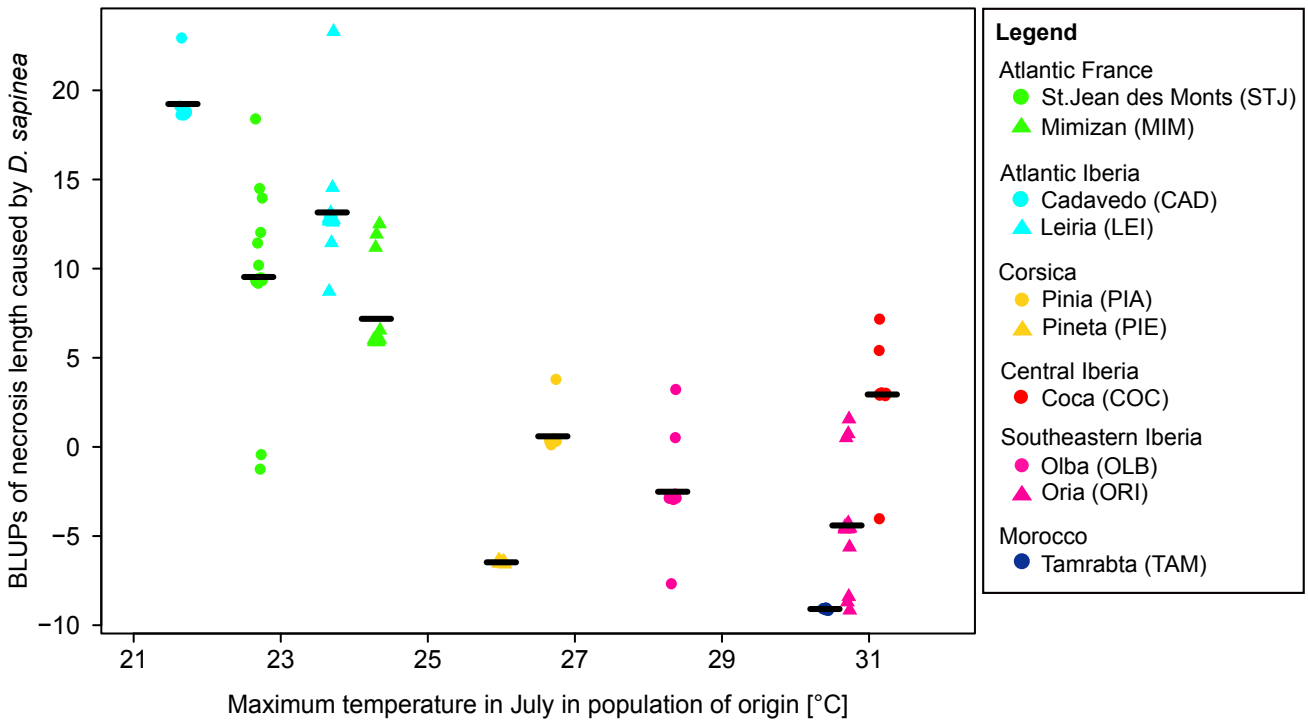
BLUPs of necrosis length caused by D. sapinea
20
15
10
5
0
−5
−10
21
23
25
27
29
31
Maximum temperature in July in population of origin [°C]
Legend
Atlantic France
St.Jean des Monts (STJ)
Mimizan (MIM)
Atlantic Iberia
Cadavedo (CAD)
Leiria (LEI)
Corsica
Pinia (PIA)
Pineta (PIE)
Central Iberia
Coca (COC)
Southeastern Iberia
Olba (OLB)
Oria (ORI)
Morocco
Tamrabta (TAM)

Figure 3

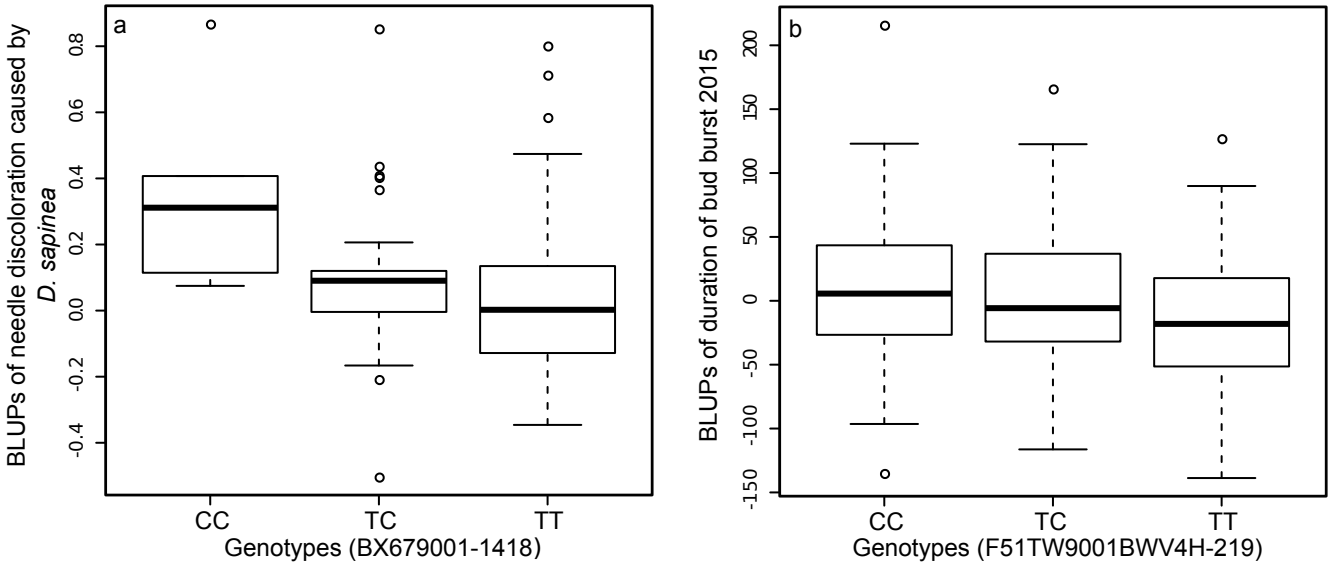
a
BLUPs of needle discoloration caused by
D. sapinea
-0.4
-0.2
0.0
0.2
0.4
0.6
0.8
CC
TC
TT
Genotypes (BX679001-1418)
b
BLUPs of duration of bud burst 2015
-150
-100
-50
0
50
100
150
200
CC
TC
TT
Genotypes (F51TW9001BWV4H-219)

Figure 4

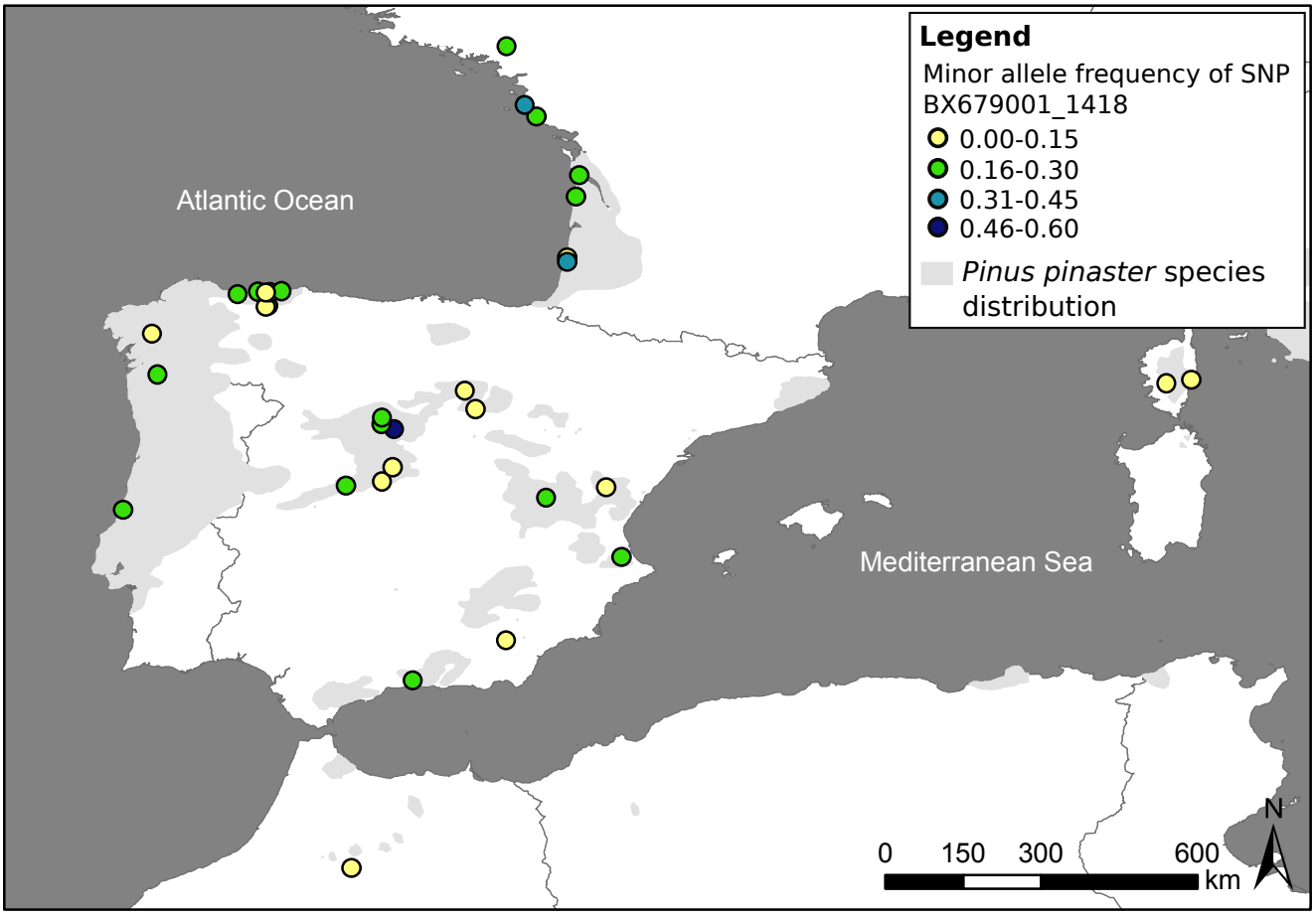
Legend
Minor allele frequency of SNP BX679001_1418
0.00-0.15
0.16-0.30
0.31-0.45
0.46-0.60
Pinus pinaster species distribution
Atlantic Ocean
Mediterranean Sea
0
150
300
600
km
N

Figure 5