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

Agathe Hurel[1], Marina de Miguel[1], Cyril Dutech[1], Marie-Laure Desprez-Loustau[1], Christophe Plomion[1], Isabel Rodríguez-Quilón[2], Agathe Cyrille[1], Thomas Guzman[1], Ricardo Alía[2], Santiago C. González-Martínez[1], Katharina B. Budde[1]

[1]BIOGECO, INRA, Univ. Bordeaux, 33610 Cestas, France.

[2]CIFOR, INIA, 28040 Madrid, Spain.

*Running title:*

Genetics of pathogen susceptibility in maritime pine

*Corresponding author:*

Katharina B. Budde

BIOGECO, INRA, 69 route d'Arcachon, 33610 Cestas, France

Ph: +33 535385343, E-mail: katharina.budde@inra.fr

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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.

## 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). Genotypes and phenotypes are often highly structured throughout the species' distribution, despite extensive gene flow across populations. 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 forest trees (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). Interactions between pathogens and their host species can lead to changes in their abundance and distribution, and to modifications of the genetic composition in both partners (Woolhouse *et al.*, 2005). 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 given 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 show 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 forest pathogens have been identified, e.g. in *Pinus taeda* against the fusiform rust disease (Kuhlman *et al.*, 2002) and in several other North American pine species against white pine blister rust (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. A widely used mixed model approach developed by Yu *et al.* (2006) allows to associate phenotypes and genotypes while accounting for population genetic structure as covariate and relatedness between individuals as random factor. 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 coast of southern France and the west coast 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, especially in the Iberian Peninsula, possibly due to its long term persistence in this region (Salvador *et al.*, 2000; Bucci *et al.*, 2007), and it is highly 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 (Schvester, 1982; 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, for example, 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 natural forests and plantations, 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). Moreover, the presence of *D. sapinea* in the wood provides a suitable resource for the pine wood nematode, *Bursaphelenchus xylophilus*, the causal agent of pine wilt disease (Futai *et al.*, 2007). 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*. Among the six species compared by Iturritxa *et al.*, (2013), *P. radiata* was most susceptible, *P. halepensis* was most resistant and *P. pinaster* was moderately susceptible. The species was first described in Europe in 1823 under the name *Sphaeria sapinea*, and then received many synonyms (Piou *et al.*, 1991). 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. One single genotype can reach a size as big as 965 ha, infesting trees by root contact, rhizomorphs and spores (Ferguson *et al.*, 2003), and can reach an estimated age of thousands of years. 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 have a strong effect on the impact of *A. ostoyae* on conifer forests in the coming years (Kubiak *et al.*, 2017).

*Thaumetopoea pityocampa* 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). Moreover, processionary caterpillars release urticating hairs that can cause anaphylactic reactions to both humans and animals, making *T. pityocampa* a health threat (Battisti *et al.*, 2011).

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 using the Illumina Infinium single nucleotide polymorphism (SNP) array described in Plomion *et al.*, (2016). Altogether, our approach, combining the evaluation of a clonal common garden and a genotyping array, produced relevant insights on the evolution, genetic basis and architecture of adaptive traits in maritime pine, an ecologically and economically important forest tree species.

## 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 and genotypes, and population coordinates of 33 populations included in this study), representing all known differentiated gene pools (Central Spain, Southeastern Spain, Iberian Atlantic, French Atlantic, Corsica 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 (*Thaumetopoea pityocampa*) 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). Pathogen susceptibility was assessed in a subset of genotypes using excised branches collected from the clonal trial (sample size of 180-453 branches, see Table S1.1, Supporting Information and below). 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 to 5 (0: bud without elongation during winter, 1: elongation of the bud, 2: emergence of brachyblast, 3: brachyblast begins to space, 4: elongation of the needles, 5: total

elongation of the needles (see Figure S2.1, Supporting Information). The Julian day of entry in each stage (S1 to S5) 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 (*Thaumetopoea pityocampa*) in the tree crowns was assessed in March 2018.

*Experimental evaluation of susceptibility to Diplodia sapinea*

Inoculations were carried out on excised shoots taken from pines in the common garden (for a detailed laboratory protocol see Supporting Information S3.1). 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 sequencing the ITS region, amplified using the primers ITS1-F and ITS4 (Gardes and Bruns 1993), and blasting it against the NCBI nucleotide data base (Benoît Laurent, personal communication). Only current-year shoots at the phenological stage 3 to 5, i.e. with fully elongated buds but not fully mature, were sampled (see Supporting Information S2.1). For the inoculation, we removed a needle fascicle in the middle of each shoot with a scalpel. A 5 mm diameter plug of malt-agar taken at the active margin of a *D. sapinea* culture was put on the wound, mycelium side down, and then wrapped in cellophane. Control shoots were treated in the same manner but with plugs of sterile rather than colonized malt-agar. 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. The shoots were not lignified and the lesions were visible. However, the surface was superficially stripped to see the limit of the lesion when it was not visible otherwise. Needle discoloration was also observed, and evaluated using a scale from 0: no discoloration to 3: all needles along the necrosis showed discoloration (see Figure S3.1, Supporting Information). To confirm that discoloration was caused by the pathogen, one discolored needle from one branch per population was placed on a malt-agar Petri dish to grow. After 3 days, *D. sapinea* could be visually identified in each Petri dish.

We sampled a total of 453 branches, from 151 genotypes (i.e. one branch from each of three replicate trees per genotype) representing all differentiated gene pools known in maritime pine (see Jaramillo-Correa *et al.*, 2015). Every day between June 12$^{th}$ and July 31$^{st}$ 2018, one lateral branch per tree was cut from the previous year whorl on 30 randomly selected trees included in our experimental design and taken to the laboratory for inoculation. Inoculations were performed on the leader shoot of the current whorl of the excised branch.

*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, two plugs of 5 mm diameter of malt agar with the *A. ostoyae* mycelium were put on the top of a mixture of industrial vegetable soup (Knorr 9 légumes©, Heilbronn, Germany), malted water and hazelnut wood chips in a 180 mL plastic jar (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 during three months in a heat chamber set at 23°C and 80% humidity 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 3$^{rd}$-4$^{th}$ 2018.

The basal part of the shoots (ca. 8 cm) was placed in the center of the 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. Only the jars showing a minimum jar occupation by *A. ostoyae* of 60% were used. 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) was used for genotyping. Apart from potentially neutral genetic polymorphisms, this array comprises SNPs from candidate genes that showed signatures of natural selection (Eveno *et al.*, 2008; Grivet *et al.*, 2011), significant environmental associations with climate at the range-wide spatial scale (Jaramillo-Correa *et al.*, 2015) or differential expression under biotic and abiotic stress in maritime pine (Plomion *et al.*, 2016). 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.

*Estimation of genetic variance, heritability, population genetic differentiation and genetic correlations*

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

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

$$y_{ijk} = \mu + block_i + cov + pop_j + pop(genotype)_{jk} + \varepsilon_{ijk} \quad (2)$$

where for any trait *y*, $\mu$ 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* and ε is the residual effect. In model 2, *cov* represents the covariates 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). All analyzed traits presented a Gaussian distribution with the exception of presence of pine processionary moth nests and needle discoloration caused by *D. sapinea* infection that followed a binomial distribution and were modeled with *logit* and *probit* link functions, respectively. Multivariate-normal prior distribution with mean centered around zero and large variance matrix ($10^8$) were used for fixed effects with the exception of the model for needle discoloration caused by *D. sapinea* where a gelman prior for V was set, as suggested by Gelman *et al.* (2008) for ordinal traits. Inverse Wishart non-informative priors were used for the variances and covariances with a matrix parameter V set to 1 and a parameter n set to 0.002 (Hadfield, 2010). Parameter expanded priors were used to improve the convergence and mixing properties of the chain as suggested by Gelman (2006) for models on the presence of pine processionary moth nests, needle discoloration caused by *D. sapinea*, and necrosis caused by *A. ostoyae*. Parameter estimates were not sensitive to change in the priors. The models were run for at least 750,000 iterations, including a burn-in of 50,000 iterations and a thinning interval of 500 iterations. Four chains per model were run to test for parameter estimates convergence. Gelman-

Rubin criterion Potential Scale Reduction Factor (psrf) was consistently below 1.01 (Gelman & Rubin, 2007) (see Table S4.1, Supporting Information 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 ($H^2$) :

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

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

where $\sigma^2_{genotype}$ is the variance among genotypes within populations, $\sigma^2_{pop}$ is the variance between populations and $\sigma^2_e$ 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).

$$Q_{ST} = \frac{\sigma^2_{pop}}{\sigma^2_{pop}+2\sigma^2_{genotype}} \quad (5)$$

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 (BLUPs accounting for both population and genotype effects). Ancestry proportions of each sample were computed using STRUCTURE (Falush *et al.*, 2007). 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 and blast results were retrieved from Plomion *et al.*, (2016) for each significantly associated SNP. The minimum allele frequency of significantly associated SNPs was then estimated in each population using SPAGeDi 1.5 and plotted in a geographic map.

## 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 (Figure 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 (Figure 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 (Figure 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, Figure 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, Figure 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 (Figure 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, Figure 4 and Figure 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 usually not reflect the population genetic structure of the species (Figure 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 variation in maritime pine for response to two pathogenic fungi, *D. sapinea* and *A. ostoyae*, at the range-wide scale, by 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 of pathogen susceptibility, height and needle phenology in maritime pine*

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 these regions, such as from 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). A mathematical model 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 needles, 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 under study. 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.

**ORCID**

Agathe Hurel https://orcid.org/0000-0002-4853-9123
Marina de Miguel https://orcid.org/0000-0001-6398-2660
Marie-Laure Desprez-Loustau https://orcid.org/0000-0001-6457-1089
Christophe Plomion https://orcid.org/0000-0002-3176-2767
Isabel Rodríguez-Quilón https://orcid.org/0000-0002-1296-8313
Ricardo Alía https://orcid.org/0000-0002-9426-0967
Santiago C. González-Martínez https://orcid.org/0000-0002-4534-3766
Katharina B. Budde https://orcid.org/0000-0002-5361-2815

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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; mean$T^a$, mean temperature; $T^a$max, 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 Figure 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.

**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<br>1 - low: 123<br>2 - medium: 141<br>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<br>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 | meanT$^{a}$July | maxT$^{a}$July | maxT$^{a}$Aug | 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

1089 **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 |

1090

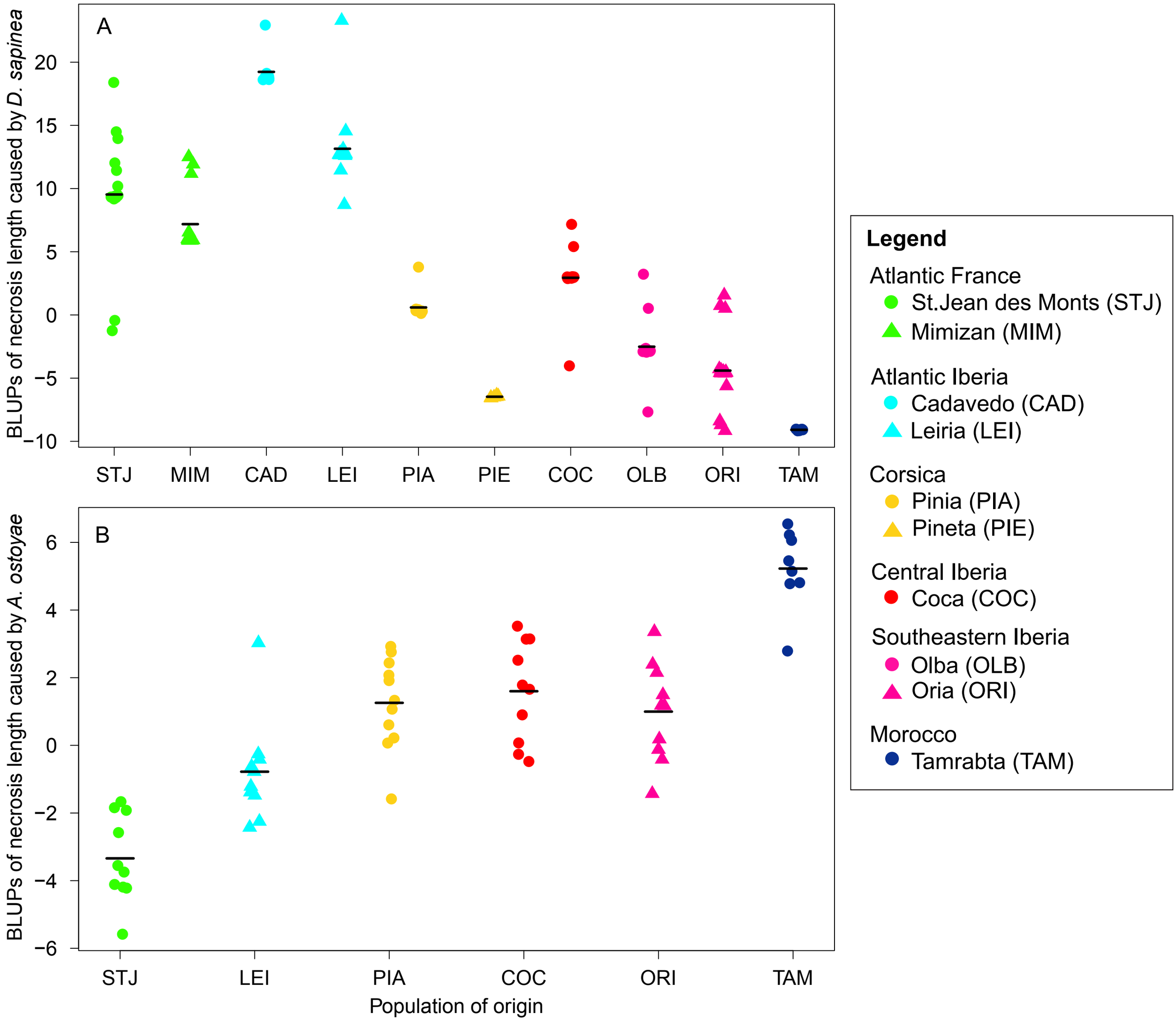
A
BLUPs of necrosis length caused by D. sapinea
20
15
10
5
0
−5
−10
STJ
MIM
CAD
LEI
PIA
PIE
COC
OLB
ORI
TAM
B
BLUPs of necrosis length caused by A. ostoyae
6
4
2
0
−2
−4
−6
STJ
LEI
PIA
COC
ORI
TAM
Population of origin
Legend
Atlantic France
St.Jean des Monts (STJ)
Mimizan (MIM)
Atlantic Iberia
Cadavedo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

Figure 1

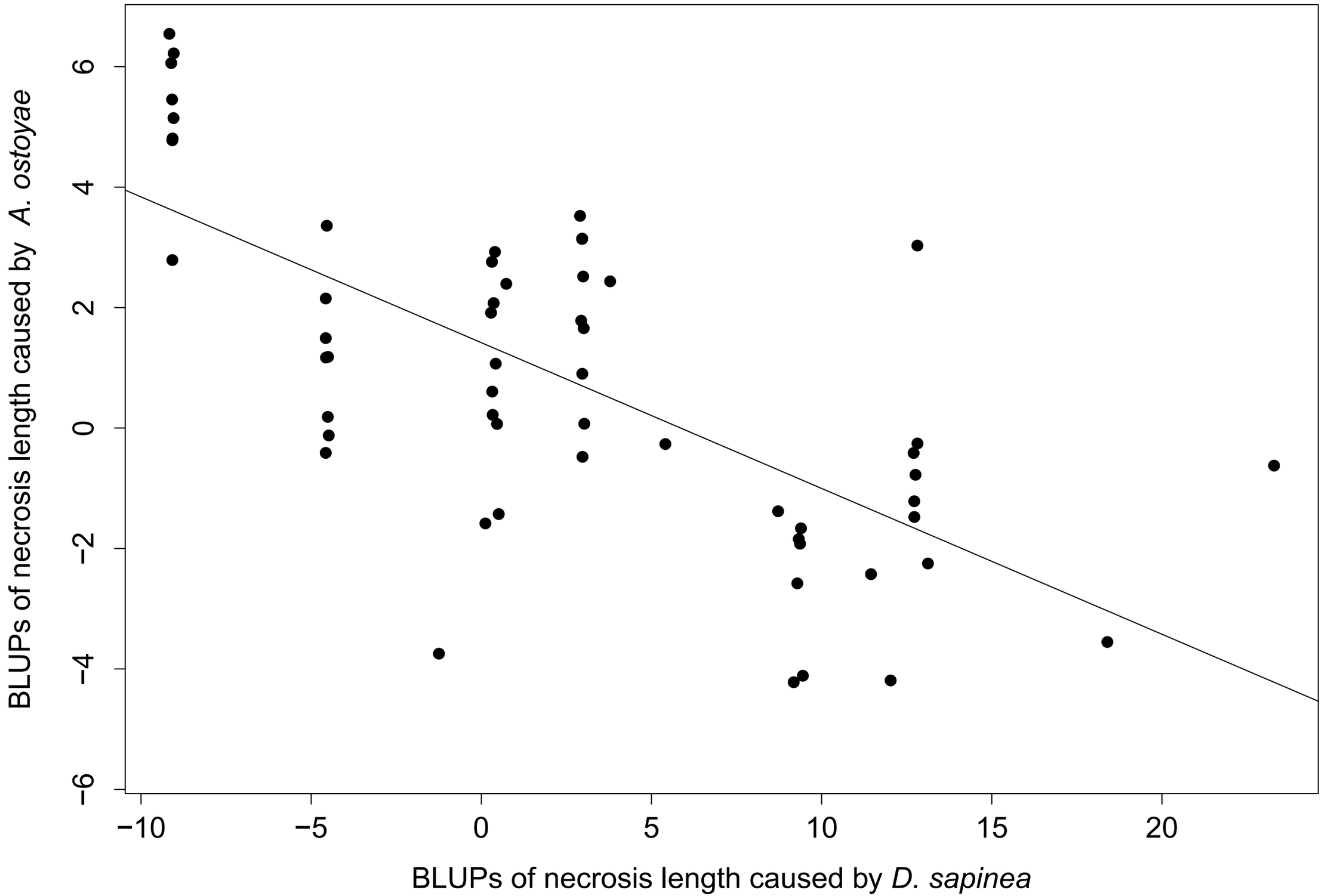
BLUPs of necrosis length caused by *A. ostoyae*
6
4
2
0
−2
−4
−6
−10
−5
0
5
10
15
20
BLUPs of necrosis length caused by *D. sapinea*

Figure 2

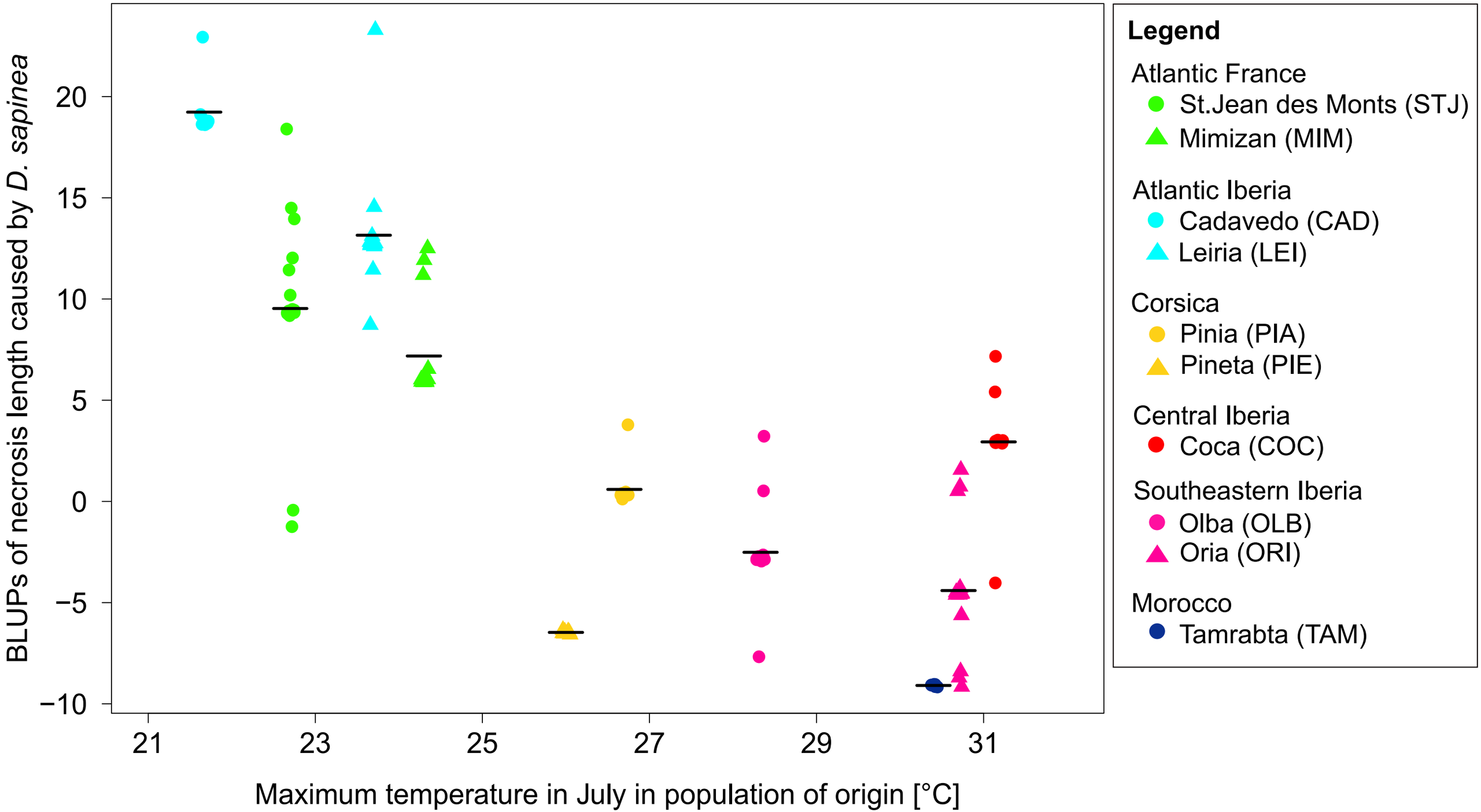
BLUPs of necrosis length caused by D. sapinea
20
15
10
5
0
−5
−10
21
23
25
27
29
31
Maximum temperature in July in population of origin [°C]
Legend
Atlantic France
St.Jean des Monts (STJ)
Mimizan (MIM)
Atlantic Iberia
Cadavedo (CAD)
Leiria (LEI)
Corsica
Pinia (PIA)
Pineta (PIE)
Central Iberia
Coca (COC)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

Figure 3

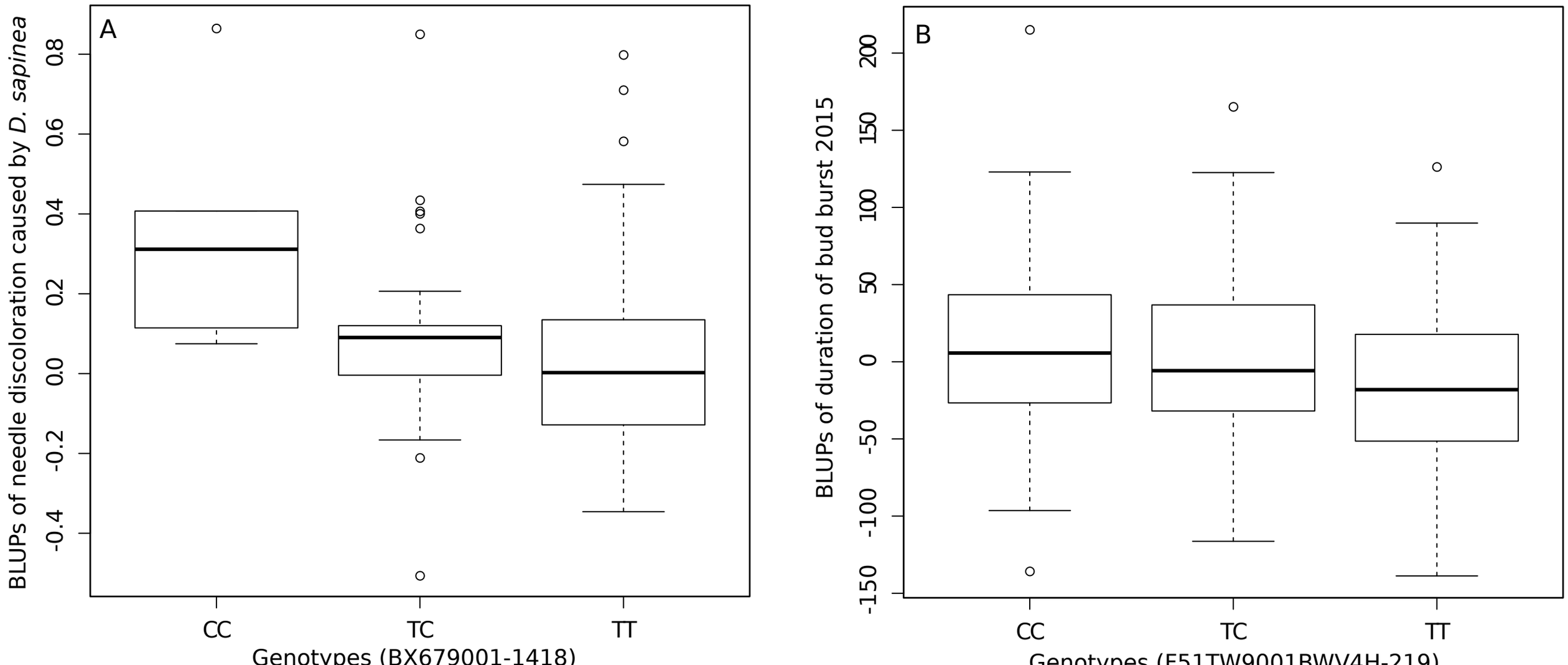
A
BLUPs of needle discoloration caused by D. sapinea
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
CC
TC
TT
Genotypes (BX679001-1418)
B
BLUPs of duration of bud burst 2015
200
150
100
50
0
-50
-100
-150
CC
TC
TT
Genotypes (F51TW9001BWV4H-219)

Figure 4

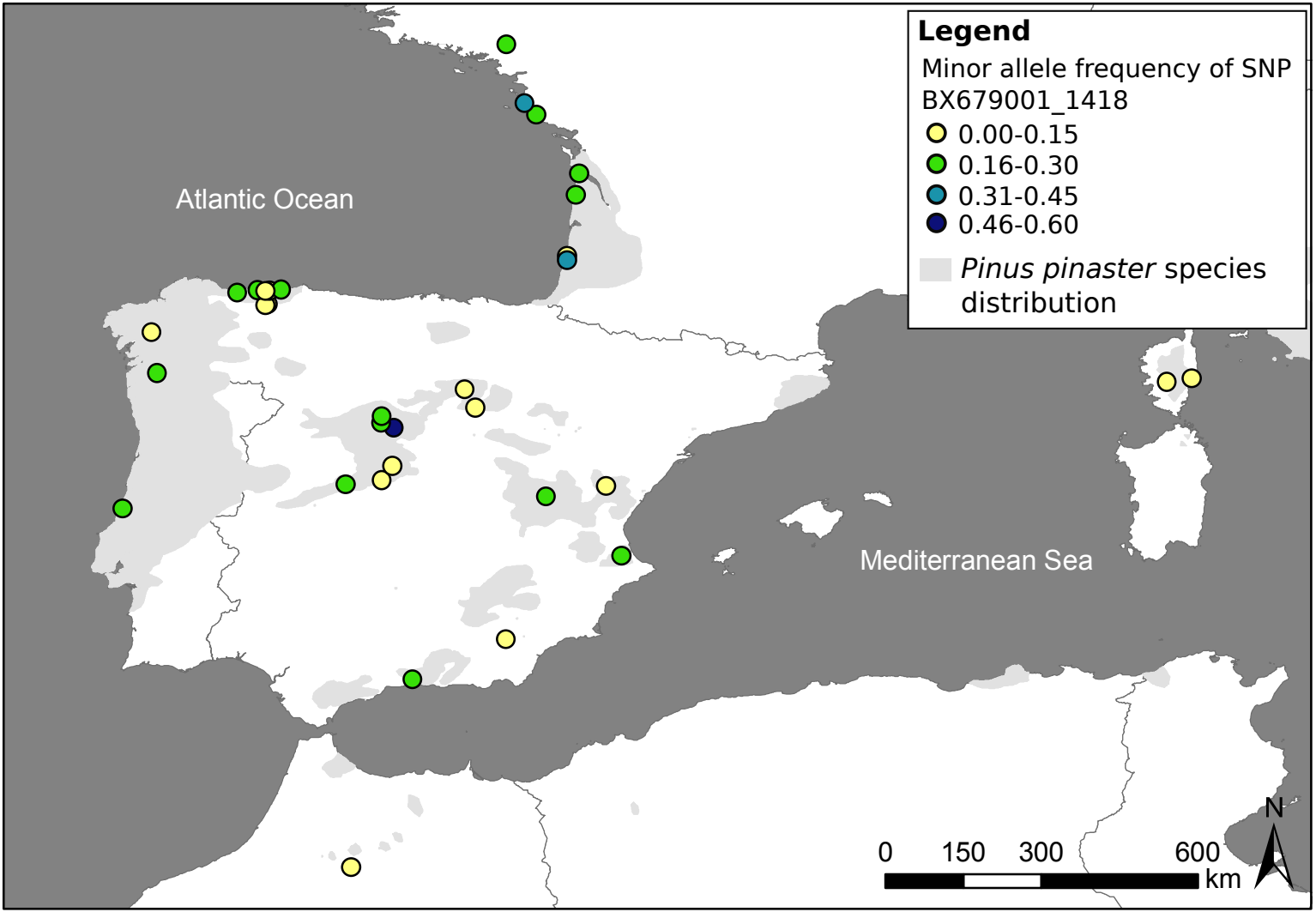
Legend
Minor allele frequency of SNP BX679001_1418
0.00-0.15
0.16-0.30
0.31-0.45
0.46-0.60
Pinus pinaster species distribution
Atlantic Ocean
Mediterranean Sea
0
150
300
600
km
N

Figure 5