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Ecological genomics of local adaptation in maritime pine (*Pinus pinaster* Aiton)

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“I speak for the trees. I speak for the trees for the trees have no tongues.”

Dr Seuss

À Emma

SUMMARY

In the current context of global change, natural ecosystems are threatened worldwide. Predictions show that climate change will cause a value loss for the European forest of 21 to 50% by 2071-2100. In France, the Nouvelle-Aquitaine region depends on forests, which occupy 34% of the territory. Maritime pine (*Pinus pinaster*) is of fundamental importance for this region: it represents 40% of the wood resources. This emblematic conifer of the Mediterranean basin and the southwestern Atlantic area has a discontinuous range distribution, which makes studying its genetic adaptation especially interesting.
This PhD thesis aims to study maritime pine genetic adaptation to environmental conditions at various temporal scales and at different tree life-stages.

The first chapter explores the susceptibility of different maritime pine populations to two pathogens: *Armillaria ostoyae*, a root pathogen and *Diplodia sapinea*, a systemic one. For this study, we used populations from CLONAPIN, a clonal collection representing all the gene-pools of maritime pine. We estimated H^2 (broad-sense heritability) and Q_{st} (quantitative genetic differentiation) for pest susceptibility, as well as H^2 and Q_{st} of other adaptive traits: height and phenology. The CLONAPIN collection having been genotyped, we were able to do a genotype-phenotype association study with all the mentioned traits. Finally, correlations were established between the genetic component of the traits and climatic variables. We observed moderate H^2 for most traits, whereas Q_{st} was generally high, showing a strong population differentiation. Susceptibility to *D. sapinea* was strongly correlated to high temperatures. SNPs (Single Nucleotide Polymorphisms) associated with the traits had a small genotype effect, pointing to a polygenic nature of the traits.

The second chapter is set within the European project GenTree, aiming at studying genetic adaptation and evolutionary potential of natural tree populations. For our study, we were interested in maritime pine populations from Spain, Italy and France and in Scots pine (*Pinus sylvestris*) populations from Spain, Germany, Lithuania and Finland. As part of this project, 25 trees from each population were phenotyped for height, diameter, wood density, specific leaf area (SLA) and carbon isotopic discrimination. Seeds were sampled on the phenotyped trees, while conserving the

family structure. Common gardens were established with these seeds in Spain and France for maritime pine, and in Spain, Germany, Lithuania and Finland for Scots pine. In each garden, all of the species' sampled populations were sowed, trying to imitate natural regeneration. Germination, survival and growth stages were monitored during one (*P. pinaster*) and two years (*P. sylvestris*). Thanks to these data, we estimated different components of fitness, which demonstrate a strong population effect, and detect significant selection gradients in these populations. Most adult variables are significant in selection gradients, though SLA was predominant in both species.

The third chapter is based on Corsican populations. This island has the particularity of representing a single gene-pool of *P. pinaster*, which was able to adapt locally to the different environments of the territory. We took advantage of the common garden PINCORSE, composed of families issued from 33 Corsican populations. These different populations were phenotyped over several years for height, and a subset for phenology and carbon isotopic discrimination too. With these data, we computed h^2 (narrow-sense heritability) and Q_{st}, and conducted an association study based on over 50k newly generated SNPs.

These studies present an innovative work bringing new insights on the adaptive capacities of maritime pine. Genetic data on performance of tree populations are essential to the genetic improvement program of maritime pine, tree-breeding and forest genetic resource conservation strategies in environments facing major changes.

Keywords: adaptation, climat change, common gardens, genetic correlation, genetic association, differentiation

RÉSUMÉ

Dans le contexte actuel de changement global, les écosystèmes naturels mondiaux sont menacés. Des prédictions montrent que le changement climatique causera une perte de valeur économique des forêts européennes de 21 à 50% d'ici 2071-2100. En France, la région Nouvelle-Aquitaine dépend de ses forêts, qui occupent 34% du territoire. Le pin maritime (*Pinus pinaster*) est d'une importance cruciale pour la région dont il représente 40% de la ressource en bois. Ce pin emblématique du bassin méditerranéen et de la zone Atlantique Sud-Ouest possède une distribution discontinue, rendant son adaptation génétique particulièrement intéressante.
Cette thèse étudie l'adaptation génétique du pin maritime aux conditions environnementales à des échelles temporelles variées et à différents stades de vie de l'arbre.

La première partie explore la susceptibilité de différentes origines de pin maritime à deux pathogènes : *Armillaria ostoyae*, pathogène des racines et *Diplodia sapinea*, pathogène systémique. Nous avons utilisé des populations de CLONAPIN, un jardin clonal représentant tous les gene-pools du pin maritime. Le H^2 (héritabilité au sens large) et le Q_{st} (différenciation génétique quantitative) de cette susceptibilité ont été estimés ainsi que ceux d'autres traits adaptatifs: la hauteur et la phénologie. La collection CLONAPIN ayant été génotypée, nous avons pu faire une étude d'association avec les traits étudiés. Enfin, des corrélations ont été établies entre les composants génétiques des traits et des variables climatiques.
Pour la majorité des traits un H^2 modéré a été observé, alors que le Q_{st} élevé indique ici une forte différenciation entre populations. La susceptibilité à *D. sapinea* est corrélée aux fortes températures. Les SNPs (Single Nucleotide Polymorphisms) associés aux traits ont un faible effet génotype, signe de la nature polygénique de ces traits.

La deuxième partie s'inscrit dans le projet européen GenTree, destiné à étudier l'adaptation génétique et le potentiel évolutif des populations naturelles d'arbres. Nous nous sommes intéressés aux populations de pin maritime d'Espagne, Italie et France, et de pin sylvestre (*Pinus sylvestris*) d'Espagne, Allemagne, Lituanie et Finlande. Vingt-cinq arbres par population ont été phénotypés pour la hauteur, diamètre, densité du bois, surface des aiguilles (SLA) et discrimination isotopique

du carbone. Des graines récoltées sur les arbres phénotypés, en conservant la structure familiale, ont servi à établir des jardins en Espagne et en France pour le pin maritime et dans les quatre pays d'origine du pin sylvestre. Dans chacun des jardins, toutes les populations de l'espèce ont été plantées, de façon à mimer la régénération naturelle. Les germinations, survie et stades ontologiques ont été évalués durant un (*P. pinaster*) et deux ans (*P. sylvestris*). Nous avons estimé les valeurs de performance, qui possèdent fort un effet population, et les gradients de sélection. La plupart des traits adultes sont significatifs pour ces gradients, et on observe une tendance entre les espèces : *P. sylvestris* présente majoritairement des gradients relatifs à la taille de la mère (hauteur et diamètre), alors que les gradients de *P. pinaster* sont relatifs à la SLA et à la discrimination isotopique du carbone.

La dernière partie s'intéresse aux populations corses. Cette île a la particularité de présenter un seul gene-pool du pin maritime qui a réussi à s'adapter aux environnements très divers de ce territoire. Nous avons bénéficié de la collection PINCORSE, composée de familles issues de 33 populations corses, lesquelles ont été phénotypées sur plusieurs années pour la hauteur, et certaines aussi la discrimination isotopique du carbone. Nous avons pu estimer h^2 (héritabilité au sens restreint) et le Q_{st} de ces traits, et l'utilisation de 50k nouveaux SNPs nous a permis d'indentifier les populations marginales.

Ces études novatrices apportent de nouvelles données sur les capacités adaptatives du pin maritime, lesquelles sont essentielles au programme d'amélioration génétique de l'espèce et aux stratégies de production et de conservation des ressources génétiques dans des environnements en plein bouleversement.

Mots-clefs : adaptation, changement climatique, jardins communs, corrélations génétiques, associations génétiques, différenciation

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General Introduction

Little doubt is left regarding the causes of the ongoing global changes: anthropogenic actions are the actors of an unprecedented natural crisis. Greenhouse gas emissions and changes in land use are responsible for climate change and the disruption of biodiversity (Vitousek, 1992). The consequences affect the equilibrium of natural elements, causing a raise of temperatures on Earth (IPCC, 2014), perturbations of sea levels and high flood-risks for coastal habitats (Kulp & Strauss, 2019), extreme weather events such as hurricanes, provoked by changes in atmospheric conditions (Dale *et al.*, 2001), and heavy rain, which, coupled with deforestation, cause landslides (Buma & Dehn, 1998). Global change also affects human society, changing the paradigm in agricultural exploitations and highlighting social injustice, as people whose resources come from nature find themselves greatly challenged (Thomas & Twyman, 2005). The crisis is mainly ecological, and we are facing a mass extinction comparable to the Cretaceous-Tertiary boundary (Vitousek, 1992). We stand witness to the important loss of biodiversity (Reusch & Wood, 2007), extinction of species, both terrestrial such as birds (Jetz *et al.*, 2007) and marine such as corals (Hoegh-Guldberg *et al.*, 2007), and most worryingly, the degradation of complex ecosystems such as forests (Seidl *et al.*, 2017).

When facing critical changes in their habitat, the first alternative to extinction adopted by many species is to modify their geographical distribution by migrating towards more favourable conditions (Aitken *et al.*, 2008). When migrating capacities fall short of their needs, as is the case with most trees (Berg *et al.*, 2010), organisms can rely on phenotypic plasticity and rapidly adjust to their new environment, by expressing different combination of traits (Benito Garzón *et al.*, 2011). Finally, species can respond to environmental pressure by going through evolutionary processes, leading to changes in the genetic composition of populations within a geographically defined space. This process, known as local adaptation, offers species the possibility to remain adapted in-situ for extended amounts of time (Kawecki & Ebert, 2004; Berg *et al.*, 2010).

These responses to changing environments are crucial to decipher so as to be able to preserve ecosystemic balances. Forest ecosystems stand out in this concern by the different roles they play and their multiple representations. Summing up to 30% of the global terrestrial vegetation (Costanza *et al.*, 1997), forest trees produce and sequester carbon, filter air and water (Lind *et al.*, 2018). Some forests are home to the richest biodiversities found on Earth, from microscopic insect species to millennial trees, and a great number of representatives of the animal reign. A good

example of this diversity is the Amazonian rainforest, but such forests are also present in Central Africa and Asia (Gentry, 1988). These forests are also inhabited by indigenous human communities that have been living off the forest for generations, with deep respect and consideration for the ecosystem's balance. This way of life has impacted the genetics, demography and evolutionary history of forest dwellers (Lopez *et al.*, 2018). The relationship they have with their environment could almost be qualified as "family-like", as is the case of the Maoris in New-Zealand and the kauri tree (Bradshaw *et al.*, 2019). Moreover, thorough knowledge of their ecosystems and their resources can confer to these communities an almost supernatural status, as it is the case with Pygmies and their farmer neighbours in Central Africa (Bahuchet & Guillaume, 1982). Forests also have an important social role in Occidental culture, as "social/psychological" value is one of the four main categories of ecosystem services attributed to forests in a public poll (Ford *et al.*, 2017). Finally, forests have a more down-to-earth, everyday-life value, as they are exploited in several industries. Challenged as they are by changing environmental conditions (Turner, 2010), forests are currently facing episodes of high tree mortality and perturbations (Castro *et al.*, 2009; Seidl *et al.*, 2017). As the southern part of Europe is already suffering from climatic constraints (Sala, 2000), a tree species emblematic of the Mediterranean basin and southwestern Atlantic area was selected as a model to study climate change response in forest trees: maritime pine (*Pinus pinaster* Aiton).

Figure 1. Adult *Pinus pinaster* stand in the Landes forest, Gironde, France

Pinus pinaster is a long-lived, monoecious species, typically established in coastal environments on sandy or poor soils, in altitudes ranging from sea level to 2600 m a.s.l., with optimal annual precipitation of 850 mm. Pollen dispersal is rather high (de-Lucas *et al.*, 2008) and seeds are wind-dispersed. It is characterized by long generation time (~30 years), and can live up to 500 years. Individuals in this species can be as high as 40 m, their bark is thick, deeply fissured and dark red-brown, needles are 15-20 cm long and dark green (Rameau *et al.*, 1989). Present in Spain, Corsica, southwestern France, western Portugal, northern Morocco, Tunisia, Algeria and northwestern Italy, this species is discontinuously distributed across its natural range, as a consequence of survival in multiple glacial refugia (Bucci *et al.*, 2007; Naydenov *et al.*, 2014). Limited gene flow across the different groups - or gene pools - and genetic drift result in a strong genetic structure in the species (Jaramillo-Correa *et al.*, 2015) (Figure 1), and led to great variation in morphological traits, including height (Alía *et al.*, 1995) and trunk straightness (Durel & Bahrman, 1995). This variation can also be observed in adaptive traits such as those related to tree physiology (Corcuera *et al.*, 2012) and resistance to fire (Fernandes & Rigolot, 2007).

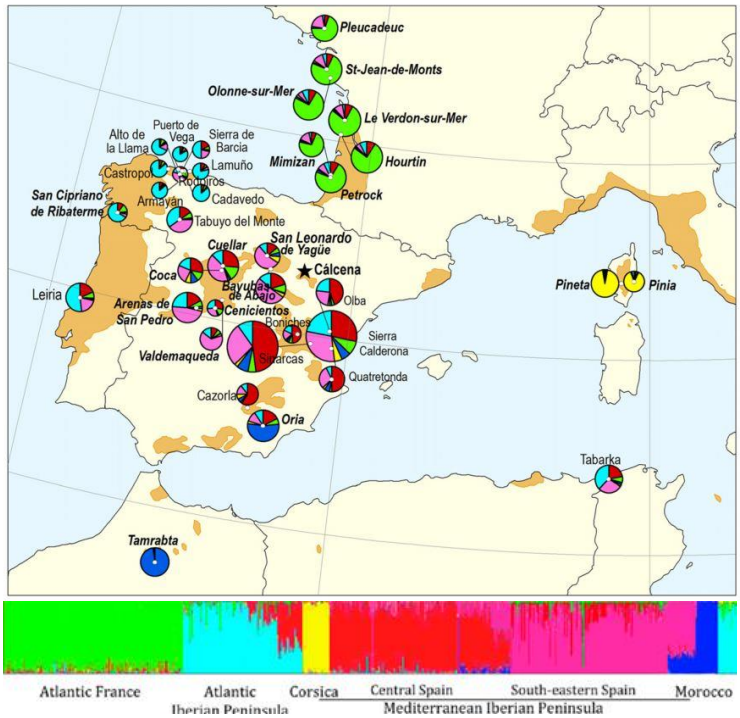

Figure 2. Genetic structure across the different gene-pools on the natural distribution *of Pinus pinaster* (from Jaramillo-Correa *et al.*, 2015)

Generally, *P. pinaster* forests are exploited for industry, as the species is involved in several sectors: wood products (timber, furniture), non-wood products (pulp, paper) and chemicals (turpentine). It is also used for landscaping and reforestation. In France, the forestry sector is very important, especially in the Nouvelle-Aquitaine region (southwestern France) where the Landes forest represents one of the main financial resources of the region, with up to 3.5 billion euros of turnover in the woodworking industry and 34 000 jobs (www.nouvelle-aquitaine.fr). Planted in 1857 to prevent the littoral dunes from moving inland and as a sanitation project in a marsh area, the Landes forest now covers 1.3 million hectares and is thus Europe's largest plantation forest (Labbé, 2015).

The importance of production and wood quality means that biotic attacks on *P. pinaster* are closely monitored. The repartition of pests and pathogens threatening *P. pinaster* populations varies, and not all gene pools suffer the same pressure. Moreover, pathogen ranges can extend quickly, due to involuntary, cautionless anthropogenic actions. Among the most common and dangerous, the following can be found:

- *Heterobasidion annosum*
- *Armillaria ostoyae*
- *Diplodia sapinea*
- *Melampsora pinitorqua*
- *Bursaphelenchus xylophilus*, transmitted via the genus *Monochamus*
- *Matsucoccus feytaudi*
- *Thaumatopea pityocampa*

Heterobasidion annosum and *A. ostoyae* are both root pathogens, with similar spreading strategies: they can spread by root contact and their spores can infect healthy stands. They provoke root rot and cause high tree mortality (Mesanza & Iturritxa, 2012; Heinzelmann *et al.*, 2018). *M. pinitorqua* is the agent of pine rust and causes cankers on branches, resulting in tissue asphyxiation, which can lead to deformation, branch mortality and individual mortality. The cycle of the pathogen involves another tree species, the aspen (*Populus tremula*) (Desprez-Loustau & Baradat, 1991). *D. sapinea* is an opportunist endophyte, able to infect the tree without symptoms until the host suffers stress (drought, hail); in which case it can infect all tissues of the tree, notably causing tip

blight and blue stain disease (Piou *et al.*, 1991). Interestingly, wood infected by *D. sapinea* seems to be favoured for infection by *B. xylophilus* (Futai *et al.*, 2007). This nematode creates subcortical cavities, enabling attacks by other agents such as *Monochamus* (Vicente *et al.*, 2012). *M. feytaudi* larvae feed of elaborated sap, leaving the host in a weakened state, suitable for other infections (Jactel *et al.*, 1998). Finally, *T. pityocampa*, the pine processionary moth, is a severe defoliator (Régolini *et al.*, 2014) and a health issue for humans and animals (Battisti *et al.*, 2011).

While these threats are widespread in the current repartition of *P. pinaster*, ongoing environmental changes are already causing shifts in their range and pathogenicity (Battisti *et al.*, 2006; Desprez-Loustau *et al.*, 2006; Brodde *et al.*, 2019). The increased selective pressures make understanding the genetic basis underlying local adaptation of *P. pinaster* all the more crucial, in particular under combined abiotic and biotic stress that can lead to trade-offs between adaptive traits.

A first step to understanding local adaptation is studying the phenotypic outcome of selective pressure, that is to say adaptive traits, such as growth and phenology. As adaptive traits are often complex, it is hard to study them in natural populations where too many confounding effects are in action (for instance, phenotypic plasticity, demographic history, genetic drift) (De Villemereuil *et al.*, 2016). Key tools to study these traits in ecology and genetics are therefore the experiments in common gardens (Morgenstern, 2011). These structures gather large numbers of individuals in a single environment, unifying environmental pressure and easing field observations. Most of the time, common gardens are designed according to a focus, like representing the whole distribution of a species or to studying populations along a gradient (Rellstab *et al.*, 2015). Furthermore, while half-sib gardens allow to compute the narrow sense heritability of the studied traits, clonal gardens give more statistical power to the observations made. Though incredibly informative, common gardens only allow studies on a single generation, so they do not provide evolutionary perspectives. To study evolutionary potential, the implementation of cross-generational studies is of paramount importance. For example, monitoring the growth of seedlings in sowing experiments to record progeny survival and fitness of parents can provide valuable insights on the evolutionary potential of populations (e.g. Vizcaíno-Palomar et al., 2014).

Regardless of the recent forward leaps in genomic research, molecular data is still scarce in the case of maritime pine. As most conifers, its genome is of great size and complexity (Mackay *et*

al., 2012), and it is not a model species. However, genotypic efforts have recently been made, allowing some genotype-phenotype association studies. These studies' aim is to identify factors that shape adaptive genetic variation and the gene variants driving local adaptation (Rellstab *et al.*, 2015). Recent associations studies have therefore been successful in correlating Single Nucleotide Polymorphisms (SNPs) with adaptive traits such as growth (Cabezas *et al.*, 2015), stem straightness (Bartholomé *et al.*, 2016) and even with the proportion of serotinous cones, a trait related to fire-adaptation (Budde *et al.*, 2014). Most interestingly, Yeaman *et al.* (2016) used a combination of 47 genes and 17 diverse phenotypic traits to detect convergent local adaptation in two different conifers species. In addition, as genotyping possibilities increase with Next Generation Sequencing (NGS) techniques, data on complex genomes are becoming easier to obtain.

The goal of this PhD thesis is to contribute to our understanding of the genetic basis of local adaptation in maritime pine using integrated research methods. Together, the three chapters explore the evolutionary potential of *P. pinaster*. Big datasets were produced, both on genotyping and phenotyping levels which, if they are not fully exploited in these chapters, will be used in similar projects and therefore are going to produce more results to be integrated in this research. Overall, the objectives of this PhD were to investigate several adaptive traits using quantitative genetics approaches (e.g. estimation of heritability), and compare signatures of local adaptation at different levels (within and across gene-pools, and across generations) and in different environments across the geographical distribution of maritime pine. More specifically:

Chapter 1 aims to untangle the genetic correlations between height, phenology and susceptibility to pests and pathogens. This study benefited from the CLONAPIN common garden, a clonal garden representing 512 genotypes of maritime pine all across its distribution range. Moreover, 6100 SNPs (Single Nucleotide Polymorphisms) were available for all the clones. One pest, *Thaumetopoea pityocampa*, and two pathogens, *Armillaria ostoyae* and *Diplodia sapinea*, were targeted for susceptibility assessments. Height and phenology were measured in CLONAPIN in 2015 and 2017, presence or absence of *T. pityocampa* nests evaluated in spring 2018 and finally, two novel protocols on excised branches were developed to observe the susceptibility to *A. ostoyae* and *D. sapinea*. Best Linear Unbiased Predictors (BLUPs) were computed for each trait, as well as broad-sense heritability H^2 and quantitative genetic differentiation, Q_{ST}. A genotype/phenotype

association was conducted based on trait BLUPs and the available SNP set. There was a strong population effect for all the traits and very little variation within-populations (i.e. among clones). The SNPs associated with the traits had small genotype effects (< 5%), as it is typical in these studies, indicating a polygenic nature for the studied traits. Most interestingly, there was a strong negative correlation between susceptibility to *D. sapinea* and maximum temperature in the stand of origin. Moreover, a strong negative correlation was observed between susceptibility to each pathogen, possibly reflecting different defence mechanisms.

Chapter 2 translates the question of local adaptation to the first stages of establishment (germination and early survival). It gives a temporal perspective as I evaluated a main component of phenotypic trait change across generations, i.e. the selection gradient. This study consisted in evaluating fitness and detecting selection gradients across European populations of *P. pinaster* and *P. sylvestris* (Scots pine). Reciprocal regeneration gardens were sowed all across Europe and monitored for one year (*P. pinaster*) and two years (*P. sylvestris*) for germination, survival and height. In addition, the mother-trees were phenotyped on the sampling sites for height, diameter at breast height (DBH), wood density (WD), specific leaf area (SLA) and carbon isotope discrimination ($\delta^{13}C$). First, mixed models were used to estimate components of fitness, which revealed a strong population effect. Selection gradients were then tested by running linear models using the family estimates of the components of fitness. Though most of the mother-trait variables were involved in significant selection gradients, both relevance and strength of selection gradients are highly variable across species and populations. A trend was detected between the two species: selection gradients in *P. pinaster* were mainly correlated with needle traits, whereas those in *P. sylvestris* are more related to mother size trait. This Chapter is set in the context of the H2020 European project GenTree (http://www.gentree-h2020.eu).

Chapter 3 narrowed the study to focus on a single gene-pool of maritime pine, the one present on the French island of Corsica. This particularity allowed to avoid the confounding effects of wide-range population structure when searching for adaptation signals. Despite forming a single gene pool due to extensive gene flow, pines from this area had to adapt to a high number of contrasted environments, meaning that populations are expected to have evolved under different selective pressure. Thirty of these populations are represented in the common garden PINCORSE, which presents the asset of being build using families and thus allows for estimation of narrow-sense

heritability. All families were phenotyped for height over several years, and a restricted number of them also for $\delta^{13}C$. Narrow-sense heritability, h^2, along with quantitative genetic differentiation, Q_{ST}, were estimated for these traits. Moreover, ~100k novel SNPs enabled us to test for genetic structure between populations and detect marginal population of interest for conservation on the island.

By associating classical quantitative genetics with novel protocols and considerable genotyping effort, this PhD brings valuable information for selection in the objective of breeding programs and conservation of maritime pine forest genetic resources. These programs are essential to the preservation of an ecosystemic balance in the current context of global change.

References

Aitken SN, Yeaman S, Holliday JA, Wang T, Curtis-McLane S. **2008**. Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evolutionary Applications* **1**: 95–111.

Alía R, Gil L, Pardos J. **1995**. Performance of 43 *Pinus pinaster* Ait. provenances on 5 locations in Central Spain. *Silvae genetica* **44**: 75–81.

Bahuchet S, Guillaume H. **1982**. Aka-farmer relations in the northwest Congo Basin. In: Leacock E, Lee R, eds. Politics and history in band societies. 198–210.

Bartholomé J, Bink MC, van Heerwaarden J, Chancerel E, Boury C, Lesur I, Isik F, Bouffier L, Plomion C. **2016**. Linkage and Association Mapping for Two Major Traits Used in the Maritime Pine Breeding Program: Height Growth and Stem Straightness (T Yin, Ed.). *PLOS ONE* **11**: e0165323.

Battisti A, Holm G, Fagrell B, Larsson S. **2011**. Urticating Hairs in Arthropods: Their Nature and Medical Significance. *Annual Review of Entomology* **56**: 203–220.

Battisti A, Stastny M, Buffo E, Larsson S. **2006**. A rapid altitudinal range expansion in the pine processionary moth produced by the 2003 climatic anomaly. *Global Change Biology* **12**: 662–671.

Benito Garzón M, Alía R, Robson TM, Zavala MA. **2011**. Intra-specific variability and plasticity influence potential tree species distributions under climate change. *Global Ecology and Biogeography* **20**: 766–778.

Berg MP, Kiers ET, Driessen G, van der Heijden M, Kooi BW, Kuenen F, Liefting M, Verhoef H, Ellers J. **2010**. Adapt or disperse: understanding species persistence in a changing world. *Global Change Biology* **16**: 587–598.

Bradshaw RE, Bellgard SE, Black A, Burns BR, Gerth ML, McDougal RL, Scott PM, Waipara NW, Weir BS, Williams NM, *et al.* 2019. *Phytophthora agathidicida* : Research progress, cultural perspectives and knowledge gaps in the control and management of kauri dieback in New Zealand. *Plant Pathology*: ppa.13104.

Brodde L, Adamson K, Julio Camarero J, Castaño C, Drenkhan R, Lehtijärvi A, Luchi N, Migliorini D, Sánchez-Miranda Á, Stenlid J, *et al.* 2019. Diplodia Tip Blight on Its Way to the North: Drivers of Disease Emergence in Northern Europe. *Frontiers in Plant Science* **9**.

Bucci G, González-Martínez SC, Le Provost G, Plomion C, Ribeiro MM, Sebastiani F, Alía

R, Vendramin GG. **2007**. Range-wide phylogeography and gene zones in *Pinus pinaster* Ait. revealed by chloroplast microsatellite markers. *Molecular Ecology* **16**: 2137–2153.

Budde KB, Heuertz M, Hernández-Serrano A, Pausas JG, Vendramin GG, Verdú M, González-Martínez SC. **2014**. In situ genetic association for serotiny, a fire-related trait, in Mediterranean maritime pine (*Pinus pinaster*). *New Phytologist* **201**: 230–241.

Buma J, Dehn M. **1998**. A method for predicting the impact of climate change on slope stability. *Environmental Geology* **35**: 190–196.

Cabezas JA, González-Martínez SC, Collada C, Guevara MA, Boury C, de María N, Eveno E, Aranda I, Garnier-Géré PH, Brach J, *et al.* 2015. Nucleotide polymorphisms in a pine ortholog of the *Arabidopsis* degrading enzyme cellulase KORRIGAN are associated with early growth performance in *Pinus pinaster* (R Sederoff, Ed.). *Tree Physiology* **35**: 1000–1006.

Castro J, McDowell N, Bachelet D, Cobb N, Lim J-H, Running SW, Breshears DD, Gonzalez P, Vennetier M, Semerci A, *et al.* 2009. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* **259**: 660–684.

Corcuera L, Gil-Pelegrin E, Notivol E. **2012**. Differences in hydraulic architecture between mesic and xeric *Pinus pinaster* populations at the seedling stage. *Tree Physiology* **32**: 1442–1457.

Costanza R, D'Arge R, de Groot R, Farber S, Grasso M, Hannon B, Limburg K, Naeem S, O'Neill R V., Paruelo J, *et al.* 1997. The value of the world's ecosystem services and natural capital. *Nature* **387**: 253–260.

Dale VH, Joyce LA, McNulty S, Neilson RP, Ayres MP, Flannigan MD, Hanson PJ, Irland LC, Lugo AE, Peterson CJ, *et al.* 2001. Climate Change and Forest Disturbances. *BioScience* **52**: 723–734.

Desprez-Loustau M-L, Baradat PH. **1991**. Variation in susceptibility to twisting rust of maritime pine. *Annals of Forest Science* **48**: 497–511.

Desprez-Loustau M-L, Marçais B, Nageleisen L-M, Piou D, Vannini A. **2006**. Interactive effects of drought and pathogens in forest trees. *Annals of Forest Science* **63**: 597–612.

Durel C-E, Bahrman N. **1995**. Analyse de la diversité génétique des peuplements de pin maritime de Corse. Gestion et exploitation de la ressource génétique. *Revue Forestière Française* **5**: 509–522.

Fernandes PM, Rigolot E. **2007**. The fire ecology and management of maritime pine (*Pinus pinaster* Ait.). *Forest Ecology and Management* **241**: 1–13.

Ford RM, Anderson NM, Nitschke C, Bennett LT, Williams KJH. **2017**. Psychological values and cues as a basis for developing socially relevant criteria and indicators for forest management. *Forest Policy and Economics* **78**: 141–150.

Futai K, Sriwati R, Takemoto S. **2007**. Cohabitation of the pine wood nematode, *Bursaphelenchus xylophilus*, and fungal species in pine trees inoculated with *B. xylophilus*. *Nematology* **9**: 77–86.

Gentry AH. **1988**. Tree species richness of upper Amazonian forests. *Proceedings of the National Academy of Sciences* **85**: 156–159.

Heinzelmann R, Dutech C, Tsykun T, Labbé F, Soularue J-P, Prospero S. **2018**. Latest advances and future perspectives in *Armillaria* research. *Canadian Journal of Plant Pathology* **41**: 1–23.

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, *et al.* 2007. Coral Reefs Under Rapid Climate Change and Ocean Acidification. *Science* **318**: 1737–1742.

Intergovernmental Panel on Climate Change. **2014**. *Climate Change 2013 - The Physical Science Basis* (Intergovernmental Panel on Climate Change, Ed.). Cambridge: Cambridge University Press.

Jactel H, Menassieu P, Ceria A, Burban C, Regad J, Normand S, Carcreff E. **1998**. Une pullulation de la cochenille *Matsucoccus feytaudi* provoque un début de dépérissement du Pin maritime en Corse. *Revue Forestière Française* **50**: 33.

Jaramillo-Correa JP, Rodríguez-Quilón I, Grivet D, Lepoittevin C, Sebastiani F, Heuertz M, Garnier-Géré P, Alía R, Plomion C, Vendramin GG, *et al.* 2015. Molecular proxies for climate maladaptation in a long-lived tree (*Pinus pinaster* Aiton, Pinaceae). *Genetics* **199**: 793–807.

Jetz W, Wilcove DS, Dobson AP. **2007**. Projected Impacts of Climate and Land-Use Change on the Global Diversity of Birds (GM Mace, Ed.). *PLoS Biology* **5**: e157.

Kawecki TJ, Ebert D. **2004**. Conceptual issues in local adaptation. *Ecology Letters* **7**: 1225–1241.

Kulp SA, Strauss BH. **2019**. New elevation data triple estimates of global vulnerability to sea-

level rise and coastal flooding. *Nature Communications* **10**: 4844.

Labbé F. **2015**. Étude de l'émergence et de la dynamique évolutive d'*Armillaria ostoyae*, agent pathogène du pin maritime.

Lind BM, Menon M, Bolte CE, Faske TM, Eckert AJ. **2018**. The genomics of local adaptation in trees: are we out of the woods yet? *Tree Genetics & Genomes* **14**: 29.

Lopez M, Kousathanas A, Quach H, Harmant C, Mouguiama-Daouda P, Hombert JM, Froment A, Perry GH, Barreiro LB, Verdu P, *et al.* 2018. The demographic history and mutational load of African hunter-gatherers and farmers. *Nature Ecology and Evolution* **2**: 721–730.

Mackay J, Dean JFD, Plomion C, Peterson DG, Cánovas FM, Pavy N, Ingvarsson PK, Savolainen O, Guevara MÁ, Fluch S, *et al.* 2012. Towards decoding the conifer giga-genome. *Plant Molecular Biology* **80**: 555–569.

Mesanza N, Iturritxa E. **2012**. Root and butt rot caused by Heterobasidion annosum in Atlantic coniferous ecosystems of Spain (P Lakomy, Ed.). *Forest Pathology* **42**: 514–520.

Morgenstern M. **2011**. *Geographic Variation in Forest Trees: Genetic Basis and Application of Knowledge in Silviculture*. Vancouver: University of British Columbia Press.

Naydenov KD, Alexandrov A, Matevski V, Vasilevski K, Naydenov MK, Gyuleva V, Carcaillet C, Wahid N, Kamary S. **2014**. Range-wide genetic structure of maritime pine predates the last glacial maximum: evidence from nuclear DNA. *Hereditas* **151**: 1–13.

Piou D, Chandelier P, Morelet M. **1991**. *Sphaeropsis sapinea*, un nouveau problème sanitaire des Pins en France ? *Revue Forestière Française* **43**: 203.

Rameau JC, Mansion D, Dumé G. **1989**. Flore Forestière Française (guide écologique illustré), tomes 1 « Plaines et collines » et 2 « Montagnes ». *édition IDF*.

Régolini M, Castagneyrol B, Dulaurent-Mercadal A-M, Piou D, Samalens J-C, Jactel H. **2014**. Effect of host tree density and apparency on the probability of attack by the pine processionary moth. *Forest Ecology and Management* **334**: 185–192.

Rellstab C, Gugerli F, Eckert AJ, Hancock AM, Holderegger R. **2015**. A practical guide to environmental association analysis in landscape genomics. *Molecular Ecology* **24**: 4348–4370.

Reusch TBH, Wood TE. **2007**. Molecular ecology of global change. *Molecular Ecology* **16**: 3973–3992.

Sala OE. **2000**. Global Biodiversity Scenarios for the Year 2100. *Science* **287**: 1770–1774.

Seidl R, Thom D, Kautz M, Martin-Benito D, Peltoniemi M, Vacchiano G, Wild J, Ascoli D, Petr M, Honkaniemi J, *et al.* 2017. Forest disturbances under climate change. *Nature Climate Change* **7**: 395–402.

Thomas DSG, Twyman C. **2005**. Equity and justice in climate change adaptation amongst natural-resource-dependent societies. *Global Environmental Change* **15**: 115–124.

Turner MG. **2010**. Disturbance and landscape dynamics in a changing world. *Ecology* **91**: 2833–2849.

Vicente C, Espada M, Vieira P, Mota M. **2012**. Pine Wilt Disease: A threat to European forestry. *European Journal of Plant Pathology* **133**: 89–99.

De Villemereuil P, Gaggiotti OE, Mouterde M, Till-Bottraud I. **2016**. Common garden experiments in the genomic era: New perspectives and opportunities. *Heredity* **116**: 249–254.

Vitousek PM. **1992**. Global Environmental Change: An Introduction. *Annual Review of Ecology and Systematics* **23**: 1–14.

Yeaman S, Hodgins KA, Lotterhos KE, Suren H, Nadeau S, Degner JC, Nurkowski KA, Smets P, Wang T, Gray LK, *et al.* 2016. Convergent local adaptation to climate in distantly related conifers. *Science* **353**: 1431–1433.

Chapter 1: 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. The maritime pine (*Pinus pinaster*), a conifer widely distributed in south-western Europe and, to a lesser extent, in North Africa, grows under contrasted environmental conditions promoting local adaptation.

Genetic variation at 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 pines (*Pinus pinaster* Aiton).

Broad-sense heritability was significant for height (0.497), needle phenology (0.231-0.468) and pathogen symptoms (necsosis length caused by *D. sapinea*, 0.413 and by *A. ostoyae*, 0.066), measured after inoculation under controlled conditions, but not for pine processionary moth incidence assessed in the common garden. Genetic correlations among populations between traits revealed contrasting trends for pathogen susceptibility to *D. sapinea* and *A. ostoyae*. Higher trees showed longer necrosis length, caused by *D. sapinea*, while smaller trees showed longer necrosis length caused by *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 species' 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, drought and insect outbreaks, while warmer and wetter climates will probably increase storm and pathogen incidence on forests (Seidl *et al.*, 2017), leading to episodes of high tree mortality (Castro *et al.*, 2009) and consequently, 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. Knowledge about genetic variation in a trait and its heritability determine the potential of human mediated or naturally selected change in this trait. Genes underlying adaptive traits can 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 on 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). 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 have 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 significant 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. This 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 on 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 variations 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 for 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's* 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 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 six millions years, 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* has co-existed for a long time with maritime pines in Europe (Labbé *et al.*, 2017a) and has consequently 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 several thousand years. In maritime pines, the severity of the symptoms is related to 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).

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. For the pathogen inoculation experiments we choose samples from populations in the clonal common garden representing each of the six gene pools. However, due to higher logistical effort, it was not possible to include all genotypes in these experiments (see Table S1.1, Supporting Information).

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 the temperature variability between years into account. 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 database (Benoît Laurent, personal communication). Only current-year shoots at 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 three 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 12th and July 31st 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 in a heat chamber set at 23°C and 80% humidity, during three months 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 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 at least 60% jar occupation by *A. ostoyae* 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 onthe 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 the 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 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*, μ denotes the 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). During preliminary data analyses for height, we also tested the "gene pool" effect while populations were nested within gene pools and genotypes were nested within populations.

However, the gene pool level did not show any significant effect and was therefore not included in the final models.

All models were fitted in a Bayesian framework using Markov chain Monte Carlo (MCMC) methods implemented in the R package *MCMCglmm* (Hadfield, 2010) using R v.3.4.1 (R Core Team, 2017). 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, which followed a binomial distribution and were respectively modeled with *logit* and *probit* link functions. 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) :

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

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

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 presented in the following formula (Spitze, 1993):

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

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 on 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 of > 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 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 the 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 on 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 those from the French Atlantic gene pool (Figure 1B). Incidence of pine processionary moth nests in the common garden was not significantly heritable (H^2_p: 0.031 [0.000-0.246]).

The importance of population effect in several traits was also highlighted by high Q_{ST} values (ranging from 0.191 for bud burst in 2017 and duration of bud burst in 2015 to 0.636 for necrosis length caused by *D. sapinea*) 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*.

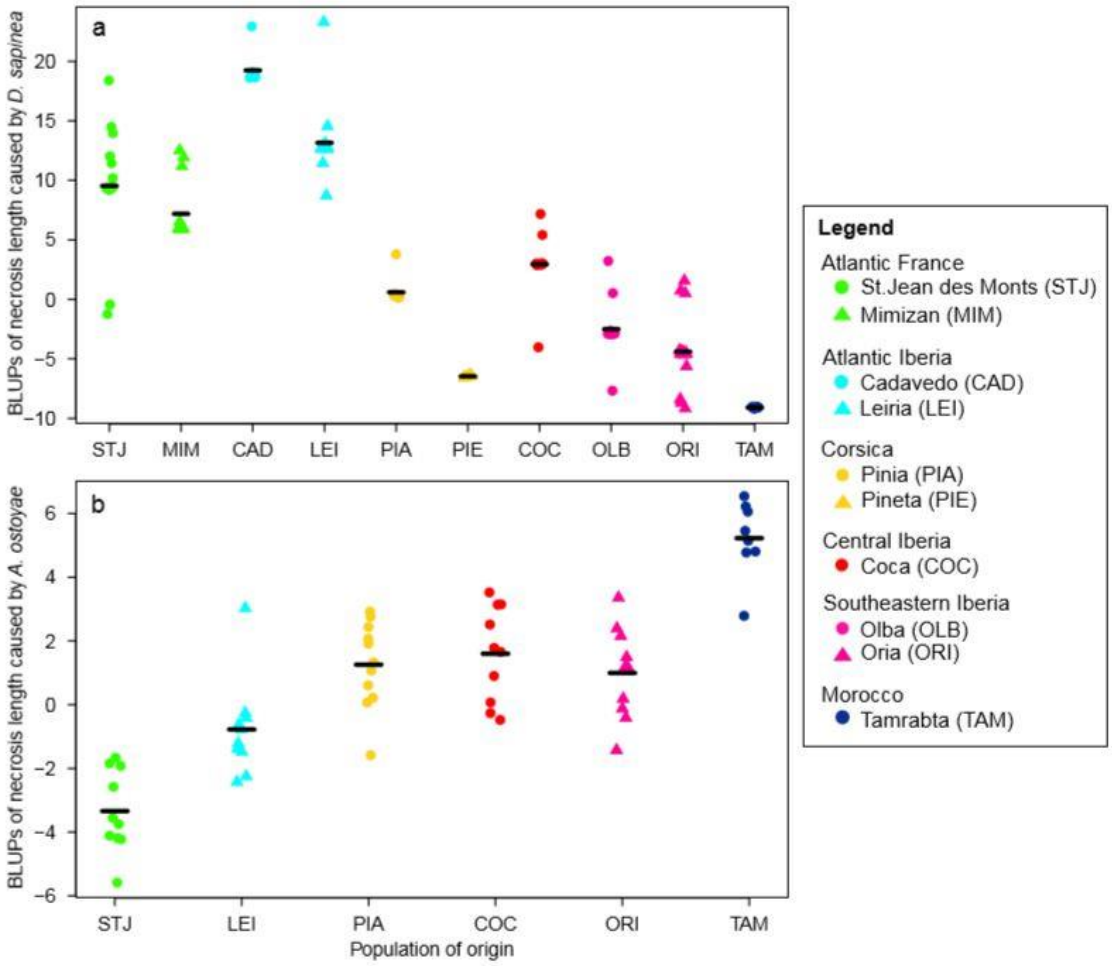

Figure 1. Stripchart of the best linear unbiased predictors (BLUPs, including both genotype and population effect) of necrosis length caused by *D. sapinea* (A) and *A. ostoyae* (B) for each of the *Pinus pinaster* populations included in each experiment. Populations were assigned to one of six gene 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.

	Phenotypic mean	Variability	H^2	H^2_p	Q_{ST}	Evolvability
height2015 (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
Armillaria 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
Diplodia 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
Diplodia 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
Processionary2015	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 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.

Correlations between traits and with environmental variables

The genetic correlation (including the population and genotype effect) between necrosis lengths 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 on the genotype level (without the population effects) 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 during 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. Although necrosis length caused by *A. ostoyae* showed a longitudinal cline (-0.895, p-value=0.031) no significant correlation with climate factors was found.

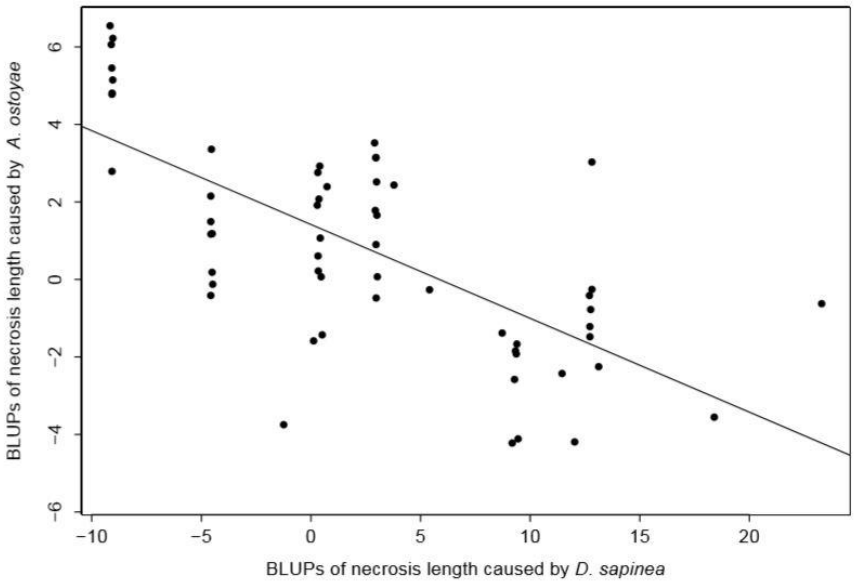

Figure 2. Genetic correlation of necrosis length caused by *Diplodia sapinea* and *Armillaria ostoyae* based on best linear unbiased predictors (BLUPs, including both clone and population effect). A linear trend line is also shown.

	bb2015	dbb2015	bb2017	dbb2017	Diplodia necrosis	Diplodia disc.	Armillaria necrosis
height2015	0.533**	0.426*	0.192	0.902***	0.770**	0.551	-0.845*
bb2015		0.889***	0.735***	0.533**	0.828**	0.768**	-0.534
dbb2015			0.687***	0.404*	0.782**	0.821**	-0.432
bb2017				0.214	0.410	0.492	-0.056
dbb2017					0.792**	0.611	-0.724
Diplodia necrosis						0.854**	-0.859*
Diplodia disc.							-0.426

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

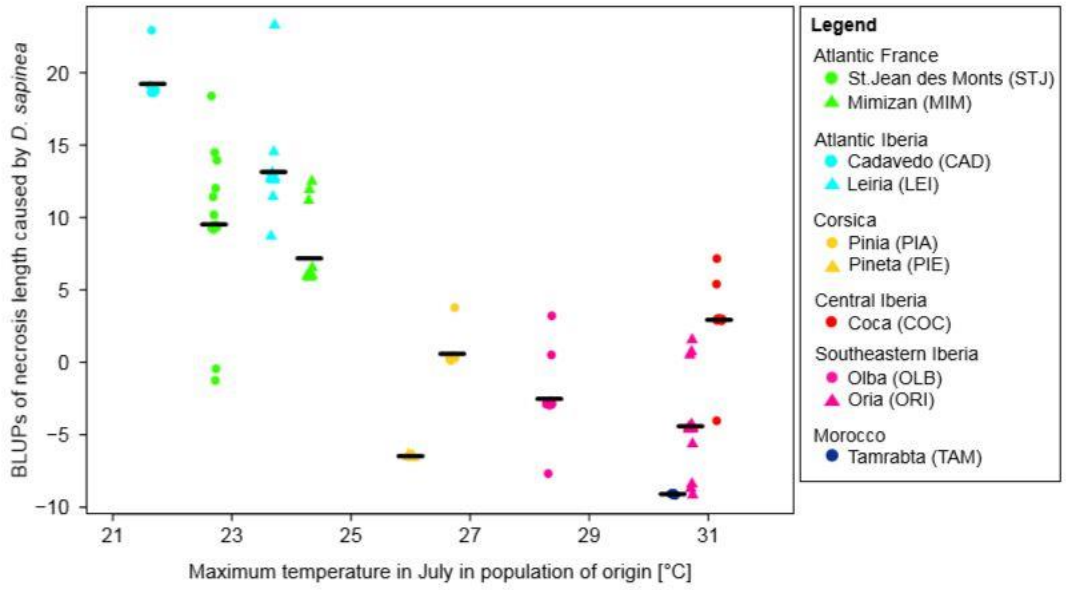

Figure 3. Stripchart of necrosis length caused by *Diplodia sapinea* (BLUPs, including both clone and population effect) plotted against the maximum temperature in July in each *Pinus pinaster* population of origin. Black lines indicate the average necrosis length in each population.

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 the different phenology traits and measurement years altogether), and eight with pathogen susceptibility (Table 4). In total, four significantly associated SNPs showed non-synonymous changes. Two non-synonymous SNPs were significantly associated with bud burst in 2017 (Figure S6.1, Supporting Information) and one non-synonymous SNP was associated with each needle discoloration caused by *D. sapinea* and duration of bud burst in 2015 (Table 5, Figure 4 and Figure S6.2, Supporting Information).

Trait	*N*	Latitude	Longitude	Altitude	meanT^{a}July	maxT^{a}July	maxT^{a}Aug	precJan	precFeb	precAug	precSep
height2015	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***
Diplodia necrosis	10	-0.483	0.618	-0.762*	-0.827**	-0.78*	-0.769*	0.741*	0.671	0.546	0.569
Diplodia disc.	10	-0.485	0.327	-0.536	-0.738*	-0.691*	-0.69*	0.733*	0.733*	0.265	0.302
Armillaria necrosis	6	-0.011	-0.895*	0.832	0.594	0.782	0.746	-0.373	-0.129	-0.678	-0.712

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, mean temperature; Tmax, 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.

Trait	SNP name	SNP motif	Site annotation	LG	MAF	MLMs			BMLMs		
						F	*P*	R^2	Mean	(95% CIs)	
height2015	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
Armillaria 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
Diplodia 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
Diplodia 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 4. SNPs significantly associated to height, spring 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). Diplodia disc., Diplodia needle discoloration.

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
Diplodia disc.	BX679001-1418	[T/C]	Isoleucine - Valine	Translation initiation factor eIF-5

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). Diplodia disc., Diplodia needle discoloration

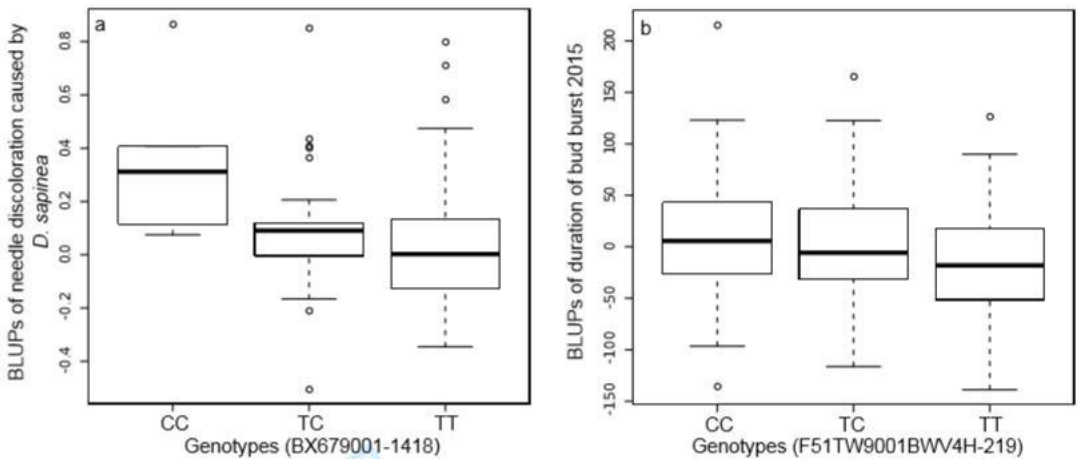

Figure 4. Genotypic effects (box plots) for two exemplary single nucleotide polymorphisms (SNPs) showing significant association with needle discoloration caused by *Diplodia sapinea* (a) and duration of bud burst in 2015 (b) in *Pinus pinaster*.

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 usually reflect the species' population genetic structure (Figure 5).

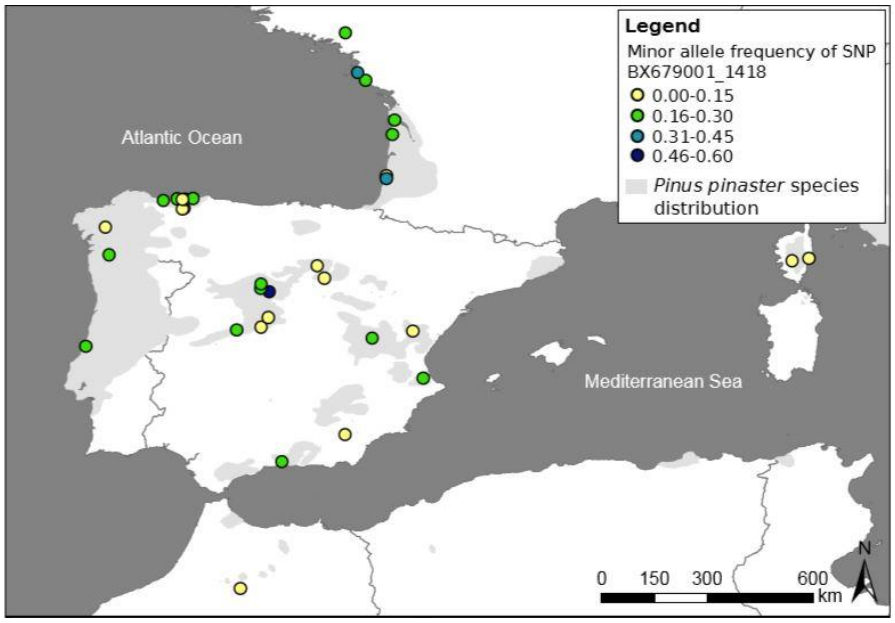

Figure 5. Minor allele frequency distribution of SNP BX679001_1418 in natural populations of *Pinus pinaster*. This locus was significantly associated to needle discoloration caused by *Diplodia sapinea*.

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 pines for incidence of pine processionary moth nests and response to two pathogenic fungi, *D. sapinea* and *A. ostoyae*, on a range-wide scale, by using trees grown in a clonal common garden and novel inoculation protocols 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 pathogens. We found a strong population effect for height, needle phenology and infection-related traits. Susceptibility variations 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 that several loci were significantly associated with pathogen susceptibility, height and phenological traits in maritime pines. 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

Population level heritability reflects the genetic differences between populations, which are not necessarily due to selection but might also reflect other processes, e.g. drift. Nevertheless, it provides important insights on trait variation among populations. Genetic correlations among populations 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. Notably, maximum temperatures during the 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 caused by drought (Luchi *et al.*, 2014). Maritime pine populations growing in these regions - such as Morocco and southern Spain - would then be more likely to have developped 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 the 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, whilst 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 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, on both population and genotype levels. Intra-population variability of susceptibility to *A. ostoyae* was higher than for *D. sapinea*, where no significant variability on the intra-population level was found. Our results indicated that maritime pine trees 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 on *A. ostoyae* from the southern 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* on 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, which might be key to 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, even 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 it 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 the high susceptibility of this population of maritime pine. In the case of *A. ostoyae*, the main threat resides in the host's condition. 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 temperature 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 has shown 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 promotes 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 elapsed 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 took advantage of a range-wide clonal common garden of maritime pine to provide estimates of the genetic variability and heritability within and among populations for pathogen response, height and needle phenology traits. We revealed strong divergence of several adaptive traits, especially height and necrosis length caused by *D. sapinea* across maritime pine populations. We have shown that several adaptive traits in maritime pines 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, as well as emergent pests and pathogens expanding their range (Seidl *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 of genetic responses to emerging pests and pathogens will help ensure the health of forests in the future.

References

Adamson K, Klavina D, Drenkhan R, Gaitnieks T, Hanso M. **2015**. *Diplodia sapinea* is colonizing the native Scots pine (*Pinus sylvestris*) in the northern Baltics. *European Journal of Plant Pathology* **143**: 343–350.

Agosta SJ, Klemens JA. **2008**. Ecological fitting by phenotypically flexible genotypes: implications for species associations, community assembly and evolution. *Ecology Letters* **11**: 1123–1134.

Alberto FJ, Derory J, Boury C, Frigerio J-M, Zimmermann NE, Kremer A. **2013**. Imprints of natural selection along environmental gradients in phenology-related genes of *Quercus petraea*. *Genetics* **195**: 495–512.

Alía R, Chambel R, Notivol E, Climent J, González-Martínez SC. **2014**. Environment-dependent microevolution in a Mediterranean pine (*Pinus pinaster* Aiton). *BMC Evolutionary Biology* **14**: 200.

Aranda I, Alía R, Ortega U, Dantas ÂK, Majada J. **2010**. Intra-specific variability in biomass partitioning and carbon isotopic discrimination under moderate drought stress in seedlings from four *Pinus pinaster* populations. *Tree Genetics & Genomes* **6**: 169–178.

Badeck F-W, Bondeau A, Bottcher K, Doktor D, Lucht W, Schaber J, Sitch S. **2004**. Responses of spring phenology to climate change. *New Phytologist* **162**: 295–309.

Bartholomé J, Bink MC, van Heerwaarden J, Chancerel E, Boury C, Lesur I, Isik F, Bouffier L, Plomion C. **2016**. Linkage and association mapping for two major traits used in the Maritime Pine breeding program: Height growth and stem straightness. *PLoS ONE* **11**: e0165323.

Battisti A, Stastny M, Buffo E, Larsson S. **2006**. A rapid altitudinal range expansion in the pine processionary moth produced by the 2003 climatic anomaly. *Global Change Biology* **12**: 662–671.

Beaulieu J, Doerksen T, Boyle B, Clément S, Deslauriers M, Beauseigle S, Blais S, Poulin P-L, Lenz P, Caron S, *et al.* 2011. Association genetics of wood physical traits in the conifer white spruce and relationships with gene expression. *Genetics* **188**: 197–214.

Blodgett JT, Bonello P. **2003**. The aggressiveness of *Sphaeropsis sapinea* on Austrian pine varies with isolate group and site of infection. *Forest Pathology* **33**: 15–19.

Boutte B. 2018. *Chronologie et localisation des dégâts Indice de sévérité*. Note de service DSF, France

Bradbury PJ, Zhang Z, Kroon DE, Casstevens TM, Ramdoss Y, Buckler ES. **2007**. TASSEL: Software for association mapping of complex traits in diverse samples. *Bioinformatics* **23**: 2633–2635.

Brodde L, Adamson K, Julio Camarero J, Castaño C, Drenkhan R, Lehtijärvi A, Luchi N, Migliorini D, Sánchez-Miranda Á, Stenlid J, *et al.* 2019. Diplodia tip blight on its way to the north: drivers of disease emergence in northern Europe. *Frontiers in Plant Science* **9**.

Bucci G, González-Martínez SC, Le Provost G, Plomion C, Ribeiro MM, Sebastiani F, Alía R, Vendramin GG. **2007**. Range-wide phylogeography and gene zones in *Pinus pinaster* Ait. revealed by chloroplast microsatellite markers. *Molecular Ecology* **16**: 2137–2153.

Budde KB, Heuertz M, Hernández-Serrano A, Pausas JG, Vendramin GG, Verdú M, González-Martínez SC. **2014**. In situ genetic association for serotiny, a fire-related trait, in Mediterranean maritime pine (*Pinus pinaster*). *New Phytologist* **201**: 230–241.

Burban C, Petit RJ, Carcreff E, Jactel H. **1999**. Rangewide variation of the maritime pine bast scale *Matsucoccus feytaudi* Duc. (*Homoptera: Matsucoccidae*) in relation to the genetic structure of its host. *Molecular Ecology* **8**: 1593–1602.

Burdon JJ, Thrall PH. **2000**. Coevolution at multiple spatial scales: *Linum marginale-Melampsora lini* - From the individual to the species. *Evolutionary Ecology* **14**: 261–281.

Burgess TI, Wingfield MJ, Wingfield BD. **2004**. Global distribution of *Diplodia pinea* genotypes revealed using simple sequence repeat (SSR) markers. *Australasian Plant Pathology* **33**: 513.

Cabezas JA, González-Martínez SC, Collada C, Guevara MA, Boury C, de María N, Eveno E, Aranda I, Garnier-Géré PH, Brach J, *et al.* 2015. Nucleotide polymorphisms in a pine ortholog of the *Arabidopsis* degrading enzyme cellulase KORRIGAN are associated with early growth performance in *Pinus pinaster*. *Tree Physiology* **35**: 1000–1006.

Castro J, McDowell N, Bachelet D, Cobb N, Lim J-H, Running SW, Breshears DD, Gonzalez P, Vennetier M, Semerci A, *et al.* 2009. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* **259**: 660–684.

Chapin FS, Autumn K, Pugnaire F. **1993**. Evolution of Suites of Traits in Response to Environmental Stress. *The American Naturalist* **142**: S78–S92.

Chou CKS. **1978**. Penetration of young stems of *Pinus radiata* by *Diplodia pinea*. *Physiological Plant Pathology* **13**: 189–192.

Cobb FW, Kristic M, Zavarin E. **1968**. Inhibitory effects of volatile oleoresin components on *Fomes annosus* and four *Ceratocystis* species. *Phytopathology* **58**: 1327–1335.

Corcuera L, Gil-Pelegrin E, Notivol E. **2012**. Differences in hydraulic architecture between mesic and xeric *Pinus pinaster* populations at the seedling stage. *Tree Physiology* **32**: 1442–1457.

Cornelius J. **1994**. Heritabilities and additive genetic coefficients of variation in forest trees. *Canadian Journal of Forest Research* **24**: 372–379.

Correia I, Almeida MH, Aguiar A, Alía R, David TS, Pereira JS. **2008**. Variations in growth, survival and carbon isotope composition (delta^{13}C) among *Pinus pinaster* populations of different geographic origins. *Tree Physiology* **28**: 1545–1552.

Cruickshank MG, Morrison D, Punja ZK. **1997**. Incidence of *Armillaria* species in precommercial thinning stumps and spread of *Armillaria ostoyae* to adjacent Douglas-fir trees. *Canadian Journal of Forest Research* **27**: 481–490.

Delzon S, Bresson CC, Michalet R, Vitasse Y, Kremer A. **2009**. Altitudinal differentiation in growth and phenology among populations of temperate-zone tree species growing in a common garden. *Canadian Journal of Forest Research* **39**: 1259–1269.

Desprez-Loustau M-L, Aguayo J, Dutech C, Hayden KJ, Husson C, Jakushkin B, Marçais B, Piou D, Robin C, Vacher C. **2016**. An evolutionary ecology perspective to address forest pathology challenges of today and tomorrow. *Annals of Forest Science* **73**: 45–67.

Desprez-Loustau M-L, Baradat PH. **1991**. Variation in susceptibility to twisting rust of maritime pine. *Annals of Forest Science* **48**: 497–511.

Desprez-Loustau M-L, Marçais B, Nageleisen L-M, Piou D, Vannini A. **2006**. Interactive effects of drought and pathogens in forest trees. *Annals of Forest Science* **63**: 597–612.

Eckert AJ, Bower AD, Wegrzyn JL, Pande B, Jermstad KD, Krutovsky K V., St. Clair JB, Neale DB. **2009**. Association genetics of coastal Douglas fir (*Pseudotsuga menziesii* var. *menziesii*, Pinaceae). I. Cold-hardiness related traits. *Genetics* **182**: 1289–1302.

Elvira-Recuenco M, Iturritxa E, Majada J, Alía R, Raposo R. **2014**. Adaptive potential of maritime pine (*Pinus pinaster*) populations to the emerging pitch canker pathogen, *Fusarium circinatum*. *PLoS ONE* **9**: e114971.

Elzinga JA, Atlan A, Biere A, Gigord L, Weis AE, Bernasconi G. **2007**. Time after time: flowering phenology and biotic interactions. *Trends in Ecology and Evolution* **22**: 432–439.

Ennos RA. **2015**. Resilience of forests to pathogens: An evolutionary ecology perspective. *Forestry* **88**: 41–52.

Eveno E, Collada C, Guevara MÁ, Léger V, Soto A, Díaz L, Léger P, González-Martínez SC, Cervera MT, Plomion C, *et al.* 2008. Contrasting patterns of selection at *Pinus pinaster* Ait. drought stress candidate genes as revealed by genetic differentiation analyses. *Molecular Biology and Evolution* **25**: 417–437.

Falush D, Stephens M, Pritchard JK. **2007**. Inference of population structure using multilocus genotype data: Dominant markers and null alleles. *Molecular Ecology Notes* **7**: 574–578.

Ferguson BA, Dreisbach TA, Parks CG, Filip GM, Schmitt CL. **2003**. Coarse-scale population structure of pathogenic *Armillaria* species in a mixed-conifer forest in the Blue Mountains of northeast Oregon. *Canadian Journal of Forest Research* **33**: 612–623.

Freeman JS, Hamilton MG, Lee DJ, Pegg GS, Brawner JT, Tilyard PA, Potts BM. **2019**. Comparison of host susceptibilities to native and exotic pathogens provides evidence for pathogen-imposed selection in forest trees. *New Phytologist* **221**: 2261–2272.

Robinson GK. **1991**. That BLUP is a good thing: The estimation of random effects. *Statistical Science* **6**: 15–32.

Gardes M, Bruns TD. **1993**. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**: 113–118.

Gaspar MJ, Velasco T, Feito I, Alía R, Majada J. **2013**. Genetic variation of drought tolerance in *Pinus pinaster* at three hierarchical levels: A comparison of induced osmotic stress and field testing. *PLoS ONE* **8**: e79094.

Gelman A. **2006**. Prior distribution for variance parameters in hierarchical models. *Bayesian Analysis* **1**: 515–533.

Gelman A, Van Dyk DA, Huang Z, Boscardin WJ. **2008**. Using redundant parameterizations to fit hierarchical models. *Journal of Computational and Graphical Statistics* **17**: 95–122.

Gelman A, Rubin DB. **2007**. Inference from iterative simulation using multiple sequences. *Statistical Science* **7**: 457–472.

Georgieva M, Hlebarska S. **2017**. A review of *Sphaeropsis sapinea* occurrence on *Pinus* species in Bulgaria. *Journal of Bioscience and Biotechnology* **5**: 247–250.

Ghelardini L, Santini A. **2009**. Avoidance by early flushing: a new perspective on Dutch elm disease research. *iForest - Biogeosciences and Forestry* **2**: 143–153.

Goldfarb B, Neale DB, Wegrzyn JL, Davis JM, Lee JM, Liechty JD, Quesada T, Langley CH, Cumbie WP, Eckert AJ, *et al.* 2013. The evolutionary genetics of the genes underlying phenotypic associations for loblolly pine (*Pinus taeda*, Pinaceae). *Genetics* **195**: 1353–1372.

González-Martínez SC, Alía R, Gil L. **2002**. Population genetic structure in a Mediterranean pine (*Pinus pinaster* Ait.): A comparison of allozyme markers and quantitative traits. *Heredity* **89**: 199–206.

Gonzalo J. **2007**. *Phytoclimatic analysis of the Spanish Peninsula: update and geostatistical analysis.* PhD thesis, University of Valladolid, Palencia, Spain.

Grivet D, Sebastiani F, Alía R, Bataillon T, Torre S, Zabal-Aguirre M, Vendramin GG, González-Martínez SC. **2011**. Molecular footprints of local adaptation in two mediterranean conifers. *Molecular Biology and Evolution* **28**: 101–116.

Guillaumin J-J, Lung-Escarmant B, Legrand P. **2005**. Sensibilité des hôtes ligneux à l'armillaire et sélection pour la tolérance. In: Guillaumin J-J ed. *L'armillaire et le pourridié-agraric des végétaux ligneux*. Paris: INRA, 377–388.

Hadfield JD. **2010**. MCMC Methods for multi-response generalized linear mixed models: The MCMCglmm R Package. *Journal of Statistical Software* **33**.

Hamilton MG, Williams DR, Tilyard PA, Pinkard EA, Wardlaw TJ, Glen M, Vaillancourt RE, Potts BM. **2013**. A latitudinal cline in disease resistance of a host tree. *Heredity* **110**: 372–379.

Hanewinkel M, Cullmann DA, Schelhaas M-J, Nabuurs G-J, Zimmermann NE. **2013**. Climate change may cause severe loss in the economic value of European forest land. *Nature Climate Change* **3**: 203–207.

Hanna JW, Warwell MV, Maffei H, Fairweather ML, Blodgett JT, Zambino PJ, Worral J, Burns KS, Jacobs JJ, Ashiglar SM, *et al.* 2016. Bioclimatic modeling predicts potential distribution of *Armillaria solipides* and *Pseudotsuga menziesii* (Douglas-Fir) under contemporary and changing climates in the interior Western USA. In: Proceedings of the 63rd Annual Western International Forest Disease Work Conference.

Hardy OJ, Vekemans X. **2002**. SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Molecular Ecology Notes* **2**: 618–620.

Heinzelmann R, Dutech C, Tsykun T, Labbé F, Soularue J-P, Prospero S. **2018**. Latest advances and future perspectives in *Armillaria* research. *Canadian Journal of Plant Pathology* **41**: 1–23.

Heinzelmann R, Rigling D. **2016**. Mycelial fan formation of three sympatric *Armillaria* species on excised stem segments of *Picea abies*. *Forest Pathology* **46**: 187–199.

Henderson CR. **1973**. Sire evaluation and genetic trends. *Journal of Animal Science* **1973**: 10–41.

Herms DA, Mattson WJ. **2004**. The Dilemma of Plants: To Grow or Defend. *The Quarterly Review of Biology* **67**: 283–335.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. **2005**. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* **25**: 1965–1978.

Holliday J a., Ritland K, Aitken SN. **2010**. Widespread, ecologically relevant genetic markers developed from association mapping of climate-related traits in Sitka spruce (*Picea sitchensis*). *New Phytologist* **188**: 501–514.

Hopkins MT, Lampi Y, Wang T-W, Liu Z, Thompson JE. **2008**. Eukaryotic Translation Initiation Factor 5A Is Involved in Pathogen-Induced Cell Death and Development of Disease Symptoms in *Arabidopsis*. *Plant Physiology* **148**: 479–489.

Houle D. **1992**. Comparing evolvability and variability of quantitative traits. *Genetics* **130**: 195–204.

IPCC. **2014**. *Climate Change 2014: Synthesis Report*. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp

Iturritxa E, Ganley R, Raposo R, García-Serna I, Mesanza N, Kirkpatrick SC, Gordon TR. **2013**. Resistance levels of Spanish conifers against *Fusarium circinatum* and *Diplodia pinea*. *Forest Pathology* **43**: 488–495.

Jacquet J-S, Bosc A, O'Grady AP, Jactel H. **2013**. Pine growth response to processionary moth defoliation across a 40-year chronosequence. *Forest Ecology and Management* **293**: 29–38.

Jactel H, Barbaro L, Battisti A, Bosc A, Branco M, Brockerhoff E, Castagneyrol B, Dulaurent AM, Hódar JA, Jacquet J-S, *et al.* 2015. Insect-tree interactions in *Thaumetopoea pityocampa*. In: Roque A ed. *Processionary Moths and Climate Change: An Update*. 265–310.

Jaramillo-Correa JP, Rodríguez-Quilón I, Grivet D, Lepoittevin C, Sebastiani F, Heuertz M, Garnier-Géré P, Alía R, Plomion C, Vendramin GG, *et al.* 2015. Molecular proxies for climate maladaptation in a long-lived tree (*Pinus pinaster* Aiton, Pinaceae). *Genetics* **199**: 793–807.

Kawecki TJ, Ebert D. **2004**. Conceptual issues in local adaptation. *Ecology Letters* **7**: 1225–1241.

Kenis M, Li H, Fan J, Courtial B, Auger-Rozenberg M-A, Yart A, Eschen R, Roques A. **2018**. Sentinel nurseries to assess the phytosanitary risks from insect pests on importations of live plants. *Scientific Reports* **8**: 11217.

Koch RA, Wilson AW, Séné O, Henkel TW, Aime MC. **2017**. Resolved phylogeny and biogeography of the root pathogen *Armillaria* and its gasteroid relative, *Guyanagaster*. *BMC Evolutionary Biology* **17**: 33.

Kremer A, Lascoux M. **1988**. Genetic architecture of height growth in maritime pine (*Pinus pinaster* Ait.). *Silvae Genetica* **37**: 1–8.

Krokene P, Lahr E, Dalen LS, Skrøppa T, Solheim H. **2012**. Effect of phenology on susceptibility of Norway spruce (*Picea abies*) to fungal pathogens. *Plant Pathology* **61**: 57–62.

Kubiak K, Żółciak A, Damszel M, Lech P, Sierota Z. **2017**. *Armillaria* pathogenesis under climate changes. *Forests* **8**: 100.

Kuhlman EG, Amerson H V., Wilcox PL, Liu BH, O'Malley DM, Sederoff RR. **2002**. Detection of a major gene for resistance to fusiform rust disease in loblolly pine by genomic mapping. *Proceedings of the National Academy of Sciences* **93**: 3859–3864.

de la Mata R, Merlo E, Zas R. **2014**. Among-population variation and plasticity to drought of Atlantic, Mediterranean, and interprovenance hybrid populations of maritime pine. *Tree Genetics & Genomes* **10**: 1191–1203.

de la Torre AR, Puiu D, Crepeau MW, Stevens K, Salzberg SL, Langley CH, Neale DB. **2019**. Genomic architecture of complex traits in loblolly pine. *New Phytologist* **221**: 1789–1801.

Labbé F, Fontaine MC, Robin C, Dutech C. **2017a**. Genetic signatures of variation in population size in a native fungal pathogen after the recent massive plantation of its host tree. *Heredity* **119**: 402–410.

Labbé F, Lung-Escarmant B, Fievet V, Soularue J-P, Laurent C, Robin C, Dutech C. **2017b**. Variation in traits associated with parasitism and saprotrophism in a fungal root-rot pathogen invading intensive pine plantations. *Fungal Ecology* **26**: 99–108.

Labbé F, Marçais B, Dupouey J-L, Bélouard T, Capdevielle X, Piou D, Robin C, Dutech C. **2015**. Pre-existing forests as sources of pathogens? The emergence of *Armillaria ostoyae* in a recently planted pine forest. *Forest Ecology and Management* **357**: 248–258.

Lamy J-B, Bouffier L, Burlett R, Plomion C, Cochard H, Delzon S. **2011**. Uniform selection as a primary force reducing population genetic differentiation of cavitation resistance across a species range. *PLoS ONE* **6**: e23476.

Leimu R, Koricheva J. **2006**. A meta-analysis of genetic correlations between plant resistances to multiple enemies. *The American Naturalist* **168**: E15–E37.

Lepoittevin C, Harvengt L, Plomion C, Garnier-Géré P. **2012**. Association mapping for growth, straightness and wood chemistry traits in the *Pinus pinaster* Aquitaine breeding population. *Tree Genetics and Genomes* **8**: 113–126.

Li Z, Gopal V, Li X, Davis JM, Casella G. **2012**. Simultaneous SNP identification in association studies with missing data. *Annals of Applied Statistics* **6**: 432–456.

Lind BM, Friedline CJ, Wegrzyn JL, Maloney PE, Vogler DR, Neale DB, Eckert AJ. **2017**. Water availability drives signatures of local adaptation in whitebark pine (*Pinus*

albicaulis Engelm.) across fine spatial scales of the Lake Tahoe Basin, USA. *Molecular Ecology* **26**: 3168–3185.

Lind BM, Menon M, Bolte CE, Faske TM, Eckert AJ. **2018**. The genomics of local adaptation in trees: are we out of the woods yet? *Tree Genetics & Genomes* **14**: 29.

Lindner M, Maroschek M, Netherer S, Kremer A, Barbati A, Garcia-Gonzalo J, Seidl R, Delzon S, Corona P, Kolström M, *et al.* 2010. Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. *Forest Ecology and Management* **259**: 698–709.

Liu J-J, Sniezko RA, Sturrock RN, Chen H. **2014**. Western white pine SNP discovery and high-throughput genotyping for breeding and conservation applications. *BMC Plant Biology* **14**: 380.

Loiselle BA, Sork VL, Nason J, Graham C. **1995**. Spatial genetic structure of a tropical understory shrub, *Psychotria officinalis* (Rubiaceae). *American Journal of Botany* **82**: 1420–1425.

López-Goldar X, Villari C, Bonello P, Borg-Karlson AK, Grivet D, Zas R, Sampedro L. **2018**. Inducibility of plant secondary metabolites in the stem predicts genetic variation in resistance against a key insect herbivore in maritime pine. *Frontiers in Plant Science* **9**.

Luchi N, Oliveira Longa CM, Danti R, Capretti P, Maresi G. **2014**. *Diplodia sapinea*: The main fungal species involved in the colonization of pine shoots in Italy. *Forest Pathology* **44**: 372–381.

Lung-Escarmant B, Guyon D. **2004**. Temporal and spatial dynamics of primary and secondary infection by *Armillaria ostoyae* in a *Pinus pinaster* plantation. *Phytopathology* **94**: 125–131.

Lung-Escarmant B, Maugard F, Giraud A, Escrivant M, Molinier F, Merilleau F, Vida G. **2002**. Infectious cycle of *Armillaria ostoyae*. In: Laflamme G, Bérubé JA, Bussières G, eds. Proceedings of the 10th International Conference on Root and Butt Rot. Natural Resources Canada, Canadian Forest Service. Québec, CA, 428–431.

Marxmüller H, Guillaumin J-J. **2005**. Description et distribution des armillaires européennes. In: Guillaumin J-J ed. *L'armillaire et le pourridié-agraric des végétaux ligneux*. Paris, France, 63–84.

Meijón M, Feito I, Oravec M, Delatorre C, Weckwerth W, Majada J, Valledor L. **2016**. Exploring natural variation of *Pinus pinaster* Aiton using metabolomics: Is it possible to identify the region of origin of a pine from its metabolites? *Molecular Ecology* **25**: 959–976.

Menéndez-Gutiérrez M, Alonso M, Toval G, Díaz R. **2017**. Variation in pinewood nematode susceptibility among *Pinus pinaster* Ait. provenances from the Iberian Peninsula and France. *Annals of Forest Science* **74**: 76.

de Miguel M, Guevara MÁ, Sánchez-Gómez D, de María N, Díaz LM, Mancha JA, Fernández de Simón B, Cadahía E, Desai N, Aranda I, *et al.* 2016. Organ-specific metabolic responses to drought in *Pinus pinaster* Ait. *Plant Physiology and Biochemistry* **102**: 17–26.

Mimura M, Aitken SN. **2007**. Adaptive gradients and isolation-by-distance with postglacial migration in *Picea sitchensis*. *Heredity* **99**: 224–232.

Moran E, Lauder J, Musser C, Stathos A, Shu M. **2017**. The genetics of drought tolerance in conifers. *New Phytologist* **216**: 1034–1048.

Moreira X, Mooney KA, Rasmann S, Petry WK, Carrillo-Gavilán A, Zas R, Sampedro L. **2014**. Trade-offs between constitutive and induced defences drive geographical and climatic clines in pine chemical defences. *Ecology Letters* **17**: 537–546.

Morgenstern M. **2011**. *Geographic variation in forest trees: Genetic basis and application of knowledge in silviculture*. Vancouver, CA: University of British Columbia Press.

Nakagawa S, Schielzeth H. **2010**. Repeatability for Gaussian and non-Gaussian data: A practical guide for biologists. *Biological Reviews* **85**: 935–956.

Neale DB, Ersoz E, Wheeler NC, Nelson CD, González-Martínez SC. **2006**. Association Genetics in *Pinus taeda* L. I. Wood Property Traits. *Genetics* **175**: 399–409.

Neale DB, Kremer A. **2011**. Forest tree genomics: Growing resources and applications. *Nature Reviews Genetics* **12**: 111–122.

Nielsen LR, McKinney LV, Kjær ED. **2017**. Host phenological stage potentially affects dieback severity after Hymenoscyphus fraxineus infection in *Fraxinus excelsior* seedlings. *Baltic Forestry* **23**: 229–232.

Perry A, Brown AV, Cavers S, Cottrell JE, Ennos RA. **2016**. Has Scots pine (*Pinus sylvestris*) co-evolved with *Dothistroma septosporum* in Scotland? Evidence for spatial heterogeneity in the susceptibility of native provenances. *Evolutionary Applications* **9**: 982–993.

Petit RJ, Bahrman N, Baradat PH. **1995**. Comparison of genetic differentiation in maritime pine (*Pinus pinaster* ait.) estimated using isozyme, total protein and terpenic loci. *Heredity* **75**: 382–389.

Petit RJ, Bialozyt R, Garnier-Géré P, Hampe A. **2004**. Ecology and genetics of tree invasions: from recent introductions to Quaternary migrations. *Forest Ecology and Management* **197**: 117–137.

Petit RJ, Hampe A. **2006**. Some Evolutionary Consequences of Being a Tree. *Annual Review of Ecology, Evolution, and Systematics* **37**: 187–214.

Phillips MA, Croteau RB. **1999**. Resin-based defenses in conifers. *Trends in Plant Science* **4**: 184–190.

Piou D, Chandelier P, Morelet M. **1991**. *Sphaeropsis sapinea*, un nouveau problème sanitaire des Pins en France ? *Revue Forestière Française* **43**: 203.

Plomion C, Bartholomé J, Lesur I, Boury C, Rodríguez-Quilón I, Lagraulet H, Ehrenmann F, Bouffier L, Gion J-M, Grivet D, *et al.* 2016. High-density SNP assay development for genetic analysis in maritime pine (*Pinus pinaster*). *Molecular Ecology Resources* **16**: 574–587.

Pot D, McMillan L, Echt C, Le Provost G, Garnier-Géré P, Cato S, Plomion C. **2005**. Nucleotide variation in genes involved in wood formation in two pine species. *New Phytologist* **167**: 101–112.

Prospero S, Holdenrieder O, Rigling D. **2004**. Comparison of the virulence of *Armillaria cepistipes* and *Armillaria ostoyae* on four Norway spruce provenances. *Forest Pathology* **34**: 1–14.

Quesada T, Casella G, Goldfarb B, Cumbie WP, Gopal V, Eckert AJ, Huber D a., Davis JM, Wegrzyn JL, Neale DB. **2010**. Association Mapping of Quantitative Disease Resistance in a Natural Population of Loblolly Pine (*Pinus taeda* L.). *Genetics* **186**: 677–686.

R Development Core Team. **2017**. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org/.

Reich PB, Oleksyn J, Tjoelker MG. **1996**. Needle respiration and nitrogen concentration in scots pine populations from a broad latitudinal range: A common garden test with field-grown trees. *Functional Ecology* **10**: 768.

Resende RT, Resende MDV, Silva FF, Azevedo CF, Takahashi EK, Silva-Junior OB, Grattapaglia D. **2017**. Regional heritability mapping and genome-wide association identify loci for complex growth, wood and disease resistance traits in Eucalyptus. *New Phytologist* **213**: 1287–1300.

Rishbeth J. **2006**. Resistance to fungal pathogens of tree roots. *Proceedings of the Royal Society of London. Series B. Biological Sciences* **181**: 333–351.

Rodríguez-Quilón I. **2017**. *Ecological and association genetics in two Mediterranean pine species*. PhD Thesis, Universidad Politécnica de Madrid, Spain.

Rodríguez-Quilón I, Santos-del-Blanco L, Serra-Varela MJ, Koskela J, González-Martínez SC, Alía R. **2016**. Capturing neutral and adaptive genetic diversity for conservation in a highly structured tree species. *Ecological Applications* **26**: 2254–2266.

Salvador L, Alía R, Agúndez D, Gil L. **2000**. Genetic variation and migration pathways of maritime pine (*Pinus pinaster* Ait) in the Iberian Peninsula. *Theoretical and Applied Genetics* **100**: 89–95.

Savolainen O, Lascoux M, Merilä J. **2013**. Ecological genomics of local adaptation. *Nature Reviews Genetics* **14**: 807–820.

Savolainen O, Pyhäjärvi T, Knürr T. **2007**. Gene Flow and Local Adaptation in Trees. *Annual Review of Ecology, Evolution, and Systematics* **38**: 595–619.

Schvester D. **1982**. Incidence of *Matsucoccus feytaudi* Duc. on maritime pine of different origins (*Pinus pinaster* Ait.) in the Mediterranean region. *Comptes Rendus des Séances de l'Académie d'Agriculture de France* **68**: 1324–1333.

Seidl R, Thom D, Kautz M, Martin-Benito D, Peltoniemi M, Vacchiano G, Wild J, Ascoli D, Petr M, Honkaniemi J, *et al.* 2017. Forest disturbances under climate change. *Nature Climate Change* **7**: 395–402.

Shain L. **1967**. Resistance of sapwood in stems of loblolly pine to infection by *Fomes annosus*. *Phytopath.* **57**: 1034–1045.

Sniezko RA. **2010**. Resistance breeding against nonnative pathogens in forest trees — current successes in North America. *Canadian Journal of Plant Pathology* **28**: S270–S279.

Solla A, Aguín O, Cubera E, Sampedro L, Mansilla JP, Zas R. **2011**. Survival time analysis of *Pinus pinaster* inoculated with *Armillaria ostoyae*: genetic variation and relevance of seed and root traits. *European Journal of Plant Pathology* **130**: 477–488.

Solla A, Tomlinson F, Woodward S. **2002**. Penetration of *Picea sitchensis* root bark by *Armillaria mellea*, *Armillaria ostoyae* and *Heterobasidion annosum*. *Forest Pathology* **32**: 55–70.

Spitze K. **1993**. Population structure in *Daphnia obtusa*: quantitative genetic and allozymic variation. *Genetics* **135**: 367–74.

Stanosz GR, Blodgett JT, Smith DR, Kruger EL. **2002**. Water stress and *Sphaeropsis sapinea* as a latent pathogen of red pine seedlings. *New Phytologist* **149**: 531–538.

Stanosz GR, Trobaugh J, Guthmiller MA, Stanosz JC. **2004**. *Sphaeropsis* shoot blight and altered nutrition in red pine plantations treated with paper mill waste sludge. *Forest Pathology* **34**: 245–253.

Swart WJ, Wingfield MJ. **1991**. Biology and Control of *Sphaeropsis sapinea* on *Pinus* Species in South Africa. *Plant Disease* **75**: 761.

Swedjemark G, Stenlid J, Karlsson B. **1998**. Genetic variation among clones of *Picea abies* in resistance to growth of *Heterobasidion annosum*. *Silvae Genetica* **46**: 369–374.

Toïgo M, Barraquand F, Barnagaud JY, Piou D, Jactel H. **2017**. Geographical variation in climatic drivers of the pine processionary moth population dynamics. *Forest Ecology and Management* **404**: 141–155.

Tsykun T, Rigling D, Prospero S. **2013**. A new multilocus approach for a reliable DNA-based identification of *Armillaria* species. *Mycologia* **105**: 1059–1076.

Turner MG. **2010**. Disturbance and landscape dynamics in a changing world. *Ecology* **91**: 2833–2849.

Whitlock MC, Guillaume F. **2009**. Testing for spatially divergent selection: Comparing Q_{ST} to F_{ST}. *Genetics* **183**: 1055–1063.

Woolhouse MEJ, Haydon DT, Antia R. **2005**. Emerging pathogens: The epidemiology and evolution of species jumps. *Trends in Ecology and Evolution* **20**: 238–244.

Xu H, Cao D, Chen Y, Wei D, Wang Y, Stevenson RA, Zhu Y, Lin J. **2016**. Gene expression and proteomic analysis of shoot apical meristem transition from dormancy to activation in *Cunninghamia lanceolata* (Lamb.) Hook. *Scientific Reports* **6**: 19938.

Yu J, Pressoir G, Briggs WH, Bi IV, Yamasaki M, Doebley JF, McMullen MD, Gaut BS, Nielsen DM, Holland JB, *et al.* 2006. A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nature Genetics* **38**: 203–208.

Zas R, Solla A, Sampedro L. **2007**. Variography and kriging allow screening *Pinus pinaster* resistant to *Armillaria ostoyae* in field conditions. *Forestry* **80**: 201–209.

Zohner CM, Renner SS. **2014**. Common garden comparison of the leaf-out phenology of woody species from different native climates, combined with herbarium records, forecasts long-term change. *Ecology Letters* **17**: 1016–1025.

Zwolinski JB, Swart WJ, Wingfield MJ. **1990**. Intensity of dieback induced by *Sphaeropsis sapinea* in relation to site conditions. *Forest Pathology* **20**: 167–174.

Supplementary Information to Chapter 1

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

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S1 Samples included in this study from the CLONAPIN clonal common garden in Cestas

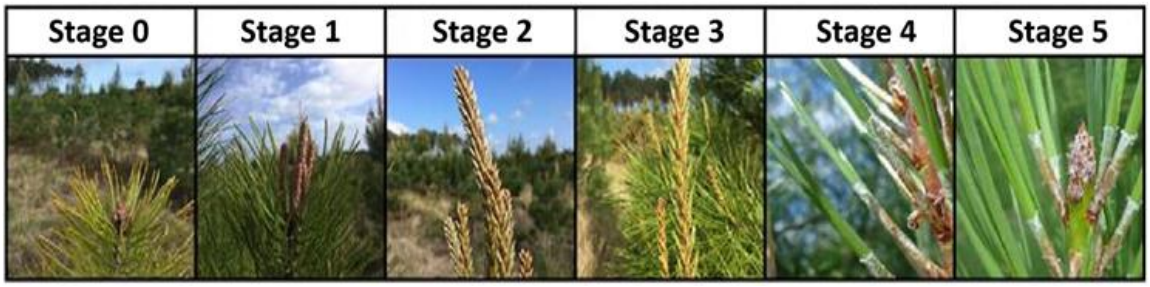
Table S1.1. Number of genotypes from the CLONAPIN clonal common garden used to study adaptive traits in *Pinus pinaster*. Bb, bud burst; dbb, duration of bud burst; proces., processionary moth nest; necr., necrosis length; disc., needle discoloration;

Population	Latitude	Longitude	Altitude (m)	Country	Height	Bb 2015	Dbb 2015	Bb 2017	Dbb 2017	Proces.	*D. sapinea* necr.	D. sapinea disc.	*A. ostoyae*. necr.
					n# genotypes								
Hourtin	45.18	-1.15	26	France	21	21	21	21	21	21			
Le Verdon	45.55	-1.09	11	France	21	21	21	18	21	21			
Mimizan	44.13	-1.3	37	France	16	16	16	14	16	16	16	15	
Olonne sur mer	46.57	-1.83	13	France	19	19	19	19	19	19			
Petrocq	44.06	-1.3	31	France	19	19	19	19	19	19			
Pleucadec	47.78	-2.34	80	France	18	18	18	17	18	18			
St-Jean des Monts	46.76	-2.03	6	France	23	23	23	23	23	23	23	22	10
Alto de la Llama	43.28	-6.49	503	Spain	7	7	7	6	7	7			
Armayán	43.31	-6.46	498	Spain	8	8	8	8	8	8			
Cadavedo	43.54	-6.42	210	Spain	10	10	10	9	9	10	9	9	
Sierra de Barcia	43.53	-6.49	240	Spain	6	6	6	5	6	6			
Castropol	43.5	-6.98	391	Spain	10	10	10	10	10	10			
Lamuño	43.56	-6.22	134	Spain	8	8	8	8	8	8			
Puerto de Vega	43.55	-6.63	121	Spain	7	7	7	5	7	7			
Sergude (Seed orchard)	42.82	-8.45	298	Spain	21	21	21	19	21	21			
San Cipriano de Ribaterme	42.12	-8.36	300	Spain	7	7	7	6	7	7			
Leiria	39.78	-8.96	20	Portugal	19	19	19	17	19	19	19	18	10

Pineta	41.97	9.04	750	France	7	7	7	7	7	7	7	7	
Pinia	42.02	9.47	10	France	14	14	14	14	14	14	14	13	10
Arenas de San Pedro	40.2	-5.12	733	Spain	14			14	14	14			
Valdemaqueda	40.52	-4.31	890	Spain	8	8	8	8	8	8			
Cenicientos	40.28	-4.49	1100	Spain	5	5	5	5	5	5			
Coca	41.26	-4.5	800	Spain	14	14	14	13	14	14	14	14	10
Cuellar	41.38	-4.48	830	Spain	25	25	25	20	25	25			
Carbonero el Mayor	41.17	-4.28	845	Spain	6	6	6	6	6	6			
Bayubas de Abajo	41.52	-2.88	998	Spain	19	19	19	19	19	19			
San Leonardo	41.84	-3.06	1096	Spain	10	10	10	10	10	10			
Boniches	39.99	-1.66	1104	Spain	6	6	6	6	6	6			
Olba	40.17	-0.62	1002	Spain	16	16	16	14	16	16	15	14	
Quatretonda	38.97	-0.36	435	Spain	15	15	15	13	15	15			
Cómpeta	36.83	-3.95	903	Spain	4	4	4	4	4	4			
Oria	37.53	-2.35	1223	Spain	23	23	23	23	23	23	23	23	10
Tamrabta	33.6	-5.02	1758	Morocco	9	9	9	9	9	9	9	9	8
Total number of genotypes					443	422	422	417	442	443	151	146	60
Total number of trees					3311	3146	3152	1440	1905	3330	453	438	180

S2 Phenological stages of bud burst

Figure S2.1. Phenological stages of bud burst: 0) bud without elongation, as at the end of winter, 1) elongation of the bud, 2) emergence of brachyblasts, 3) brachyblast begin to space, 4) elongation of the needles, 5) total elongation of the needles.

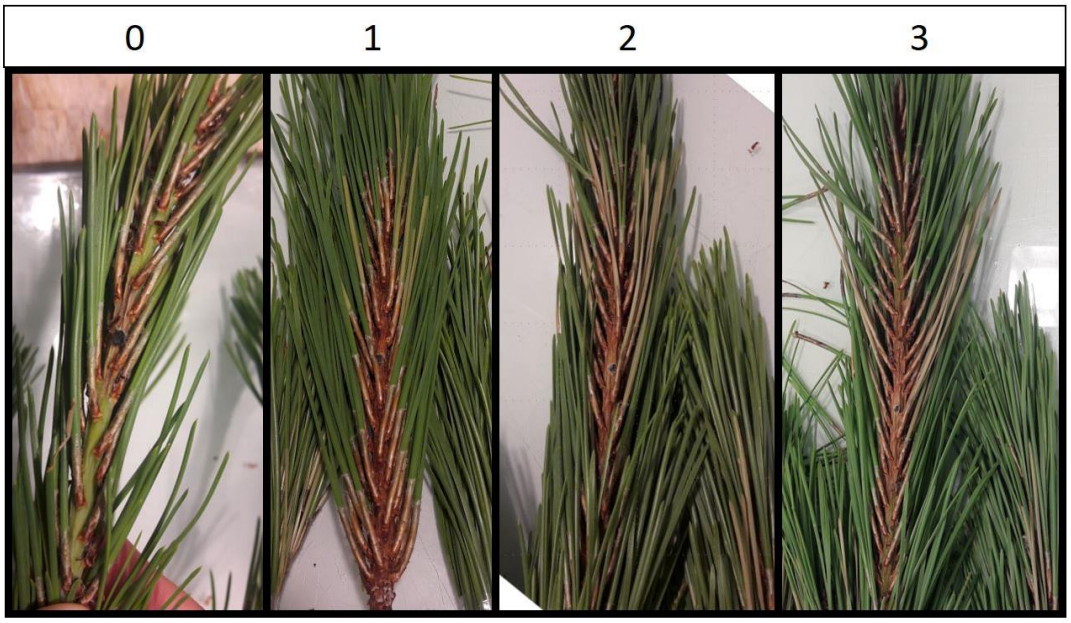


S3 Pathogen inoculations on excised branches

S3.1 Laboratory protocol for Diplodia sapinea inoculations

1. Under the fume hood with sterilized material, *Diplodia sapinea* strain Pier4 was subcultured into 15 malt-agar Petri dishes. The pathogen was left to grow at room temperature for 3 days, during which it colonized the whole surface of the malt-agar.
2. The colonized Petri dishes were kept at 4°C to stop growth.
3. Shoots were collected in the CLONAPIN common garden, the phenological stage was estimated and the diameter was measured with a caliper.
4. We removed a needle fascicle in the middle of each shoot with a scalpel, making a small wound.
5. On the wound, we placed a 5mm diameter plug of malt-agar infected with *D. sapinea*, the mycelial side of the plug on the wound.
6. To keep the plug in place, we carefully wrapped the shoot in 3cm-wide cellophane.
7. The shoots were placed each in a glass jar with water, and kept in a climatic chamber set at 20°C with a daily cycle of 12h of light and 12 of dark.
8. Six days after inoculation, we removed the cellophane and the plug. The length of the necrosis around the wound was measured with a caliper, and needle discoloration was estimated from 0 – no discoloration to 3 – all needles discoloured along the necrosis. Other observations, such as “resin at the inoculation point” and “necrosis reaches the bud” were made but not used in the analysis of this study.

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. 0) No discoloration along the necrosis 1) Up to 50% of the needles are partly of fully discoloured 2) More than 50% of the needles are partly or fully discoloured 3) All needles are discoloured along the necrosis



S3.2 Laboratory protocol for *Armillaria ostoyae* inoculations

1. For *A. ostoyae* inoculations we modified the protocols developed by Heinzelmann and Rigling (2016) and Ford et al. (2017). The liquid part of the medium consisted of 50% industrial vegetable soup (Knorr® 9 légumes) and 50% malt diluted in water (10 gr of malt for 500mL of water).
2. The mix was sterilized for 20 minutes at 120°C.
3. The solid part of the medium consisted of fresh hazelnut wood, sampled in Cestas (Nouvelle-Aquitaine, France) and chipped with an outdoor chipper. The chips were sieved then put in Sterilsop® bags (Hartmann®), sterilized a first time at 120°C for 20 min, placed in a heat chamber at 40°C to dry for 2h, then sterilized and dried a second time the same way. The bags were not opened during this process.
4. Bamboo sticks of approximatively 8cm length were cleaned following the same process (see step 3).
5. All material was sterilized under U.V light for 15 min under the fume hood before use.
6. Sterile laboratory jars (Dutscher®, 180mL) were filled with hazelnut chips placing one bamboo stick in the middle as a place holder for the excised branch. The remaining space was filled with liquid medium. The jars were sealed with sterile cotton, aluminum foil and tape and re-sterilized 20 minutes at 120°C.
7. When the medium was cold, we inoculated each jar with two plugs of malt-agar of 5 mm of diameter infected with *A.ostoyae* and closed the jar with a lid.
8. The inoculated jars where then placed in the dark with firmly closed lids. After 2 months, we could not observe significant growth of the fungus, and some of the jars had to be discarded because of important penicilium contamination inside the jar.
9. The remaining jars were placed in heat chambers at 23°C, 80% humidity with loosely closed lids to allow oxygenation (1/4 turn opened) (Lung-Escarmant, oral communication). After one month and a half, 180 jars showed satisfactory growth of *A.ostoyae*.
10. For each jar, the lid was opened, penicilium contamination estimated (on a scale from 0-no contamination to 5-very contaminated). Contaminated jars were safe to use as penicilium was only present on the surface of the mycelial culture. The bamboo stick was removed.
11. The lid was pierced with a Ø12mm drill, replaced on the jar, and a branch was put in place of the bamboo stick. All of the jars and branches were treated this way, then replaced in the heat chamber with the same settings with an additional 12h cycle of light/dark. The branches had a minimum length of 10 cm, and as the maximum length to fit inside the heat chamber

was 30 cm, some branches were shortened. The length of the branch on sampling day depended on the growth of the year.

12. After 3 weeks, observations were made. Invasion success of *A. ostoyae* in the jar was visually estimated on a scale from 0 (0%) to 5 (more than 80%). Humidity was visually estimated in each jar (from 0 - dry to 3 – very humid). We measured with a caliper the total length of the branch, the length of the branch before needles implantation, length of the necrosis, length of the mycelium under the bark

S4 Estimation of genetic variance and heritability using MCMCglmm

Table S4.1. MCMCglmm Bayesian model parametrization. Psrf stands for Gelman-Rubin criterion Potential Scale Reduction Factor, a mesure of model convergence. Good convergence of models is expected for psrf <1.02. Bb, bud burst; dbb, duration of bud burst; necr., necrosis length; disc., needle discoloration; Proc., processionary moth nests.
*Qualitative trait with 3 levels of humidity: dry, medium and very humid

	Trait distribution	**Link function**	**Covariable**	**Prior fixed effects**	**Prior random effects**	**Prior residuals**	**Nb of iterations**	**Burn-in**	**Thinning**	**psrf**
Height	Normal	identity	_	default prior	V=1; n=0.002	V=1; n=0.002	750,000	50,000	500	1.004
Bb2015	Normal	identity	_	default prior	V=1; n=0.002	V=1; n=0.002	750,000	50,000	500	1.004
Bb2017	Normal	identity	_	default prior	V=1; n=0.002	V=1; n=0.002	750,000	50,000	500	1.004
lbb2015	Normal	identity	_	default prior	V=1; n=0.002	V=1; n=0.002	750,000	50,000	500	1.003
lbb2017	Normal	identity	_	default prior	V=1; n=0.002	V=1; n=0.002	750,000	50,000	500	1.003
A. ostoyae necr.	Normal	identity	Humidity in the jar*	default prior	V=1; n=0.002	V=1; n=0.002	950,000	50,000	500	1.003
D. sapinea necr.	Normal	identity	_	default prior	V=1; n=0.002	V=1; n=0.002	1,050,000	50,000	500	1.003
D. sapinea disc.	Binomial	probit	_	gelman.prior	V=1 ; n=0.002 ; alpha.mu=0 ; alpha.v=1000	Variance fixed at 1	1,050,000	50,000	500	1.003
Proc.	Binomial	logit	Height	default prior	V=1 ;n=0.002 ; alpha.mu=0 ; alpha.v=1000	Variance fixed at 1	950,000	50,000	500	1.008

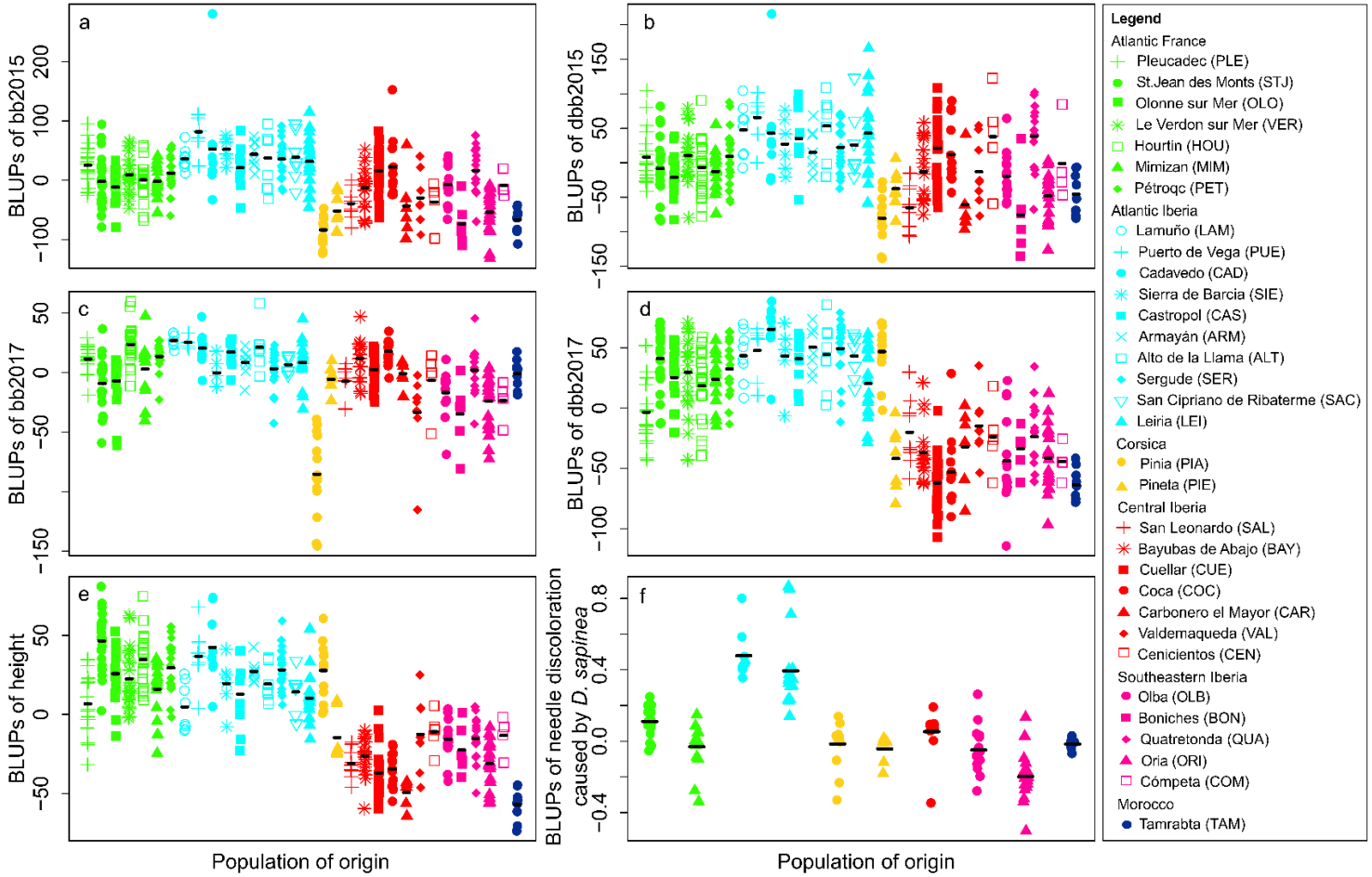


Figure S5.1. Stripcharts of the best linear unbiased predictors (BLUP) of each phenotypic trait for each genotype in each population of origin of *Pinus pinaster*. Colours represent the gene pool (see Jaramillo-Correa, *et al.* 2015) and symbols represent the population in each gene pool (see legend). The black lines indicate the average BLUP value for each population. a) Bud burst (bb) in 2015, b) Duration of bud burst (dbb) in 2015, c) Bud burst in 2017, d) Duration of bud burst in 2017, e) Height, f) Needle discoloration caused by *Diplodia sapinea*. For necrosis length caused by *D. sapinea* and *A. ostoyae* see Figure 1 in the chapter.

Table S5.1. Pearson's correlation coefficients of the best linear unbiased predictors (BLUPs) of the genotype values between adaptive traits in *Pinus pinaster*. bb, bud burst; dbb, duration of bud burst; necr., necrosis; disc., needle discoloration. Significance levels after false discovery rate (FDR) correction: *<0.05; **<0.01; ***0.001.

	bb2015	dbb2015	bb2017	dbb2017	*D. sapinea* necr.	*D. sapinea* disc.	*A. ostoyae* necr.
height	-0.172**	-0.132*	-0.178**	0.439***	0.111	0.082	-0.01
bb2015		0.798***	0.392***	-0.292***	0.118	0.041	-0.017
dbb2015			0.38***	-0.233***	0.061	0.065	-0.11
bb2017				-0.154**	-0.023	0.069	-0.092
dbb2017					-0.032	-0.093	-0.03
D. sapinea. necr.						0.231*	-0.009
D. sapinea disc.							-0.149

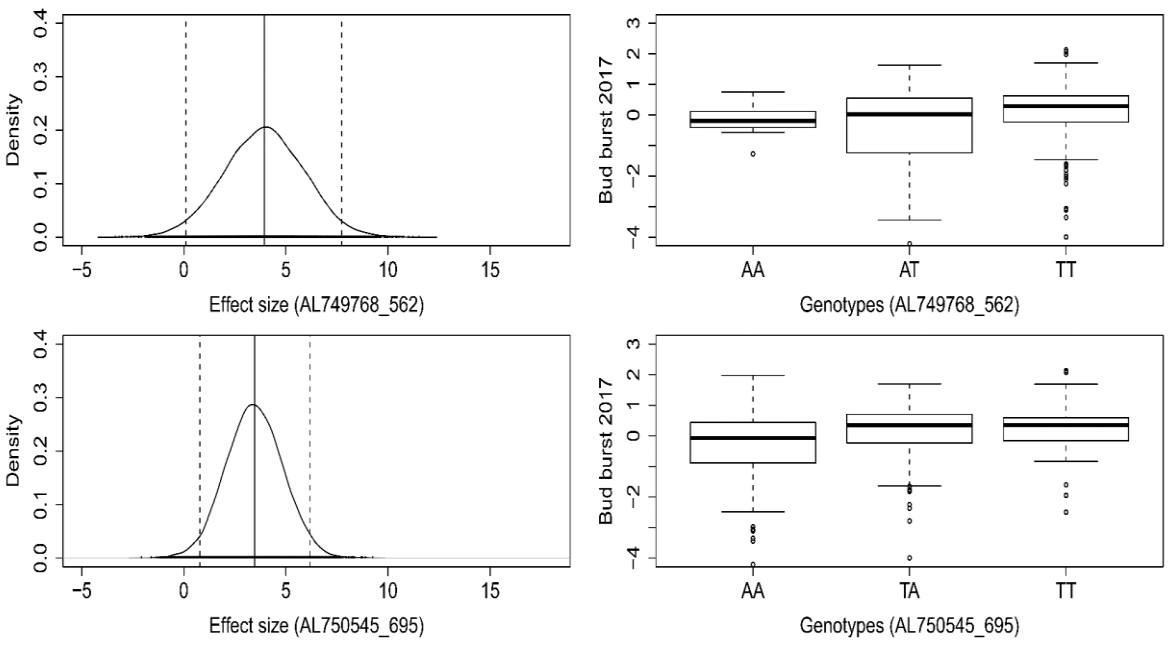


Figure S6.1. Density plots of the effect sizes based on 20,000 BAMD simulations (left) and genotypic effects (box plots, right) for three single nucleotide polymorphisms (SNPs, minor allele frequency (MAF) > 0.10) showing significant association with bud burst in 2017 and coding for a non-synonymous change in *Pinus pinaster*.

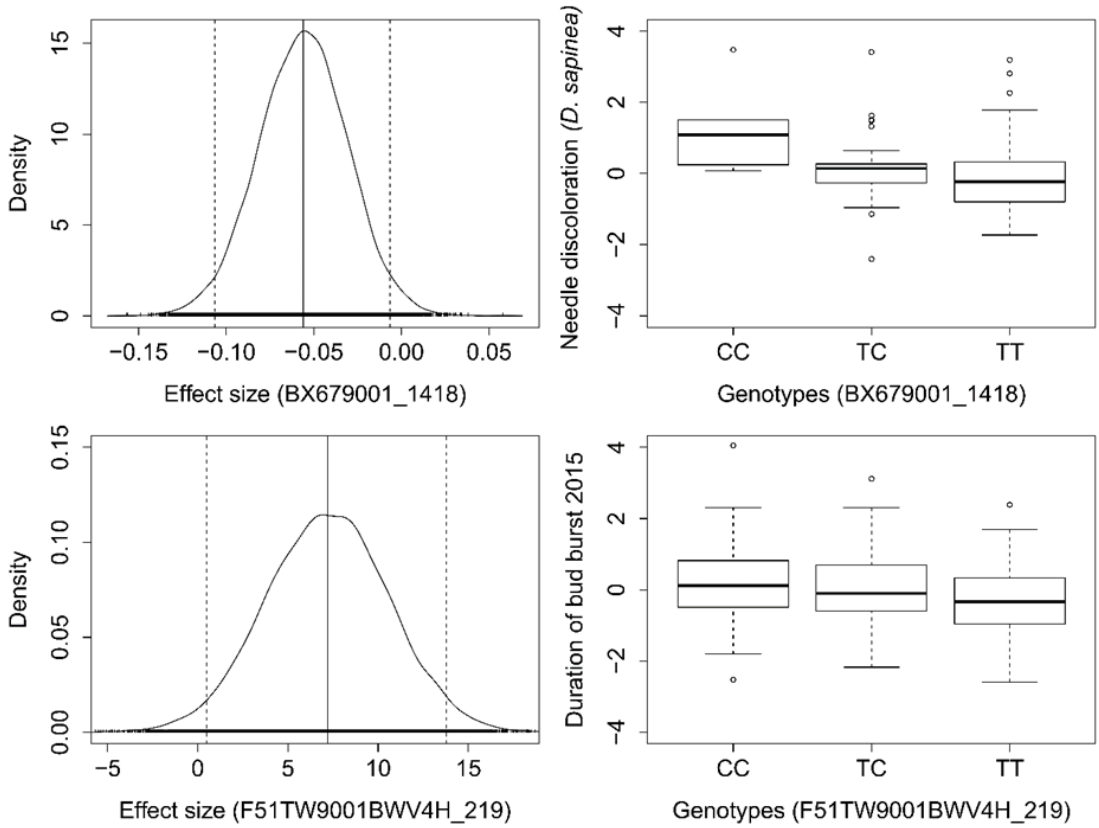


Figure S6.2. Density plots of the effect sizes based on 20,000 BAMD simulations (left) and genotypic effects (box plots, right) for two single nucleotide polymorphisms (SNPs, minor allele frequency (MAF) > 0.10) showing significant association with needle discoloration caused by *D. sapinea* (above) and bud burst in 2015 (below) and coding for a non-synonymous change in *Pinus pinaster*.

Table S6.1 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.

						Mixed linear model (TASSEL)								
Trait	SNP name	SNP motif	site annotation	LG	MAF	F	p	additive effect	additive F	additive p	dominant effect	dominant F	dominant p	MarkerR2
height	2_9280_01.Pipn_311	[T/C]	unk	5	0.2113	6.3981	0.0018	-4.5156	4.7249	0.0303	-2.9055	1.1998	0.2740	0.0237
N= 3331	AJ309108_736	[T/C]	unk		0.4190	5.7371	0.0035	0.7236	0.2357	0.6276	6.7292	11.4224	0.0008	0.0213
	AL749753_337	[C/G]	unk	2	0.1105	6.7504	0.0013	-2.3586	0.3954	0.5298	-11.4072	7.1788	0.0077	0.0250
	AL750825_659	[A/G]	unk	8	0.1460	6.5602	0.0016	-7.8050	11.2864	0.0009	-2.0895	0.4787	0.4894	0.0246
	BX249583_420	[A/G]	unk	1	0.2660	6.9502	0.0011	6.8045	12.4400	0.0005	6.4775	8.0676	0.0047	0.0259
	BX250546_705	[T/C]	unk	12	0.1489	5.3934	0.0049	-0.9646	0.1581	0.6911	8.8694	8.4661	0.0038	0.0200
	BX253184_476	[T/C]	unk	1	0.2187	7.3467	0.0007	7.8993	13.0218	0.0004	8.6990	9.7916	0.0019	0.0292
	CL3736CT1511CN1615_81	[A/G]	nc	5	0.4504	5.8334	0.0032	3.2518	4.8374	0.0284	-5.8281	8.4953	0.0038	0.0216
	CL544Contig1_03.Pipn_84	[T/G]	unk		0.1351	5.8398	0.0032	1.4908	0.2921	0.5892	6.5156	4.1267	0.0429	0.0217
	CR392177_1312	[T/C]	nc	7	0.2136	6.1008	0.0025	-4.4201	4.4520	0.0355	9.3695	12.1970	0.0005	0.0234
	CT_2714_442	[T/C]	unk		0.3809	5.7192	0.0036	4.8880	11.1002	0.0009	-3.0127	2.2980	0.1303	0.0212
	CT574626_412	[A/G]	syn		0.1501	5.6577	0.0038	6.2984	5.7170	0.0173	-0.0449	0.0002	0.9888	0.0210
	CT574626_946	[T/C]	nc		0.1492	5.7466	0.0035	-6.5618	5.8524	0.0160	-0.3037	0.0087	0.9257	0.0213
	CT575717_1382	[A/G]	nc		0.2059	5.8907	0.0030	7.7382	11.7036	0.0007	5.2416	3.4208	0.0651	0.0220
	CT576335_234	[C/G]	nc	10, 4	0.4916	5.4388	0.0047	-2.3479	2.7829	0.0960	5.3672	8.1633	0.0045	0.0202
	CT579373_506	[A/C]	unk	6	0.3440	5.4977	0.0044	-4.1241	7.1626	0.0077	5.7908	7.9591	0.0050	0.0204
	CT580064_331	[T/C]	unk		0.4417	6.3613	0.0019	3.4157	6.0084	0.0147	5.9518	9.1044	0.0027	0.0236
	CT583593_733	[T/C]	nc		0.2313	6.8637	0.0012	0.6363	0.1142	0.7356	7.4177	8.9149	0.0030	0.0255
	F51TW9001AZG2W_933	[C/G]	unk	4	0.4380	10.1377	0.0001	-5.5081	13.5768	0.0003	5.4582	7.6254	0.0060	0.0376
	F51TW9001B6MAF_1134	[T/G]	nc	4	0.2702	6.5872	0.0015	0.6597	0.1450	0.7036	7.7446	11.3327	0.0008	0.0244
	F51TW9001DBXsynZ_1463	[C/G]	nc	5	0.1816	6.6885	0.0014	5.4433	5.3356	0.0214	8.4709	8.7836	0.0032	0.0248
	F51TW9002FK37R_544	[A/G]	non-syn	10	0.1914	5.6305	0.0039	0.6530	0.0995	0.7525	8.4853	9.7803	0.0019	0.0209
	FN694775_756	[T/C]	nc		0.1320	6.1159	0.0024	-1.1487	0.1643	0.6855	7.5612	5.2641	0.0223	0.0227
	synp_v3.0_unigene17345_1191	[T/G]	nc	9	0.3443	6.2701	0.0021	5.8204	11.7265	0.0007	4.6794	4.8341	0.0285	0.0233
	synp_v3.0_unigene18547_188	[A/T]	unk	8	0.3987	5.9298	0.0029	-3.6429	6.3296	0.0123	5.9741	9.0841	0.0027	0.0220
bb2015	BX249218_322	[A/C]	nc		0.3150	6.5023	0.0017	-10.7563	11.1048	0.0009	-11.0563	6.2902	0.0125	0.0300
N=3146	BX249671_307	[T/C]	unk	7	0.3966	6.7119	0.0014	-10.7118	11.6021	0.0007	8.4206	4.4030	0.0365	0.0302
	BX252800_1728	[T/G]	unk	7	0.4558	5.9767	0.0028	-3.8299	1.4773	0.2249	-12.0286	9.2384	0.0025	0.0269
	BX253890_151	[A/C]	nc	12	0.1573	6.2960	0.0020	-16.9879	10.1618	0.0015	-7.1762	1.1811	0.2778	0.0283
	BX681281_30	[T/C]	unk	1	0.2538	5.7077	0.0036	-4.5285	1.5196	0.2184	16.3923	11.1289	0.0009	0.0259
	CL2033CT1302CN1398_513	[A/G]	nc	1	0.4081	5.3861	0.0049	-7.5170	6.0061	0.0147	-5.8633	1.9708	0.1611	0.0243
	CL544Contig1_03.Pipn_84	[T/G]	unk		0.1351	8.9814	0.0002	-18.9626	10.5690	0.0012	2.6795	0.1561	0.6929	0.0404
	CR392131_121	[A/G]	unk	3	0.4906	6.0941	0.0025	-0.1993	0.0049	0.9441	-13.3393	12.1705	0.0005	0.0275
	F51TW9001BEJOH_703	[C/G]	non-syn	11	0.4380	5.4977	0.0044	1.8449	0.3654	0.5458	-12.7260	9.7955	0.0019	0.0248
	F51TW9001C6IZ8_79	[A/T]	nc	11	0.3617	5.5760	0.0041	7.3060	4.8734	0.0278	12.9479	9.1430	0.0027	0.0255
	F51TW9001D5P2Y_1441	[A/G]	non-syn	5	0.2143	5.5133	0.0043	12.8347	8.4328	0.0039	-16.2305	8.5627	0.0036	0.0252
	FN692276_550	[T/C]	unk	12	0.4024	7.8510	0.0005	-5.8436	3.7680	0.0529	-11.8565	8.4126	0.0039	0.0353
	i09773syn1097	[T/C]	non-syn	12	0.1938	6.1923	0.0022	-2.2553	0.2868	0.5926	17.1757	10.1389	0.0016	0.0279
	i13066syn710	[T/G]	nc		0.2415	6.0333	0.0026	-14.3036	11.8484	0.0006	-7.7970	2.1398	0.1443	0.0276
	i13173syn367	[A/C]	unk		0.1255	5.3853	0.0049	20.1432	8.2374	0.0043	-26.4891	10.5920	0.0012	0.0242
	i16267syn380	[A/G]	unk	2	0.4107	7.5253	0.0006	12.3638	14.8298	0.0001	-2.4263	0.3554	0.5514	0.0339
	LP3_3_298	[C/G]	unk		0.1433	5.5667	0.0041	-1.4517	0.0708	0.7903	15.2872	5.2606	0.0223	0.0250
dbb2015	BX249539_1987	[A/G]	unk		0.1245	7.4410	0.0007	-26.3981	9.9167	0.0018	-37.0543	14.8498	0.0001	0.0348

N=3152	BX249539_2285	[T/C]	unk	3	0.1161	7.6129	0.0006	26.4595	9.9901	0.0017	-38.1504	15.1792	0.0001	0.0356
	BX253931_1781	[T/C]	unk	12	0.2955	6.9251	0.0011	-4.7474	1.3080	0.2534	19.8810	13.1589	0.0003	0.0323
	F51TW9001BWV4H_219	[A/G]	non-syn		0.4616	5.9291	0.0029	-11.6338	10.0892	0.0016	-10.5601	4.5791	0.0330	0.0277
	F51TW9001D5P2Y_1441	[A/G]	non-syn	5	0.2143	5.9220	0.0029	11.6377	5.0766	0.0248	-22.1065	11.6160	0.0007	0.0281
	F51TW9002FPGRE_170	[T/C]	nc		0.3455	5.5118	0.0043	-11.0911	8.3497	0.0041	-12.0379	5.6251	0.0182	0.0257
bb2017	0_12730_01_contig1_159	[T/G]	unk	12	0.3788	10.1191	0.0001	-6.7930	17.4543	0.0000	-1.8696	0.6145	0.4336	0.0475
N=1440	0_4105_01_contig2_279	[A/G]	syn	7	0.1004	6.7992	0.0013	-12.2342	4.7102	0.0306	-22.0465	11.6882	0.0007	0.0319
	AL749768_562	[A/T]	non-syn	1	0.1264	5.5133	0.0044	-1.4594	0.1243	0.7246	-8.6542	3.1388	0.0772	0.0259
	AL750545_695	[A/T]	non-syn	1	0.4869	5.4174	0.0048	-4.7611	7.6575	0.0059	3.8460	2.6988	0.1012	0.0254
	AL750755_1441	[A/C]	unk	2	0.4316	5.9638	0.0028	2.8199	2.3841	0.1234	-6.6499	7.8421	0.0054	0.0280
	AL750773_910	[A/T]	unk	3	0.4990	5.9364	0.0029	5.6171	9.6643	0.0020	-3.2432	1.6692	0.1972	0.0291
	BX252045_412	[A/G]	unk	12	0.1638	5.6880	0.0037	-8.7733	11.3696	0.0008	5.2693	2.5708	0.1097	0.0267
	BX676789_1926	[A/T]	nc	12	0.2726	5.5479	0.0042	7.6827	10.9173	0.0010	-4.4661	2.3289	0.1278	0.0261
	BX678760_1291	[A/G]	unk		0.3574	5.6020	0.0040	4.9199	6.3209	0.0123	2.7826	1.1852	0.2770	0.0263
	CL2640CT2248CN2410_1340	[T/G]	unk	6	0.4765	6.1088	0.0024	5.4431	10.1847	0.0015	3.1318	1.8681	0.1725	0.0287
	CT574915_594	[A/G]	unk	5	0.1901	5.7836	0.0034	7.7400	8.7632	0.0033	-9.5791	7.9988	0.0049	0.0271
	CT576106_142	[C/G]	unk	10	0.1795	6.7119	0.0014	-10.1496	13.1002	0.0003	5.7402	2.9474	0.0868	0.0316
	CT579526_269	[T/G]	non-syn	12, 2	0.3336	5.9501	0.0029	3.2120	2.7455	0.0984	-8.9487	11.7746	0.0007	0.0280
	F51TW9001A0synJD_327	[A/G]	unk	4	0.4540	5.7047	0.0036	4.0054	5.2141	0.0230	6.3929	7.4678	0.0066	0.0268
	F51TW9001AOsyn8U_342	[T/C]	unk	4	0.2298	6.3157	0.0020	-0.8448	0.1305	0.7182	-8.6175	8.2932	0.0042	0.0296
	F51TW9001AQZUF_985	[T/C]	unk	4	0.2313	7.1474	0.0009	8.1745	13.0060	0.0004	8.7471	8.5939	0.0036	0.0335
	F51TW9001B1U5X_203	[C/G]	nc	12	0.3009	5.9611	0.0028	4.4224	4.6583	0.0315	-9.0133	11.1039	0.0009	0.0281
	F51TW9001BD1TJ_1356	[A/G]	non-syn	7	0.3006	6.1680	0.0023	3.6670	3.3478	0.0681	-8.7345	10.7494	0.0011	0.0290
	F51TW9001CGV5K_406	[A/G]	syn	1	0.4897	5.8943	0.0030	-1.9242	1.2526	0.2638	-7.7219	10.5543	0.0013	0.0277
	F7JJN6E01B7BCW_157	[T/C]	syn	5	0.1170	6.1637	0.0023	-9.6424	4.3408	0.0379	-0.8816	0.0284	0.8662	0.0289
	FM945796_840	[T/G]	unk		0.2135	6.7536	0.0013	9.1748	12.6229	0.0004	-4.5296	1.9308	0.1655	0.0317
	FM945910_1660	[A/G]	non-syn	12	0.1090	5.5106	0.0044	10.6576	7.7924	0.0055	15.4982	10.4063	0.0014	0.0259
	i08906syn326pg	[A/C]	unk	8	0.1948	5.7287	0.0035	-3.4625	1.4189	0.2343	11.0762	10.6057	0.0012	0.0269
	i10996syn1211	[T/C]	unk		0.3006	7.7686	0.0005	-8.5656	15.5347	0.0001	5.5525	3.8928	0.0492	0.0365
	i11276syn420	[T/C]	unk		0.3002	5.7510	0.0035	-5.5054	6.7638	0.0097	2.1350	0.6172	0.4326	0.0271
	PFK_39	[A/G]	unk	12	0.1545	8.5039	0.0002	-11.2969	12.3245	0.0005	14.4163	15.1795	0.0001	0.0399
dbb2017	AL749850_679	[A/G]	unk		0.4017	5.4513	0.0046	6.1738	6.0240	0.0145	-7.5903	5.3517	0.0212	0.0212
N= 1905	BX251734_1732	[T/C]	syn	5	0.3955	7.2991	0.0008	2.3184	0.9395	0.3330	-10.9013	12.3434	0.0005	0.0284
	BX251919_226	[A/C]	unk		0.4092	6.1101	0.0024	6.8990	8.1794	0.0045	-8.1818	7.4517	0.0066	0.0238
	BX667542_94	[A/G]	nc		0.2013	5.7803	0.0033	8.9065	8.4491	0.0039	10.6374	6.8472	0.0092	0.0225
	CT574915_594	[A/G]	unk	5	0.1901	5.8772	0.0030	-10.1583	9.2013	0.0026	11.9718	7.8916	0.0052	0.0229
	CT582680_451	[T/G]	unk		0.2013	7.0484	0.0010	11.0369	11.3025	0.0008	0.0793	0.0004	0.9836	0.0274
	F51TW9001AGH4F_727	[T/C]	non-syn		0.2702	5.6128	0.0039	-2.7628	1.0425	0.3078	-8.1019	5.7934	0.0165	0.0218
	F51TW9001AZG2W_933	[C/G]	unk	4	0.4380	7.8379	0.0005	-4.2792	3.4294	0.0648	10.8299	12.8531	0.0004	0.0305
	F51TW9001BAW7V_405	[A/G]	unk	12	0.1629	8.9364	0.0002	-12.6132	13.1241	0.0003	2.1706	0.2355	0.6277	0.0348
	FN694219_1268	[A/G]	nc	3	0.2777	6.6511	0.0014	-8.9805	10.8592	0.0011	0.4197	0.0147	0.9036	0.0259
	FN694219_836	[A/G]	non-syn	3	0.2772	6.7911	0.0013	9.0332	11.0205	0.0010	0.3811	0.0121	0.9124	0.0264
	i17647syn350pg	[C/G]	unk		0.1573	5.6644	0.0037	-7.8474	4.6588	0.0315	-2.6844	0.3727	0.5419	0.0220
A. ostoyae	AL750513_302	[A/G]	nc	1	0.3850	7.4765	0.0014	-0.8298	8.0397	0.0066	0.9229	5.3819	0.0245	0.1041
necrosis length	BX679585_950	[A/G]	unk	8	0.4397	7.3967	0.0015	0.0830	0.0839	0.7732	-1.5460	14.3500	0.0004	0.1007
N= 180	F51TW9001AI9YZ_1847	[A/G]	unk	7	0.2731	5.9284	0.0048	1.3039	10.3043	0.0023	-0.0881	0.0272	0.8697	0.0807

	F51TW9001ANBBN_100	[T/C]	unk	11	0.1679	6.0467	0.0044	-1.0199	6.5162	0.0137	-0.4418	0.5753	0.4516	0.0823
	F51TW9001CXU1D_1264	[T/C]	unk	6	0.3644	6.5943	0.0028	1.6422	12.7859	0.0008	-0.8273	2.4751	0.1218	0.0898
D. sapinea	AL750104_316	[A/C]	unk	10	0.2917	6.0259	0.0031	0.1331	0.0836	0.7729	2.1518	10.8531	0.0013	0.0338
necrosis length	BX250531_554	[A/G]	unk		0.2136	5.8424	0.0036	1.6444	9.2414	0.0028	0.1902	0.0603	0.8063	0.0322
N= 452	CT575341_960	[A/C]	syn		0.2378	6.0576	0.0030	-2.4579	11.3262	0.0010	1.5908	2.9881	0.0861	0.0334
	CT576149_1614	[T/C]	nc	10	0.2457	8.5338	0.0003	2.6272	11.0072	0.0012	0.8469	0.8435	0.3600	0.0470
	CT578935_1350	[A/G]	unk	2	0.3912	5.7928	0.0038	1.2968	11.4601	0.0009	-0.4538	0.6473	0.4224	0.0319
	F51TW9001A3IDU_1407	[A/G]	nc		0.2317	8.0691	0.0005	1.7308	12.5898	0.0005	2.0076	9.6738	0.0023	0.0445
	F51TW9001B2RB8_159	[T/G]	unk	1	0.3264	6.0284	0.0031	-1.1889	10.3739	0.0016	1.0337	3.8862	0.0506	0.0332
	F51TW9001EIZX5_362	[T/C]	non-syn		0.4764	7.5042	0.0008	-0.2388	0.3943	0.5311	2.0860	14.6510	0.0002	0.0414
	F51TW9002FT2ZF_1060	[A/C]	unk	12	0.4849	8.2406	0.0004	1.5983	12.0217	0.0007	-1.2878	5.4194	0.0213	0.0454
	FN695885_1909	[C/G]	nc	5	0.2786	6.1666	0.0027	-0.8324	2.5020	0.1159	2.4084	12.2718	0.0006	0.0340
	i10796syn1462pg	[A/G]	nc	12	0.4082	7.0389	0.0012	0.1045	0.0734	0.7868	1.9728	14.0318	0.0003	0.0388
	PFK_39	[A/G]	unk	12	0.1545	5.5117	0.0050	1.9475	9.2267	0.0028	-0.2703	0.1469	0.7021	0.0304
D. sapinea	BX251825_986	[A/G]	non-syn	8	0.4840	6.6652	0.0017	-0.0529	10.2744	0.0017	0.0487	4.1482	0.0435	0.0592
needle														
discoloration	BX679001_1418	[A/G]	non-syn	7	0.1917	5.5515	0.0048	0.0975	10.0649	0.0019	-0.0554	2.1429	0.1454	0.0493
N= 452	CR394067_173	[T/G]	non-syn	3	0.1248	7.6361	0.0007	0.0419	2.8367	0.0943	-0.0634	4.5444	0.0347	0.0678

					Bayesian framework (BAMD)											
					Additive model			Dominant model (allele 1)			Dominant model (allele 2)			Overdominance model		
Trait	SNP name	SNP motif	site annotation	LG	Mean effect	(95% CIs)		Mean effect	(95% CIs)		Mean effect	(95% CIs)		Mean effect	(95% CIs)	
height	2_9280_01.Pipn_311	[T/C]	unk	5										-4.9763	-8.3213	-1.6539
N= 3331	AJ309108_736	[T/C]	unk					3.9648	0.0457	7.9272				3.8914	0.6446	7.1039
	AL749753_337	[C/G]	unk	2				-5.5013	-9.3520	-1.5806						
	AL750825_659	[A/G]	unk	8	5.0943	1.8647	8.2793	4.3123	0.2928	8.2567	8.5249	1.7773	15.3012			
	BX249583_420	[A/G]	unk	1	-3.8429	-6.5598	-1.1059	-3.4594	-6.7305	-0.1669						
	BX250546_705	[T/C]	unk	12							-4.5086	-8.7194	-0.3411	6.3783	2.5386	10.2334
	BX253184_476	[T/C]	unk	1				7.7109	2.3626	12.9611						
	CL3736CT1511CN1615_81	[A/G]	nc	5				-4.7063	-8.7378	-0.7036				-3.9603	-7.1197	-0.8105
	CL544Contig1_03.Pipn_84	[T/G]	unk					4.1671	0.5885	7.7462						
	CR392177_1312	[T/C]	nc	7										3.5341	0.3417	6.7276
	CT_2714_442	[T/C]	unk		2.6072	0.2398	4.9727				6.2256	1.9933	10.5214			
	CT574626_412	[A/G]	syn					4.8236	1.2275	8.4831	8.1386	1.0515	15.2388			
	CT574626_946	[T/C]	nc								-5.4057	-9.2099	-1.6110			
	CT575717_1382	[A/G]	nc		4.2584	1.3104	7.2411	8.0924	1.6046	14.5689	4.1890	0.5422	7.8841			
	CT576335_234	[C/G]	nc	10, 4							-5.6503	-9.3704	-1.9174	3.2583	0.2279	6.2939
	CT579373_506	[A/C]	unk	6				5.9153	1.6586	10.2194						
	CT580064_331	[T/C]	unk								-4.9672	-9.0403	-0.9174	3.7066	0.6243	6.7829
	CT583593_733	[T/C]	nc					3.4929	0.0547	6.8423				3.5972	0.1875	6.9767
	F51TW9001AZG2W_933	[C/G]	unk	4	-3.2096	-5.8672	-0.6074				-6.2220	-10.2356	-2.1826	3.7943	0.5929	7.0412
	F51TW9001B6MAF_1134	[T/G]	nc	4							-3.9509	-7.2918	-0.6087			
	F51TW9001DBXsynZ_1463	[C/G]	nc	5				9.7692	4.1665	15.5363						
	F51TW9002FK37R_544	[A/G]	non-syn	10										4.7019	0.8510	8.5810
	FN694775_756	[T/C]	nc		4.8086	1.3510	8.2424	5.3771	1.3944	9.3249				4.8753	1.0069	8.6930
	synp_v3.0_unigene17345_1191	[T/G]	nc	9	3.4580	0.8379	6.0616	8.1778	3.3488	12.9448						
	synp_v3.0_unigene18547_188	[A/T]	unk	8							-5.2924	-9.5724	-0.9898			
bb2015	BX249218_322	[A/C]	nc		7.3080	2.2536	12.3645				13.9284	4.5711	23.4466			
N=3146	BX249671_307	[T/C]	unk	7	6.0802	0.7338	11.4138	16.0233	6.7525	25.1571						
	BX252800_1728	[T/G]	unk	7							10.2688	2.5664	17.9765	-9.5994	-16.3355	-2.9073
	BX253890_151	[A/C]	nc	12	11.0578	4.5013	17.5928	10.4030	2.4377	18.5200	15.8857	0.8490	31.1980			
	BX681281_30	[T/C]	unk	1										8.3436	1.1859	15.5168
	CL2033CT1302CN1398_513	[A/G]	nc	1	6.9646	1.8207	12.0634				9.4766	2.4210	16.5475	-7.7204	-14.5678	-0.8756
	CL544Contig1_03.Pipn_84	[T/G]	unk		10.3545	3.0149	17.7447	16.7794	0.7687	32.9727	12.3404	4.2033	20.4323	-8.3717	-16.4057	-0.4285
	CR392131_121	[A/G]	unk	3										-9.1512	-15.6386	-2.6077
	F51TW9001BEJOH_703	[C/G]	non-syn	11				-10.2449	-18.2204	-2.4234				-8.3007	-15.2664	-1.4568
	F51TW9001C6IZ8_79	[A/T]	nc	11							-14.3556	-23.4421	-5.3698			
	F51TW9001D5P2Y_1441	[A/G]	non-syn	5				-20.4452	-34.6712	-6.3518						

	FN692276_550	[T/C]	unk	12	5.5271	0.5534	10.4575				10.0341	2.8999	17.2179	-8.8741	-15.6714	-2.1177
	i09773syn1097	[T/C]	non-syn	12							-10.7444	-18.2256	-3.3387	11.7931	4.2101	19.3184
	i13066syn710	[T/G]	nc		8.3046	2.1705	14.3741	8.5442	0.5109	16.3957	18.0386	5.6469	30.4479			
	i13173syn367	[A/C]	unk					-20.5384	-40.8147	-0.7116						
	i16267syn380	[A/G]	unk	2	-10.4358	-15.8176	-5.1493	-13.7482	-23.3141	-4.0041	-10.9525	-18.1168	-3.6861			
	LP3_3_298	[C/G]	unk		8.5705	1.8966	15.3310	11.3029	3.1066	19.7187				12.4548	4.2794	20.5661
dbb2015	BX249539_1987	[A/G]	unk								36.8330	14.4901	60.4846			
N=3152	BX249539_2285	[T/C]	unk	3				-37.0923	-65.5001	-10.2341						
	BX253931_1781	[T/C]	unk	12				22.9696	8.3963	37.4871	-14.3584	-22.7948	-5.8362			
	F51TW9001BWV4H_219	[A/G]	non-syn		7.1947	0.5013	13.7846				17.5580	5.9192	28.8799			
	F51TW9001D5P2Y_1441	[A/G]	non-syn	5				-20.3029	-38.0228	-2.4628				-12.3552	-22.6319	-2.1032
	F51TW9002FPGRE_170	[T/C]	nc		8.7035	1.6592	15.7458				19.7053	8.1752	31.3043			
bb2017	0_12730_01_contig1_159	[T/G]	unk	12	4.5331	2.0115	7.0269	7.3695	3.0071	11.7138	6.2210	2.4250	10.0145			
N=1440	0_4105_01_contig2_279	[A/G]	syn	7										-5.6235	-10.9582	-0.3789
	AL749768_562	[A/T]	non-syn	1	3.9365	0.0920	7.7265				6.3769	1.9683	10.9550	-5.0583	-9.8051	-0.3726
	AL750545_695	[A/T]	non-syn	1	3.4631	0.7855	6.1680	5.4367	1.2761	9.6166						
	AL750755_1441	[A/C]	unk	2	-3.6781	-6.4034	-0.9512	-5.8454	-9.7643	-1.9357				-5.5854	-9.3426	-1.8558
	AL750773_910	[A/T]	unk	3	-3.4815	-6.2165	-0.7566	-4.8512	-8.7256	-0.9995						
	BX252045_412	[A/G]	unk	12	3.7144	0.3232	7.1098	7.1606	0.4647	13.8000						
	BX676789_1926	[A/T]	nc	12	-5.6823	-8.6306	-2.7130	-9.5739	-15.8050	-3.2985						
	BX678760_1291	[A/G]	unk								-4.4285	-8.4159	-0.4700			
	CL2640CT2248CN2410_1340	[T/G]	unk	6	-4.0448	-6.7826	-1.3158				-4.8965	-9.1235	-0.7349			
	CT574915_594	[A/G]	unk	5				-7.5194	-14.5199	-0.6058						
	CT576106_142	[C/G]	unk	10	4.5886	1.1391	8.0506	9.7283	2.6601	16.8358						
	CT579526_269	[T/G]	non-syn	12, 2				-6.1569	-11.4451	-0.9482						
	F51TW9001A0synJD_327	[A/G]	unk	4							-6.3271	-10.9061	-1.7635			
	F51TW9001AOsyn8U_342	[T/C]	unk	4							4.4229	0.2319	8.5877			
	F51TW9001AQZUF_985	[T/C]	unk	4							-8.5700	-14.9623	-2.1947			
	F51TW9001B1U5X_203	[C/G]	nc	12				-7.7595	-13.2502	-2.2248						
	F51TW9001BD1TJ_1356	[A/G]	non-syn	7				-5.2711	-10.2811	-0.2774				-6.1960	-10.4385	-2.0469
	F51TW9001CGV5K_406	[A/G]	syn	1							4.3565	0.1041	8.6407	-5.3759	-9.2969	-1.5450
	F7JJN6E01B7BCW_157	[T/C]	syn	5	6.7047	2.8720	10.6170	8.3489	4.1057	12.5629				7.2486	2.6664	11.8138
	FM945796_840	[T/G]	unk		-4.4837	-7.6293	-1.2690	-9.1622	-16.2472	-2.2274						
	FM945910_1660	[A/G]	non-syn	12				6.3119	1.5393	11.1744				7.4802	2.2145	12.7487
	i08906syn326pg	[A/C]	unk	8							-4.3740	-8.7529	-0.0070	5.4794	1.0537	10.0000
	i10996syn1211	[T/C]	unk		3.3180	0.3730	6.2603	9.6354	3.5337	15.8055						
	i11276syn420	[T/C]	unk					5.8312	1.8950	9.7939						
	PFK_39	[A/G]	unk	12				9.1584	1.1528	17.3883				6.8694	2.3298	11.4416

dbb2017	AL749850_679	[A/G]	unk		-4.6602	-9.0130	-0.2439	-7.8516	-14.8216	-0.9592						
N= 1905	BX251734_1732	[T/C]	syn	5										-9.0730	-14.4669	-3.6657
	BX251919_226	[A/C]	unk					-7.9710	-15.1319	-0.9201						
	BX667542_94	[A/G]	nc											7.4919	0.7951	14.0701
	CT574915_594	[A/G]	unk	5				16.2664	6.1446	26.3871				8.3032	1.7300	14.9818
	CT582680_451	[T/G]	unk		-9.9823	-15.1060	-4.8771				-8.3909	-14.9959	-1.8798			
	F51TW9001AGH4F_727	[T/C]	non-syn								7.7330	2.2761	13.2584	-6.1623	-11.5966	-0.7658
	F51TW9001AZG2W_933	[C/G]	unk	4				9.0326	2.7170	15.3434				9.5757	4.2614	14.8622
	F51TW9001BAW7V_405	[A/G]	unk	12	7.6805	3.0770	12.3826	12.8138	2.0541	23.5004	9.1678	3.1476	15.1925			
	FN694219_1268	[A/G]	nc	3							7.5383	1.7981	13.2863			
	FN694219_836	[A/G]	non-syn	3				-5.9260	-11.4945	-0.4124						
	i17647syn350pg	[C/G]	unk		6.3654	1.4410	11.2070				8.3966	2.4458	14.3254	-6.1351	-12.1742	-0.2826
A. ostoyae	AL750513_302	[A/G]	nc	1				1.1731	0.4420	1.9091				0.7189	0.0095	1.4034
necrosis length	BX679585_950	[A/G]	unk	8				-0.9544	-1.7073	-0.1991				-0.9965	-1.7053	-0.2912
N= 180	F51TW9001AI9YZ_1847	[A/G]	unk	7	-0.7349	-1.3448	-0.1294				-0.9402	-1.8870	-0.0152			
	F51TW9001ANBBN_100	[T/C]	unk	11							0.8626	0.0088	1.7259			
	F51TW9001CXU1D_1264	[T/C]	unk	6	-0.9972	-1.7872	-0.2250	-1.7246	-3.0874	-0.4280						
D. sapinea	AL750104_316	[A/C]	unk	10				0.8699	0.0798	1.6505						
necrosis length	BX250531_554	[A/G]	unk		-1.3467	-2.0488	-0.6471	-1.3978	-2.2326	-0.5668						
N= 452	CT575341_960	[A/C]	syn					2.0185	0.3478	3.7400						
	CT576149_1614	[T/C]	nc	10				2.0647	0.2910	3.9765	1.3135	0.2371	2.3927			
	CT578935_1350	[A/G]	unk	2	0.7483	0.1585	1.3385	0.9696	0.1519	1.7909	1.8112	0.6644	2.9785	1.1122	0.2215	1.9957
	F51TW9001A3IDU_1407	[A/G]	nc					1.6935	0.4615	2.9206						
	F51TW9001B2RB8_159	[T/G]	unk	1	0.8452	0.2912	1.4063	1.0838	0.1542	1.9975	1.0896	0.1570	2.0369	1.1007	0.2742	1.9391
	F51TW9001EIZX5_362	[T/C]	non-syn													
	F51TW9002FT2ZF_1060	[A/C]	unk	12	-0.9744	-1.6630	-0.2956	-1.5264	-2.5262	-0.5366				1.1541	0.2842	2.0149
	FN695885_1909	[C/G]	nc	5										1.6279	0.8124	2.4289
	i10796syn1462pg	[A/G]	nc	12												
	PFK_39	[A/G]	unk	12	0.9951	0.1908	1.8024									
D. sapinea needle	BX251825_986	[A/G]	non-syn	8				0.0660	0.0028	0.1288						
discoloration	BX679001_1418	[A/G]	non-syn	7	-0.0561	-0.1064	-0.0064									
N= 452	CR394067_173	[T/G]	non-syn	3							0.0860	0.0255	0.1467			

Chapter 2: Adaptive potential of two widespread European pines with contrasted ecology

Introduction

In the ongoing context of global change, a major issue is the sustainability of forest ecosystems. When confronted to environmental cues, forest trees have the possibility to migrate to more favourable habitats or to persist in-situ (i.e. adapt) to the new conditions, as alternatives to the risk of going extinct (Aitken *et al.*, 2008). Because it has been shown that the potential for forest trees migration falls short of their needs in most cases (Petit *et al.*, 2008), they mainly have to rely on their adaptive potential when confronted to environmental changes. Given the growing concern about the fate of natural ecosystems in the last decade, mechanisms underlying plant adaptation have been thoroughly studied in a large number of species. These studies consider adaptation from numerous angles such as demographic history (Slotte *et al.*, 2010), genetic structure and diversity (Wright & Andolfatto, 2008), or effective population size (Gossmann *et al.*, 2012), but we are still far from fully understanding it, especially in non-model long-lived species such as forest trees. These organisms being both ecologically and economically important, they are increasingly surveyed in this type of studies, and as advances in genomic tools take place, they make it possible to gain new insights about tree adaptation at the molecular level (Plomion *et al.*, 2016).

Although adaptation to temporally and spatially variable environments is explored in many species, conifers have stayed behind most other plants because of the size and complexity of their genomes (Chagné *et al.*, 2002; Birol *et al.*, 2013; McKay et al. 2012). Moreover, trees are long-lived organisms with long generation time and selective pressure can vary from one generation to another, and even along the life of the tree, making short-term adaptation crucial. Studies in forest trees are also hindered by the logistic difficulties associated with the establishment of long-term experiments such as common gardens (Matesanz & Ramírez-Valiente, 2019).

An important and sometimes neglected aspect to consider when trying to forecast future forest tree distribution is recruitment at early-life stages (Vizcaíno-Palomar *et al.*, 2014). Seedlings are more affected by selective pressure, such as competition (Peet & Christensen, 1987) than adult-trees, and can suffer mortality rates up to 90% during the first year (Castro *et al.*, 2004). Studying this key phase in natural or semi-natural conditions can be tricky, as kinship is difficult to track and elements from the environment, such as predators and mammals, can bias the results. Reciprocal common garden experiments under more controlled conditions are therefore an interesting alternative to test local adaptation in early-life stages (Morgenstern, 2011).

A first step to study mechanisms underlying genetic adaptation is the estimation of fitness. One common method to evaluate fitness in the field is to estimate fecundity (seed set) or effective reproductive success (via paternity/parentage analyses), but it does not separate genetic from environmental effects (Matesanz & Ramírez-Valiente, 2019). In addition, estimates of fitness components such as germination rate are difficult to get in the field. Here again, approaches based on common gardens with progeny observations can be useful, as they also allow the estimation of different fitness components in contrasted environments. Though these observations are not based on a genuinely natural environment, reciprocal transplantation experiments can provide additional insights on mechanisms of genetic adaptation in the wild.

Fitness estimates computed based on offspring performance can be used to estimate selection gradients (*sensu* Lande & Arnold, 1983) in the populations of interest, based on the strength of selection acting on breeding value (see, e.g. Alía *et al.*, 2014). Theoretically put, a selection gradient is the regression line determining the dependence of fitness on individual traits.

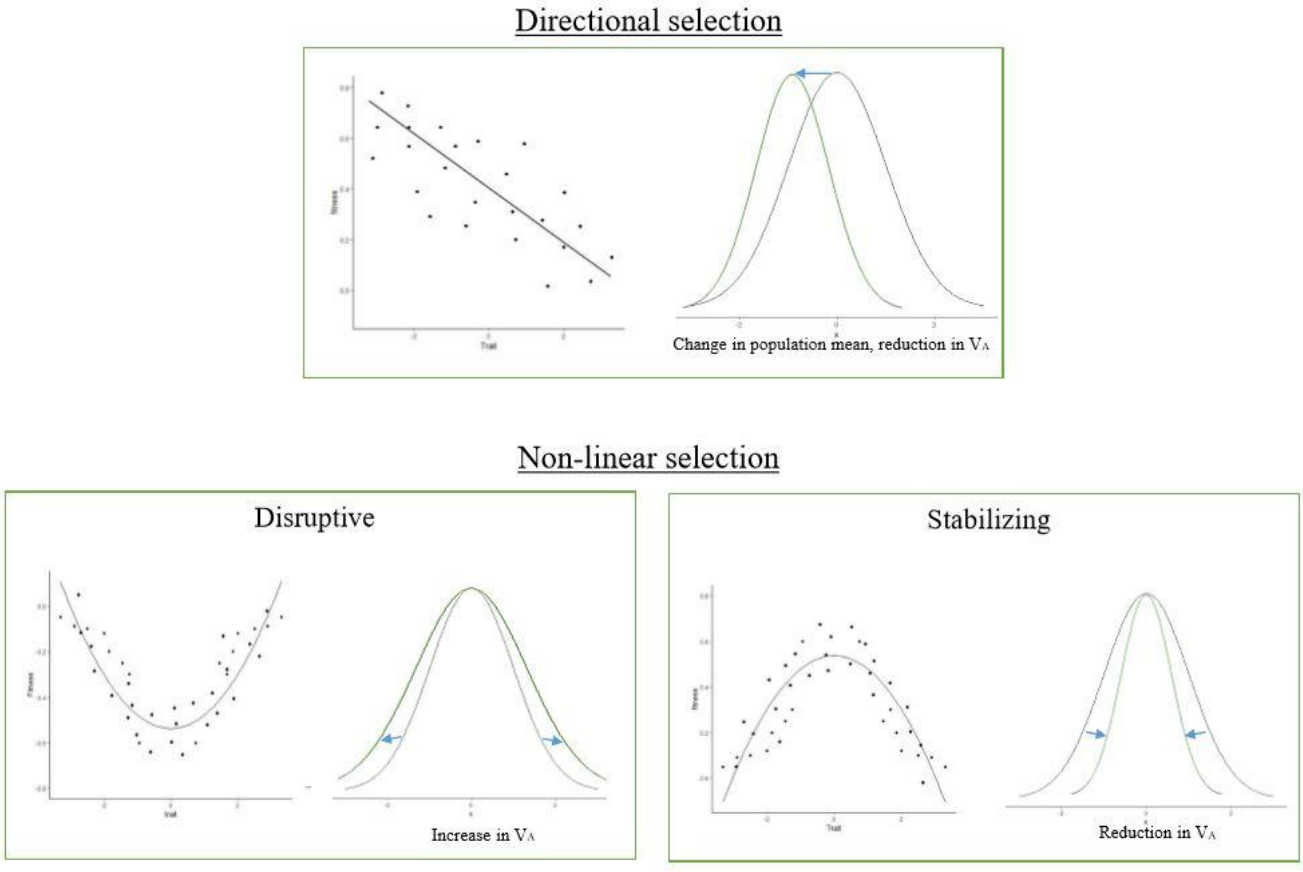


Figure 1. Directional and non-linear selection underlying linear and quadratic selection gradients, and effect in population's V_A. (Adapted from Prof. Mathias Kölliker www.evolution.unibas.ch/koelliker.)

Biologically, it allows the detection of on-going selection processes in a given population. There can be either linear or quadratic selection gradients: linear selection gradient reflects directional selection, where the optimum of fitness of the offspring is expressed for the extreme values of individual mother traits. A quadratic selection gradient reveals stabilizing selection when positive and disruptive selection when negative (Figure 1), and the optimum of fitness is reached for intermediates values of individual mother traits. When significant in a population, selection gradient underlines its adaptive potential and, combined with trait heritability and available phenotypic variance, constitute prerequisites for evolution to take place (Price, 1970). With sufficient information on adults in the populations of origin, selection gradients detected via reciprocal sowing experiments can allow identifying the main drivers of adaptation in the wild (Alía *et al.*, 2014).

To study evolutionary potential in contrasted environments, we chose two widespread conifer species with remarkably different demographic histories, ecological preferences and current distribution. Scots pine (*Pinus sylvestris*) is continuously distributed throughout Eurasia (Figure 2a), occupying a wide range of different environments to which it has locally adapted, despite substantial gene flow among populations. The current world-population is thought to have been mostly originated from an ancient bottleneck, and has a low population genetic structure (Pyhäjärvi *et al.*, 2007) except for a few populations that survived the past glaciations in cryptic glacial refugia (in the North) and the southern European Peninsulas (e.g. Iberia), where some population structure can be found. Notwithstanding the generally low genetic structure, the species has a notable variation in quantitative traits, especially at the margins of its repartition (Alía *et al.*, 2001; Notivol *et al.*, 2007; Pyhäjärvi *et al.*, 2008; Savolainen *et al.*, 2011). In contrast, maritime pine (*Pinus pinaster*) present a scattered distribution across the western Mediterranean basin and the Atlantic littoral of Portugal, Spain and southern France (Figure 2b). It is well adapted to warm and dry climates, and fire-prone environments (Fernandes & Rigolot, 2007; Budde *et al.*, 2014). This discontinuous distribution as well as the strong genetic structure among populations (Jaramillo-Correa *et al.*, 2015) are probably the results of survival in several glacial refugia and limited gene flow across them (Bucci *et al.*, 2007; Naydenov *et al.*, 2014).

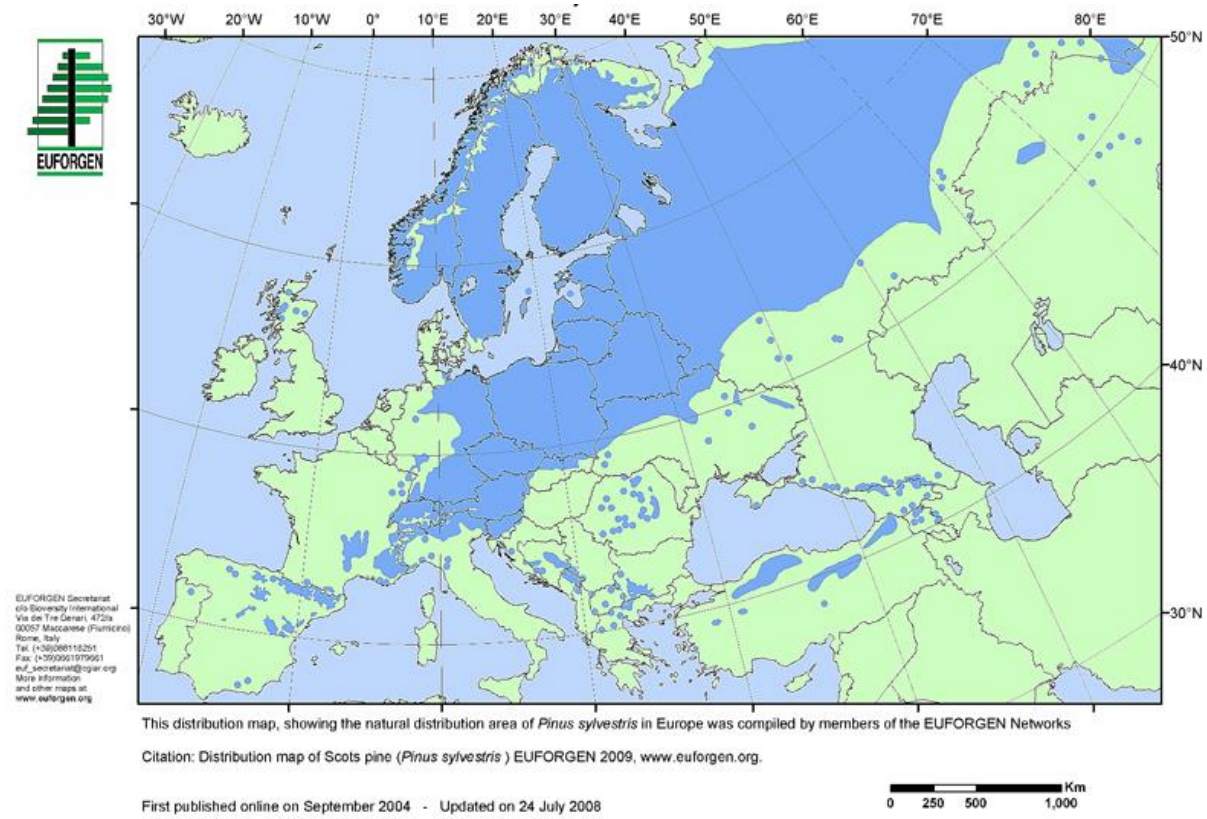


Figure 2a: EUFORGEN distribution map of *Pinus sylvestris*

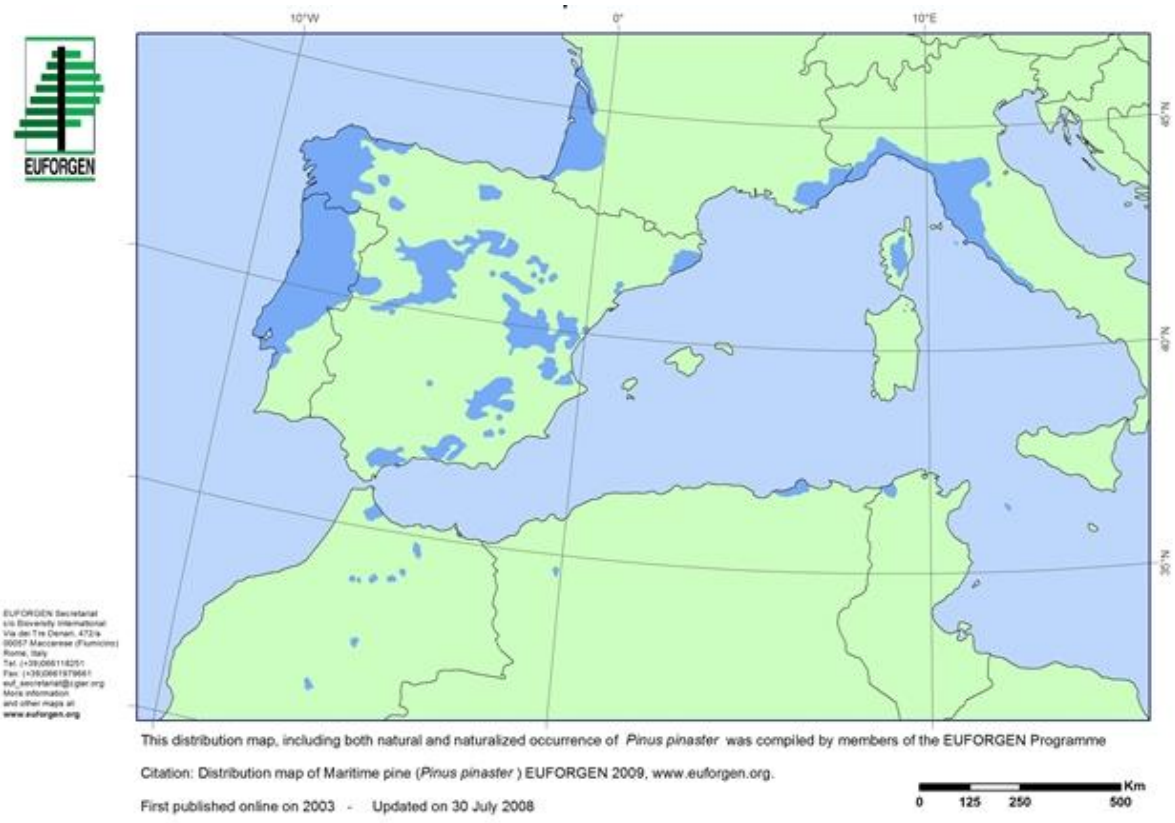


Figure 2b: EUFORGEN distribution map of *Pinus pinaster*

The current populations of maritime pine are characterized by variation in morphology (Alía *et al.*, 1995), and several adaptive traits including physiology (Lamy *et al.*, 2012, 2014; Corcuera *et al.*, 2012) and biotic stress resistance (Hurel et *al*. 2019 – *Chapter 1*), defining various ecotypes within its distribution range.

In this context, the main goal of this study is to evaluate the adaptive potential of a wide range of natural pine populations and to identify useful mother traits that act as drivers of adaptation, and thus potentially useful for tree breeding and conservation. The identification of these traits is based on the estimation of selection gradients via correlation with fitness estimates based on early-life stages in large-scale reciprocal common gardens in Europe.

Material and methods

Sampling and mother-tree phenotyping

For both *Pinus pinaster* and *P. sylvestris*, the study sites were selected to be as natural as possible, i.e. not heavily managed and not disturbed by intense and obvious natural or anthropogenic actions (Figure 3). The trees chosen for the study were adults, either dominant or co-dominant (to minimize the impact of competition), and without any widespread sign of pest or pathogen. Trees were selected at random, to collect stand variability. In some cases, the study sites were selected along latitudinal gradients (with the initial point of the sampling selected at random). The selected trees were at least 30 m apart to avoid sampling related trees. Between twenty and twenty-five mother-trees were thus selected in each population. For each tree, the diameter of the trunk at 1.30 m from the ground (DBH) and height from the ground to the tallest part of the crown were measured. Cores were drilled to estimate the age of the tree and the wood density (WD), and mature cones of the year were collected. Some needles were sampled on half the number of mother-trees to estimate specific leaf area (SLA) and carbon isotope discrimination ($\delta^{13}C$), a common estimator of Water Use Efficiency (WUE). SLA is a measure of projected leaf are per unit dry mass (Reich *et al.*, 1998) that allows an estimation of photosynthetic capacity of the plant. SLA is closely correlated to the maximum rate at which needles fix carbon. In the air, carbon is present in two isotopes, ^{12}C (98.9% of atmospheric carbon dioxide) and heavier ^{13}C (1.1%), which are discriminated during photosynthesis. $\delta^{13}C$ represents the ratio of CO_2 assimilation to stomatal conductance or transpiration, and thus reflects both plant metabolism and environment (O'Leary, 1981; Farquhar *et al.*, 1989).

In addition to mother traits, a competition index (*CI*) was computed according to Canham *et al.*(2004):

$$CI = \sum_{i=1}^{5} \left(\frac{DBH_i}{dist_i}\right) \qquad (1)$$

Where DBH_i and $dist_i$ are respectively the DBH and distance of/to the five closest neighbouring trees, all species confounded.

All these phenotypic measurements were not taken on the mother-trees from the Lithuanian and Finnish populations, and thus these populations were excluded from the study, despite being part of the reciprocal sowing common gardens.

Seed preparation

After sampling, all the *P. pinaster* cones were kept at room temperature for one month to finish maturation, then the cones were oven-dried and seeds were extracted. *P. sylvestris* cones were placed in a warm environment to encourage opening and seeds were extracted too. For both species, empty seeds were removed by floating them in water. Seeds were then weighted and kept separately at 4°C until shipment to the experimental sites. Family identification was preserved throughout this process.

Figure 3. Maritime pine provenances and experimental sites. The blue points correspond to *P. sylvestris* sites (light blue: tested provenances, where sampling and measuring were realized; dark blue: experimental sites, where all the seeds from the species where sowed). The red points correspond to *P. pinaster* sites (same colour shade code as for *P. sylvestris*).

Reciprocal sowing experiments

To keep the environment of the experiments as natural as possible, the seeds were sowed directly in the soil, regularly spaced with Guttagarden© grids (Figure 4B). The experiments was implemented following a row-column design and had three replicates (or blocks). Each family was represented by three experimental units, one experimental unit consisting in 16 seeds from the same mother tree (Figure 4). Each seed was individually sown in a Guttagarden© cell, with a total of ~4,800 seeds sown in each common garden, as populations of pooled families were also sown (data not used in the present study). The site area was chosen as flat as possible and free from direct shade. The area of each experiment was fenced and covered by a bird net to protect against cattle, rodents, small mammals and birds. In total, four common gardens were sown for *P. sylvestris* and three for *P. pinaster* (Table 1). Results for the Italian experiment in *P. pinaster* will not be shown here, as it was planted one year later than the rest.

A)

BLOCK 1	A	B	C	D	E	F	G
1	FR_09_02	ES_03_C	IT_20_02	ES_05_13	FR_09_21	FR_13_A	IT_20_27
2	FR_09_03	ES_07_B	ES_05_07	IT_20_07	ES_05_22	FR_09_30	ES_04_C
3	ES_03_A	IT_20_20	ES_04_A	FR_09_16	IT_20_22	ES_05_16	ES_05_11
4	ES_05_14	FR_09_09	FR_09_28	ES_05_17	ES_02_B	IT_20_30	ES_08_C
5	IT_20_08	FR_14_C	FR_09_10	ES_01_C	IT_20_09	ES_05_08	ES_06_A
6	ES_05_24	ES_05_23	FR_09_17	IT_20_16	ES_06_B	IT_20_13	FR_09_25
7	IT_20_03	FR_10_C	ES_05_25	ES_06_C	IT_20_23	FR_09_26	FR_09_18
8	IT_20_26	FR_09_11	FR_13_B	ES_02_C	IT_19_B	ES_05_10	FR_09_12
9	ES_07_C	FR_09_27	IT_20_04	ES_08_B	ES_05_04	FR_14_B	IT_20_06
10	FR_13_C	IT_20_28	FR_09_04	ES_05_15	FR_09_01	ES_04_B	ES_05_21
11	ES_05_01	FR_09_24	ES_07_A	IT_19_A	ES_05_02	IT_20_18	ES_01_B
12	ES_02_A	ES_05_12	FR_09_29	FR_09_14	ES_03_B	ES_08_A	IT_20_29
13	FR_10_B	IT_20_10	FR_14_A	FR_09_13	FR_09_23	ES_01_A	ES_05_06
14	FR_09_22	ES_05_09	ES_05_18	IT_20_12	FR_10_A	FR_09_15	IT_19_C

B)

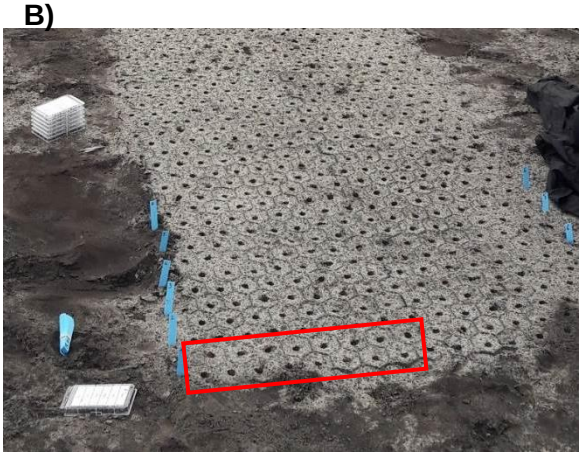


Figure 4. **A)** Design of Block 1 in the French *P. pinaster* regeneration experiment. From A to G: columns. From 1 to 14: rows. Blocks 2 and 3 (not shown) also have 14 rows each, leading up to 42 row in the complete design. Families and pools are randomized in each block. Every rectangle represents an experimental unit of 16 seeds. For instance, rectangle A1, labelled "FR_09_02" contains 16 seeds extracted from the mother-tree #02 in the French population #09, coming from Landes. In colours, structured populations (green: France, blue: Italy, orange: Spain). Left in white, pooled populations (data not used for the present study).
B) Sowing of the French experiment. The red rectangle on the picture frames an experimental unit.

Species	Country	Experimental site	Sowing dates
P. sylvestris	Spain	Segovia	16/06/2017
	Germany	Marburg	19/06/2017
	Finland	Oulu	16/06/2017
	Lithuania	Šlienava	21/06/2017
P. pinaster	Spain	Madrid	18/04/2018
	France	Cestas	31/05/2018
	Italy	Arezzo	16/03/2019

Table 1. Implementation of experimental sites

Data collection

Once germination started, the experiments were monitored every day for a month, then three times a week for the remaining of the growing season. During autumn and winter, when growth was limited, the experiments were monitored once every two weeks. On each visit, every new emergence was individually tagged and recorded, as well as ontogenic scores (Figure 4) and mortality. Height of the seedlings was measured in December 2017 for *P. sylvestris*, and December 2018 for both *P. pinaster* and *P. sylvestris*. Monitoring for this study stopped at the end of December 2018.

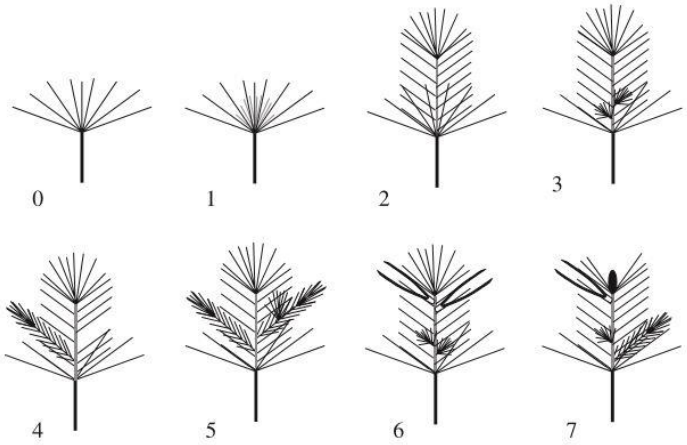


Figure 5. Ontogenic scores. 0) Cotyledonary stage, 1) emergence of the epicotyl rosette, 2) epicotyl elongation, 3) formation of axillary buds, 4) elongation of axillary long shoots, 5) formation of secondary axillary long shoot, 6) occurrence of dwarf shoots, 7) formation of a terminal bud (Chambel *et al.*, 2007).

Fitness estimates

From the experiment data, we determined five traits related to fitness components: germination (GER), numbers of degree-days to germination (GDD), height in winter (HW), survival (SUR) and "fitness" (FIT), estimated per year and in cumulated years (for *P. sylvestris*). SUR and FIT both are binomial components, but they differ in the handling of non-germinated seeds, considered an missing data "NA" for germination, and "0", same as dead, in FIT. In each site, the effect of both population and seed mass on the traits were estimated as follows, using the *lme4* package in R (Bates *et al.*, 2015).

$$Trait \sim Population + Seed\ mass + (1|Family) + (1|Row) + (1|Column) + (1|Block) \quad (2)$$

Only the best-fitted models were kept, removing fixed or random effects if needed (random effects represented in blue in the equation). Only the family estimates of the best models were used for the computation of selection gradients (i.e. adjusted to population and seed mass main effects, see Supplementary Information Figure S1).

Selection gradients

For each of the populations in each site, the dependence of seedling fitness components on mother traits (*MT*) was tested for each component as follows, using the *lme4* package in R.

$$Fitness \sim MT + CI + Age + latitude + longitude \quad (3)$$

The tested mother traits are the ones phenotyped on the original sampling sites, i.e. height, DBH, WD, $\delta^{13}C$ and SLA. In (3), all the elements but the mother-trait, *MT*, are considered as co-variates, aiming at reducing environmental noise effects. *CI* is the Competition Index described above. The co-variates "latitude" and "longitude" are the coordinates of each the mother-tree in-situ and are expected to account for within-population micro-environmental gradients as recommended by Rellstab *et al.* (2015). To avoid overestimation of effects due to correlation between variables, the variance inflation factor (VIF) was tested with an accepted threshold of VIF < 5. According to the significance of both model and variables within the models, models were adjusted by removing non-significant factors until reaching the best model. Interactions were not tested due to insufficient sample size.

As selection pressure is expected to be different in different environment, we conducted separately the analysis for each of the population tested in each of the different experimental sites.

Results

Fitness estimates and seed mass effects

Seed mass and population effects were significant in all common gardens for most fitness components. However, in the Lithuanian *P. sylvestris* experiment, GER17 was the only trait where both effects were significant, all other traits being dependant only on population effect. The trait SUR17 was also notable, as either seed mass or population effect were significant, but never both together (see Supplementary Material).

Removing seed mass effect from the family estimates was crucial to get biologically relevant results in the selection gradients analysis. Some correlations between seed mass and tested mother-traits proved to be significant, in addition to the generalised correlations between fitness components and seed mass mentioned above. In these cases, leaving seed mass in the final models to estimate selection gradients could have skewed the results by not accounting for mother effects (Bischoff & Müller-Schärer, 2010). For instance, both DBH and FIT18 were correlated with seed mass in *P. sylvestris* (0.49* and 0.60*, respectively). Then, in the case of a selection gradient such as $FIT18 \sim DBH$, it would have been impossible to distinguish the seed mass effect from the actual DBH one.

Selection gradients

Significant selection gradients were detected in both species. Overall, in *P. sylvestris*, linear selection gradients revealed an association between germination (GER) in both years of the experiment and traits related to mother-tree size and growth (height and DBH) in most experiments (Table 2). Traits related to mother-tree size and growth (height and DBH) were also correlated with other fitness components (SUR, FIT) in the German common garden. In the Spanish common garden, height in winter (HW) was also correlated to mother needle traits (SLA and $\delta^{13}C$) but were only marginally significant ($0.05 \leq$ p-value < 0.1). No significant linear selection gradient was found in the Finish common garden. Quadratic selection gradients were also significant for some combinations of mother-traits and fitness components (Table 3). Survival was associated with mother-size traits (height, wood density and DBH) in most gardens. Interestingly, cumulated survival in the Marburg site (Germany) was associated with

wood density (WD) both as linear and quadratic selection gradient, though it was only marginally significant for the quadratic gradient (Figure 6).

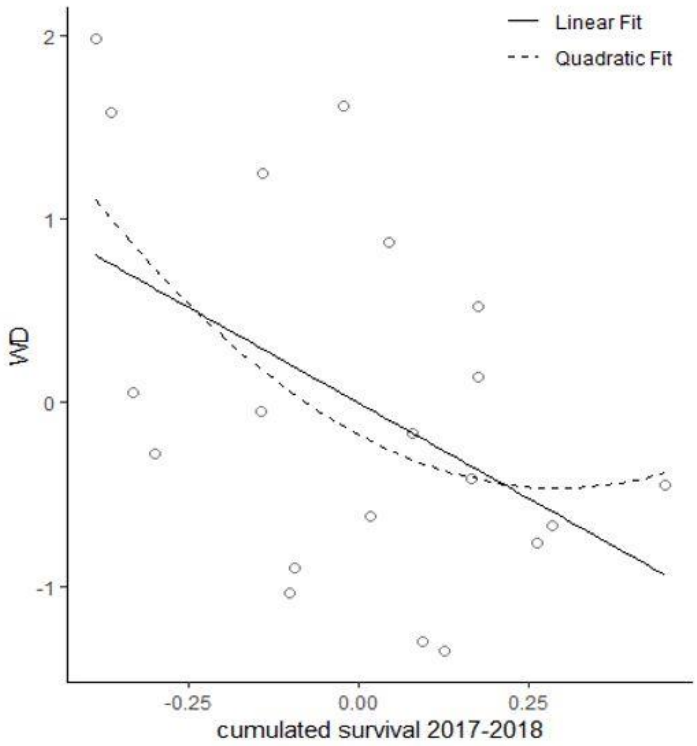


Figure 6. Relationship between mother-tree wood density (WD) and cumulated survival in 2017-2018 in the Marburg (Germany) *P. sylvestris* experiment, represented both with linear and quadratic fits.

In *P. pinaster*, all three populations revealed significant linear selection gradients in both experimental sites (Table 2). Significant selection gradients involved a wider set of fitness components than in *P. sylvestris*, but selection gradients for germination traits (both GER and GDD) and survival (SUR) were significant for most populations, and associated to both mother-size traits and needle traits. Overall, in contrast to *P. sylvestris*, the linear selection gradients for *P. pinaster* seem to be more associated to needle traits. This trend is found in the quadratic selection gradients as well, with the difference that no gradients were found for the Spanish population in the Madrid common garden (Table 3). For quadratic selection gradients, almost all significant selection gradients are associated with needle traits, with the exception of those in the Italian population. Germination (GER) and days to germination (GDD), together with survival (SUR), are the most frequent fitness components involved in significant selection gradients, with some selection gradients that were significant in linear fits found again in quadratic fits.

Pinus sylvestris **linear selection gradients**

	German site						**Lithuanian site**	
Selection	*German population*						*Spanish population*	
gradient	GER17		SUR18		FIT18		GER17	
	b	Adj-R^2	b	Adj-R^2	*b*	Adj-R^2	*b*	Adj-R^2
WD			-0.1115*	0.1888	-0.1290*	0.5148		
DBH	0.1818**	0.3249						
Height								
SLA			-0.2148*	0.5314				
δ13C							-0.6033	0.4718

	Spanish site									
Selection	*Spanish population*				*German population*					
gradient	GER18		HW17		GER18		HW17		HW18	
	b	Adj-R^2	*b*	Adj-R^2	*b*	Adj-R^2	*b*	Adj-R^2	*b*	Adj-R^2
WD										
DBH					0.2999*	0.1994				
Height	0.1973*	0.3209								
SLA			0.0368	0.2139			-0.0572	0.6046		
δ13C							0.0593	0.3103	0.3266	0.218

Pinus pinaster **linear selection gradients**

	Spanish site											
Selection	*Spanish population*				*French population*				*Italian population*			
gradient	SUR18		FIT18		GDD		HW18		SUR18		FIT18	
	b	Adj-R^2	*b*	Adj-R^2	*b*	Adj-R^2	*b*	Adj-R^2	*b*	Adj-R^2	*b*	Adj-R^2
WD												
DBH	-0.1688*	0.1713										
Height	-0.1908	0.202										
SLA							2.1355*	0.4508	-0.5612*	0.7757	-0.5948*	0.7731
δ13C			-0.1938	0.2751	96.91*	0.3237						

	French site															
Selection	*Spanish population*								*French population*						*Italian population*			
gradient	GDD		SUR18		FIT18		HW18		GER18		SUR18		HW18		GER18		GDD	
	b	Adj-R^2	b	Adj-R^2	*b*	Adj-R^2	b	Adj-R^2	*b*	Adj-R^2	*b*	Adj-R^2	*b*	Adj-R^2	*b*	Adj-R^2	*b*	Adj-R^2
WD									0.1372	0.1128					0.2793**	0.5106	-99.56*	0.3421
DBH																		
Height							-0.3856*	0.4345										
SLA	-181.7**	0.6378	-0.1421*	0.5863	-0.2112*	0.3177												
δ13C	264.4	0.2376									0.1274*	0.3475	0.3469*	0.7573				

Table 2: Linear selection gradients in *Pinus sylvestris* and *Pinus pinaster*. Significance levels of the models: *<0.05; **<0.01; ***0.001. Absence of symbol corresponds to marginally significant models.

Pinus sylvestris **quadratic selection gradients**

Selection gradient	**German site**						**Spanish site**							
	German population						*German population*				*Spanish population*			
	SUR17		SUR18		FIT18		SUR18		HW18		GER18		HW17	
	b	Adj-R^2	b	Adj-R^2	*b*	Adj-R^2	*b*	Adj-R^2	*b*	Adj-R^2	*b*	Adj-R^2	*b*	Adj-R^2
WD^2	-0.0698*	0.4365	0.1682	0.2209			0.2873**	0.4668	0.1984**	0.5491				
DBH^2											-0,3268**	0.4002	-0.047*	0.1863
$Height^2$											-0.293**	0.3771		
SLA^2					0.1483*	0.7394								
$\delta 13C^2$			-0.1489*	0.5006										

Selection gradient	**Finnish site**						**Lithuanian site**	
	Spanish population				*German population*		*German population*	
	SUR18		HW17		GER18		GER17	
	b	Adj-R^2	*b*	Adj-R^2	*b*	Adj-R^2	*b*	Adj-R^2
WD^2								
DBH^2	0.0522*	0.1921	-0.0542**	0.2816				
$Height^2$	0.0533*	0.2382						
SLA^2					0.0664*	0.6557		
$\delta 13C^2$							0.0468*	0.99985

Pinus pinaster **quadratic selection gradients**

Selection gradient	**Spanish site**													
	French population								*Italian population*					
	GER18		GDD		SUR18		FIT18		GER18		SUR18		FIT18	
	b	Adj-R^2	*b*	Adj-R^2	b	Adj-R^2	*b*	Adj-R^2	*b*	Adj-R^2	*b*	Adj-R^2	*b*	Adj-R^2
WD^2											0.1616*	0.4558	0.1894**	0.5071
DBH^2														
$Height^2$														
SLA^2	0.2511	0.5902			0.1629	0.2015	0.1956*	0.2432						
$\delta 13C^2$			79.56*	0.3185					-0.5433*	0.8904				

Selection gradient	**French site**									
	Spanish population						*French population*				*Italian population*	
	GDD		SUR18		HW18		SUR18		HW18		GER18	
	b	Adj-R^2	b	Adj-R^2	*b*	Adj-R^2	*b*	Adj-R^2	*b*	Adj-R^2	*b*	Adj-R^2
WD^2											0.137*	0.4261
DBH^2												
$Height^2$												
SLA^2	-215**	0.7538	-0.1499**	0.681					0.4332***	0.9303		
$\delta 13C^2$					0.5548**	0.9512	0.0818	0.1819				

Table 3: Quadratic selection gradients in *Pinus sylvestris* and *Pinus pinaster*. Significance levels of the models: *<0.05; **<0.01; ***0.001. Absence of symbol corresponds to marginally significant models.

Discussion

In this study, we used estimates of fitness components for two widespread conifer species, *P. sylvestris* and *P. pinaster*, in reciprocal common garden experiments set across Europe and successfully identified significant selection gradients, both linear and quadratic, for both species. These results provide useful information for tree breeding for increased resilience and assisted migration in the context of climate change. Moreover, we observed an overall pattern across species in the selection gradients: those for *P. sylvestris* were more dependent on mother size related trait, whereas needles related traits were more important for *P. pinaster*.

Local adaptation in species with contrasted features

P. sylvestris and *P. pinaster* have substantially different demographic history and continental distribution. As gene flow is limited between the different populations of *P. pinaster* across its natural range, this species is highly structured, whereas *P. sylvestris* is continuously distributed and has low genetic structure. This knowledge could lead to the assumption that when faced to environmental change, adaptation would be faster in *P. sylvestris* as low genetic structure theoretically involves more standing genetic variation (Hermisson & Pennings, 2005), therefore more adaptive potential. However, the wide range distribution of *P. sylvestris* also involves local adaptation of ecotypes to various contrasting habitats and differentiation for traits related to climate, which means that both *P. pinaster* and *P. sylvestris* may suffer strong selection in the case of strong environmental changes (Savolainen *et al.*, 2004; Grivet *et al.*, 2017). This is reflected by the similar population effect found between the two species in fitness estimates. This population effect on adaptive traits in different environments are worth looking further into (see Ramírez-Valiente et *al.*, in preparation).

If both species can be qualified as locally adapted, strategies for studying local adaptation in each species differ. On the one hand, in *P. pinaster*, these studies have seldom been conducted within a single ecotype (or gene-pool), and the high structuration can have a confounding effect on local adaptation inferences. On the other hand, the consequence of the distribution of Scots pine makes it difficult to consider all climate types in a single study. It has been shown that short-term climate change effects in *P. sylvestris* populations will be highly different according to habitat of origin across its range (Rehfeldt *et al.*, 2002). In general studies focused on different populations coming from only one region of the distribution range and it is difficult to make more general inferences (Perks & Ennos, 1999; Salmela *et al.*, 2013).

Selection gradients: overall patterns reflecting adaptive strategies

One of the most interesting outcome of our study is the pattern shown by mother traits involved in selection gradients (Supplementary Information Figure S2). Mother traits related to size (Height, DBH, WD) are more frequently found in *P. sylvestris*, whereas traits related to needles (SLA and $\delta^{13}C$) appear more often in *P. pinaster*. This striking pattern is in accordance with adaptive strategies of both species. With respect to *P. sylvestris*, the tested provenances are from the milder habitats of the natural range (Spain and Germany) and the trial sites remain in the most western part of the distribution (Finland, Lithuania, Germany and Spain). The selection gradients related to size traits are thus in accordance with the findings of Rehfeldt *et al.* (2002) who showed that *P. sylvestris* is naturally selected for growth in milder climate, and switches to selection for cold hardiness in more severe climate, which is all the more interesting considering this study shows these two traits to be negatively correlated . Though it would be logistically challenging, reciprocal common gardens such as ours would be worth implementing in the most northern/eastern part of the range to compare results. In the case of *P. pinaster*, both needle trait represented in the selection gradients are related to water use efficiency (WUE), the amount of dry matter produced per unit amount of water transpired, greatly involved in drought tolerance as high WUE in challenging conditions reflects low water requirement. It has been suggested than $\delta^{13}C$ and WUE are negatively correlated (Correia *et al.*, 2008), and $\delta^{13}C$ in often used as a way to estimate WUE in conifers (Warren *et al.*, 2001; Adams & Kolb, 2004; Correia *et al.*, 2008). SLA, the ratio of leaf area to leaf dry mass, is involved in many processes, and a critical parameter in drought resistance through stomatal density and water potential (Marshall & Monserud, 2003). *Pinus pinaster*, though demonstrating differences in drought resistance among its ecotypes (Eveno *et al.*, 2008; Aranda *et al.*, 2010; Gaspar *et al.*, 2013), is expected to be more subjected for water stress than Scots pine, given its repartition, explaining the overall trend observed in the selection gradients.

Selection gradients and mother traits

Interestingly, apart from its role in drought tolerance (Marshall & Monserud, 2003), SLA is also a main component of relative growth rate by its correlation to photosynthetic exchange (Reich *et al.*, 1998). In their study, Alía *et al.* (2014) found a positive quadratic selection gradient between fitness in *P. pinaster* (estimated as female reproductive success based on parentage analysis) and SLA breeding value for mother trees (measured in offspring). This is particularly interesting to put in perspective with heteroblasty: a trade-off exists between the

investment for establishment of saplings and photosynthesis, which is revealed by a change in needle structure between juvenile and adult stages (Kuusk *et al.*, 2018). In our study in *P. pinaster*, SLA of the mother-tree seems to influence seedling establishment too, since the most significant selection gradients are found for this trait in the French experiment, with GDD and SUR18 for the Spanish population and HW18 for the French population. There are also several significant selection gradients for this trait in the Spanish site (e.g. linear selection gradients with HW18 for the French population in this site or quadratic selection gradients with FIT18 for this same population). SLA stands out thus as an interesting trait to consider in adaptive potential studies.

The Italian *P. pinaster* population in our experiments behaves curiously: compared to the French and Spanish populations, the selection gradients associated with the Italian population involve more size-related mother traits, both in linear and quadratic fits. The origin of this difference is difficult to pinpoint. The seeds are originated from Rossiglione, a stand at an altitude of 445 m, and were planted in Madrid (Spain, 596 m) and Cestas (France, 62 m). If the altitude differences had provoked selection, it would have been more notable in the French experiment and would have also expected some significant correlations with $\delta^{13}C$, and not only size and growth traits, as isotopic discrimination increases with altitude in plants, including forest trees (Körner *et al.*, 1988; Hultine & Marshall, 2000). This might be better addressed when the results for the Italian common garden are available.

Caveats and limitations

Though the selection gradients presented in this study are statistically significant, some limitations need to be taken into account when interpreting the results. First of all, as explained by Lande & Arnold (1983), quadratic selection gradients need high sample size to be accurately detected, otherwise they must be computed after performing a principal components analysis on the offspring performances. In our study, the magnitude of the sampling campaign and the following laboratory processing did not always allow to keep high sample sizes, especially in the case of needle traits, for which the number of sampled mother trees was only 10 per population. This is translated to possible outlier effects when estimating selection gradients (i.e. selection gradients that are significant just because of the outlier values of a single mother tree; see Supplementary Information Figure S3), but dubious results were not removed from this study as the correlation they show might be proven real with increased sample size.

A third potential limitation of our approach is the need for the traits to be heritable to translate into evolutionary change. As reviewed by Lind *et al.* (2018), narrow sense heritability values

can be extremely variable, even for the same trait in a single species. For instance, DBH can present heritability values ranging from 0.02 (Danjon, 1994) to 0.57 (Zas *et al.*, 2004) and height from 0.08 (Danjon, 1994) to 1.14 (Corcuera *et al.*, 2010). This variability is also observed in physiology traits, such as $\delta^{13}C$, ranging from 0.07 (Aranda *et al.*, 2010) to 0.213 (Corcuera *et al.*, 2010). Moreover, most studies estimating narrow sense heritability take place in common garden, where heritability tends to be overestimated, as illustrated by Alía *et al.* (2014) that found narrow sense heritability of SLA to vary from 0.071 in outdoor natural conditions to 0.253 in indoor controlled-conditions. As it is, heritability of the traits involved in selection gradients must be estimated under the same environment as the gradients.

Application for breeding and conservation

Estimation of selection gradients can provide useful insights for understanding adaptive potential and foreseeing recruitment in future forests, as they allow progeny performance prediction under different environments based on mother-tree phenotypes. Some traits, such as SLA in *P. pinaster*, would be interesting to consider in modelling studies, are they are found in several selection gradients and therefore seem relevant for understanding adaptive potential. However, in our study, selection gradients detected under various environments showed substantial variability, and thus may be difficult to produce general models that can be applied to the full distribution of the species.

Additionally, the identification of the mother traits underlying progeny fitness can also be useful to inform strategies aiming at facilitating local adaptation with assisted gene-flow (Aitken & Whitlock, 2013), though this requires extended knowledge of the species climatic optima and other variables, as well as extensive experimentation, which is challenging in the case of widely distributed species such as *P. sylvestris*.

Finally, a limitation to be considered is that our study, as most studies aiming at understanding local adaptation focuses on one or few traits, wrongly suggesting that global change will only affect a few environmental factors, leaving others constant (Matesanz & Ramírez-Valiente, 2019). New modelling approaches highlights the urgent need of multi-factors models and the importance of phenotypic plasticity (Benito Garzón *et al.*, 2011).

Conclusion

This study provide new results that contribute to increase our understanding of evolutionary potential of *P. sylvestris* and *P. pinaster*, two main European forest trees. Though most of the mother-trait variables were involved in significant selection gradients, both relevance and strength of selection gradients were highly variable across species, populations and environments. In addition, trends observed in selection gradients detected in both species reflected their adaptive strategies facing contrasting environments and provide useful insights to understanding local adaptation processes in long-lived species such as forest trees.

References

Adams HD, Kolb TE. **2004**. Drought responses of conifers in ecotone forests of northern Arizona: tree ring growth and leaf δ13C. *Oecologia* **140**: 217–225.

Aitken SN, Whitlock MC. **2013**. Assisted Gene Flow to Facilitate Local Adaptation to Climate Change. *Annual Review of Ecology, Evolution, and Systematics* **44**: 367–388.

Aitken SN, Yeaman S, Holliday JA, Wang T, Curtis-McLane S. **2008**. Adaptation, migration or extirpation: climate change outcomes for tree populations. *Evolutionary Applications* **1**: 95–111.

Alía R, Chambel R, Notivol E, Climent J, González-Martínez SC. **2014**. Environment-dependent microevolution in a Mediterranean pine (*Pinus pinaster* Aiton). *BMC Evolutionary Biology* **14**: 200.

Alía R, Gil L, Pardos J. **1995**. Performance of 43 *Pinus pinaster* Ait. provenances on 5 locations in Central Spain. *Silvae genetica* **44**: 75–81.

Alía R, Moro-Serrano J, Notivol E. **2001**. Genetic variability of Scots pine (*Pinus sylvestris*) provenances in Spain: growth traits and survival. *Silva Fennica* **35**: 27–38.

Aranda I, Alía R, Ortega U, Dantas ÂK, Majada J. **2010**. Intra-specific variability in biomass partitioning and carbon isotopic discrimination under moderate drought stress in seedlings from four *Pinus pinaster* populations. *Tree Genetics & Genomes* **6**: 169–178.

Bates D, Mächler M, Bolker B, Walker S. **2015**. Fitting Linear Mixed-Effects Models Using *lme4*. *Journal of Statistical Software* **67**: 1–48.

Benito Garzón M, Alía R, Robson TM, Zavala MA. **2011**. Intra-specific variability and plasticity influence potential tree species distributions under climate change. *Global Ecology and Biogeography* **20**: 766–778.

Birol I, Raymond A, Jackman SD, Pleasance S, Coope R, Taylor GA, Yuen MM Saint, Keeling CI, Brand D, Vandervalk BP, *et al.* 2013. Assembling the 20 Gb white spruce (*Picea glauca*) genome from whole-genome shotgun sequencing data. *Bioinformatics* **29**: 1492–1497.

Bischoff A, Müller-Schärer H. **2010**. Testing population differentiation in plant species - how important are environmental maternal effects. *Oikos* **119**: 445–454.

Bucci G, González-Martínez SC, Le Provost G, Plomion C, Ribeiro MM, Sebastiani F, Alía R, Vendramin GG. **2007**. Range-wide phylogeography and gene zones in *Pinus pinaster* Ait. revealed by chloroplast microsatellite markers. *Molecular Ecology* **16**: 2137–2153.

Budde KB, Heuertz M, Hernández-Serrano A, Pausas JG, Vendramin GG, Verdú M, González-Martínez SC. **2014**. In situ genetic association for serotiny, a fire-related trait, in Mediterranean maritime pine (*Pinus pinaster*). *New Phytologist* **201**: 230–241.

Canham CD, LePage PT, Coates KD. **2004**. A neighborhood analysis of canopy tree competition: effects of shading versus crowding. *Canadian Journal of Forest Research* **34**: 778–787.

Castro J, Zamora R, Hodar JA, Gomez JM. **2004**. Seedling establishment of a boreal tree species (*Pinus sylvestris*) at its southernmost distribution limit: consequences of being in a marginal Mediterranean habitat. *Journal of Ecology* **92**: 266–277.

Chagné D, Lalanne C, Madur D, Kumar S, Frigério J-M, Krier C, Decroocq S, Savouré A, Bou-Dagher-Kharrat M, Bertocchi E, *et al.* 2002. A high density genetic map of maritime pine based on AFLPs. *Annals of Forest Science* **59**: 627–636.

Chambel MR, Climent J, Alía R. **2007**. Divergence among species and populations of Mediterranean pines in biomass allocation of seedlings grown under two watering regimes. *Annals of Forest Science* **64**: 87–97.

Corcuera L, Gil-Pelegrin E, Notivol E. **2010**. Phenotypic plasticity in *Pinus pinaster* δ13C: environment modulates genetic variation. *Annals of Forest Science* **67**: 812.

Corcuera L, Gil-Pelegrin E, Notivol E. **2012**. Differences in hydraulic architecture between mesic and xeric *Pinus pinaster* populations at the seedling stage. *Tree Physiology* **32**: 1442–1457.

Correia I, Almeida MH, Aguiar A, Alia R, David TS, Pereira JS. **2008**. Variations in growth, survival and carbon isotope composition (δ13C) among *Pinus pinaster* populations of different geographic origins. *Tree Physiology* **28**: 1545–1552.

Danjon F. **1994**. Heritabilities and genetic correlations for estimated growth curve parameters in maritime pine. *Theoretical and Applied Genetics* **89**–**89**: 911–921.

Eveno E, Collada C, Guevara MÁ, Léger V, Soto A, Díaz L, Léger P, González-Martínez SC, Cervera MT, Plomion C, *et al.* 2008. Contrasting patterns of selection at *Pinus pinaster* Ait. Drought stress candidate genes as revealed by genetic differentiation analyses. *Molecular Biology and Evolution* **25**: 417–437.

Farquhar GD, Ehleringer JR, Hubick KT. **1989**. Carbon Isotope Discrimination and Photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **40**: 503–537.

Fernandes PM, Rigolot E. **2007**. The fire ecology and management of maritime pine (*Pinus pinaster* Ait.). *Forest Ecology and Management* **241**: 1–13.

Gaspar MJ, Velasco T, Feito I, Alía R, Majada J. **2013**. Genetic Variation of Drought Tolerance in *Pinus pinaster* at Three Hierarchical Levels: A Comparison of Induced Osmotic Stress and Field Testing (PCG Ferreira, Ed.). *PLoS ONE* **8**: e79094.

Gossmann TI, Keightley PD, Eyre-Walker A. **2012**. The Effect of Variation in the Effective Population Size on the Rate of Adaptive Molecular Evolution in Eukaryotes. *Genome Biology and Evolution* **4**: 658–667.

Grivet D, Avia K, Vaattovaara A, Eckert AJ, Neale DB, Savolainen O, González-Martínez SC. **2017**. High rate of adaptive evolution in two widespread European pines. *Molecular Ecology* **26**: 6857–6870.

Hermisson J, Pennings PS. **2005**. Soft Sweeps: Molecular Population Genetics of Adaptation From Standing Genetic Variation. *Genetics* **169**: 2335–2352.

Hultine KR, Marshall JD. **2000**. Altitude Trends in Conifer Leaf Morphology and Stable Carbon Isotope Composition. *Oecologia* **123**: 32–40.

Jaramillo-Correa JP, Rodríguez-Quilón I, Grivet D, Lepoittevin C, Sebastiani F, Heuertz M, Garnier-Géré P, Alía R, Plomion C, Vendramin GG, *et al.* 2015. Molecular proxies for climate maladaptation in a long-lived tree (*Pinus pinaster* Aiton, Pinaceae). *Genetics* **199**: 793–807.

Körner C, Farquhar GD, Roksandic Z. **1988**. A global survey of carbon isotope discrimination in plants from high altitude. *Oecologia* **74**: 623–632.

Kuusk V, Niinemets Ü, Valladares F. **2018**. A major trade-off between structural and photosynthetic investments operative across plant and needle ages in three Mediterranean pines. *Tree Physiology* **38**: 543–557.

Lamy J-B, Delzon S, Bouche PS, Alía R, Vendramin GG, Cochard H, Plomion C. **2014**. Limited genetic variability and phenotypic plasticity detected for cavitation resistance in a Mediterranean pine. *New Phytologist* **201**: 874–886.

Lamy J-B, Lagane F, Plomion C, Cochard H, Delzon S. **2012**. Micro-evolutionary patterns of juvenile wood density in a pine species. *Plant Ecology* **213**: 1781–1792.

Lande R, Arnold SJ. **1983**. The Measurement of Selection on Correlated Characters. *Evolution* **37**: 1210.

Lind BM, Menon M, Bolte CE, Faske TM, Eckert AJ. **2018**. The genomics of local adaptation in trees: are we out of the woods yet? *Tree Genetics & Genomes* **14**: 29.

Marshall JD, Monserud RA. **2003**. Foliage height influences specific leaf area of three conifer species. *Canadian Journal of Forest Research* **33**: 164–170.

Matesanz S, Ramírez-Valiente JA. **2019**. A review and meta-analysis of intraspecific

differences in phenotypic plasticity: Implications to forecast plant responses to climate change (P Morellato, Ed.). *Global Ecology and Biogeography* **28**: 1682–1694.

Morgenstern M. **2011**. *Geographic Variation in Forest Trees: Genetic Basis and Application of Knowledge in Silviculture*. Vancouver: University of British Columbia Press.

Naydenov KD, Alexandrov A, Matevski V, Vasilevski K, Naydenov MK, Gyuleva V, Carcaillet C, Wahid N, Kamary S. **2014**. Range-wide genetic structure of maritime pine predates the last glacial maximum: evidence from nuclear DNA. *Hereditas* **151**: 1–13.

Neale DB, Savolainen O. **2004**. Association genetics of complex traits in conifers. *Trends in Plant Science* **9**: 325–330.

Notivol E, García-Gil MR, Alía R, Savolainen O. **2007**. Genetic variation of growth rhythm traits in the limits of a latitudinal cline in Scots pine. *Canadian Journal of Forest Research* **37**: 540–551.

O'Leary MH. **1981**. Carbon isotope fractionation in plants. *Phytochemistry* **20**: 553–567.

Peet RK, Christensen NL. **1987**. Competition and Tree Death. *BioScience* **37**: 586–595.

Perks MP, Ennos RA. **1999**. Analysis of genetic variation for quantitative characters between and within four native populations of Scots pine (*Pinus sylvestris*). *Botanical Journal of Scotland* **51**: 103–110.

Petit RJ, Hu FS, Dick CW. **2008**. Forests of the Past: A Window to Future Changes. *Science* **320**: 1450–1452.

Plomion C, Bastien C, Bogeat-Triboulot M-B, Bouffier L, Déjardin A, Duplessis S, Fady B, Heuertz M, Le Gac A-L, Le Provost G, *et al.* 2016. Forest tree genomics: 10 achievements from the past 10 years and future prospects. *Annals of Forest Science* **73**: 77–103.

Price GR. **1970**. Selection and Covariance. *Nature* **227**: 520–521.

Pyhäjärvi T, García-Gil MR, Knürr T, Mikkonen M, Wachowiak W, Savolainen O. **2007**. Demographic History Has Influenced Nucleotide Diversity in European *Pinus sylvestris* Populations. *Genetics* **177**: 1713–1724.

Pyhäjärvi T, Salmela MJ, Savolainen O. **2008**. Colonization routes of *Pinus sylvestris* inferred from distribution of mitochondrial DNA variation. *Tree Genetics & Genomes* **4**: 247–254.

Rehfeldt GE, Tchebakova NM, Parfenova YI, Wykoff WR, Kuzmina NA, Milyutin LI. **2002**. Intraspecific responses to climate in *Pinus sylvestris*. *Global Change Biology* **8**: 912–929.

Reich PB, Ellsworth DS, Walters MB. **1998**. Leaf structure (specific leaf area) modulates

photosynthesis-nitrogen relations: evidence from within and across species and functional groups. *Functional Ecology* **12**: 948–958.

Rellstab C, Gugerli F, Eckert AJ, Hancock AM, Holderegger R. **2015**. A practical guide to environmental association analysis in landscape genomics. *Molecular Ecology* **24**: 4348–4370.

Salmela MJ, Cavers S, Cottrell JE, Iason GR, Ennos RA. **2013**. Spring phenology shows genetic variation among and within populations in seedlings of Scots pine (*Pinus sylvestris* L.) in the Scottish Highlands. *Plant Ecology & Diversity* **6**: 523–536.

Savolainen O, Bokma F, García-Gil R, Komulainen P, Repo T. **2004**. Genetic variation in cessation of growth and frost hardiness and consequences for adaptation of *Pinus sylvestris* to climatic changes. *Forest Ecology and Management* **197**: 79–89.

Savolainen O, Kujala ST, Sokol C, Pyhajarvi T, Avia K, Knurr T, Karkkainen K, Hicks S. **2011**. Adaptive Potential of Northernmost Tree Populations to Climate Change, with Emphasis on Scots Pine (*Pinus sylvestris* L.). *Journal of Heredity* **102**: 526–536.

Slotte T, Foxe JP, Hazzouri KM, Wright SI. **2010**. Genome-Wide Evidence for Efficient Positive and Purifying Selection in *Capsella grandiflora*, a Plant Species with a Large Effective Population Size. *Molecular Biology and Evolution* **27**: 1813–1821.

Vizcaíno-Palomar N, Revuelta-Eugercios B, Zavala MA, Alía R, González-Martínez SC. **2014**. The Role of Population Origin and Microenvironment in Seedling Emergence and Early Survival in Mediterranean Maritime Pine (*Pinus pinaster* Aiton) (S Delzon, Ed.). *PLoS ONE* **9**: e109132.

Warren CR, McGrath JF, Adams MA. **2001**. Water availability and carbon isotope discrimination in conifers. *Oecologia* **127**: 476–486.

Wright SI, Andolfatto P. **2008**. The Impact of Natural Selection on the Genome: Emerging Patterns in *Drosophila* and *Arabidopsis*. *Annual Review of Ecology, Evolution, and Systematics* **39**: 193–213.

Zas R, Merlo E, Fernández-López J. **2004**. Genetic parameter estimates for maritime pine in the Atlantic coast of north-west Spain. *Forest Genetics* **11**: 45–53.

Supplementary Information to Chapter 2

Fitness estimates						
	ES_PP	FR_PP	DE_PS	ES_PS	FI_PS	LI_PS
GER17			Pop + Seed	Pop + Seed	Pop + Seed	Pop + Seed
GER1718				Pop + Seed	Pop + Seed	
GER18	Seed	Pop + Seed				
GDD	Pop + Seed	Pop				
SUR17			Pop	Seed	Seed	Pop
SUR1718			Pop	Pop + Seed	Pop	Pop
SUR18	Pop + Seed	Pop + Seed				
FIT18	Pop + Seed	Pop + Seed	Pop + Seed	Pop + Seed	Pop + Seed	Pop
HW17				Pop + Seed	Pop + Seed	
HW18	Seed	Pop + Seed	Pop + Seed	Pop + Seed	Pop + Seed	Pop

Figure S1. Significative fixed effects in best fitted models, used to compute family estimates in each trait. Pop: population, Seed: seed mass, GER17: germination in 2017, GER1718: cumulated germination in 2017-2018, GER18: germination 2018, GDD: days to germination, SUR17: survival 2017, SUR1718: cumulated survival in 2017-2018, SUR18: survival in 2018, FIT18: fitness 2018, HW17: height in winter 2017, HW18: height in winter 2018.

Linear

Populations	Site: Spain	Site: Germany	Site: Lithuania	Site: Finland
Spain	GER18, HW17		GER17	
Germany	GER18 HW17 HW18	GER17 SUR18 FIT18		

Quadratic

Populations	Site: Spain	Site: Germany	Site: Lithuania	Site: Finland
Spain	GER18 HW17 HW18			SUR18 HW17
Germany	SUR18 HW18	SUR17, SUR18 FIT18	GER17	GER18

Figure S2A: Schematic representation of selection gradients in *P. sylvestris*. In blue, traits related to mother-tree size (Height, DBH and wood density) and in orange, traits related to needles (δ13C and SLA). The codes inside the cells list the fitness components involved in significant selection gradients. Traits written in black in a grey cell involve both categories of mother-traits.

B.

Linear

Populations	Sites: Spain	Sites: France
Spain	SUR18 FIT18	GDD FIT18 SUR18 HW
France	GDD HW	GER18 SUR18 HW
Italy	SUR18 FIT18	GER18 GDD

Quadratic

Populations	Sites: Spain	Sites: France
Spain		GDD SUR18 HW
France	GER18 GDD SUR18 FIT18	SUR18 HW
Italy	GER18 FIT18 SUR18	GER18

Figure S2B: Schematic representation of selection gradients in *P. pinaster*, with the same colour code.

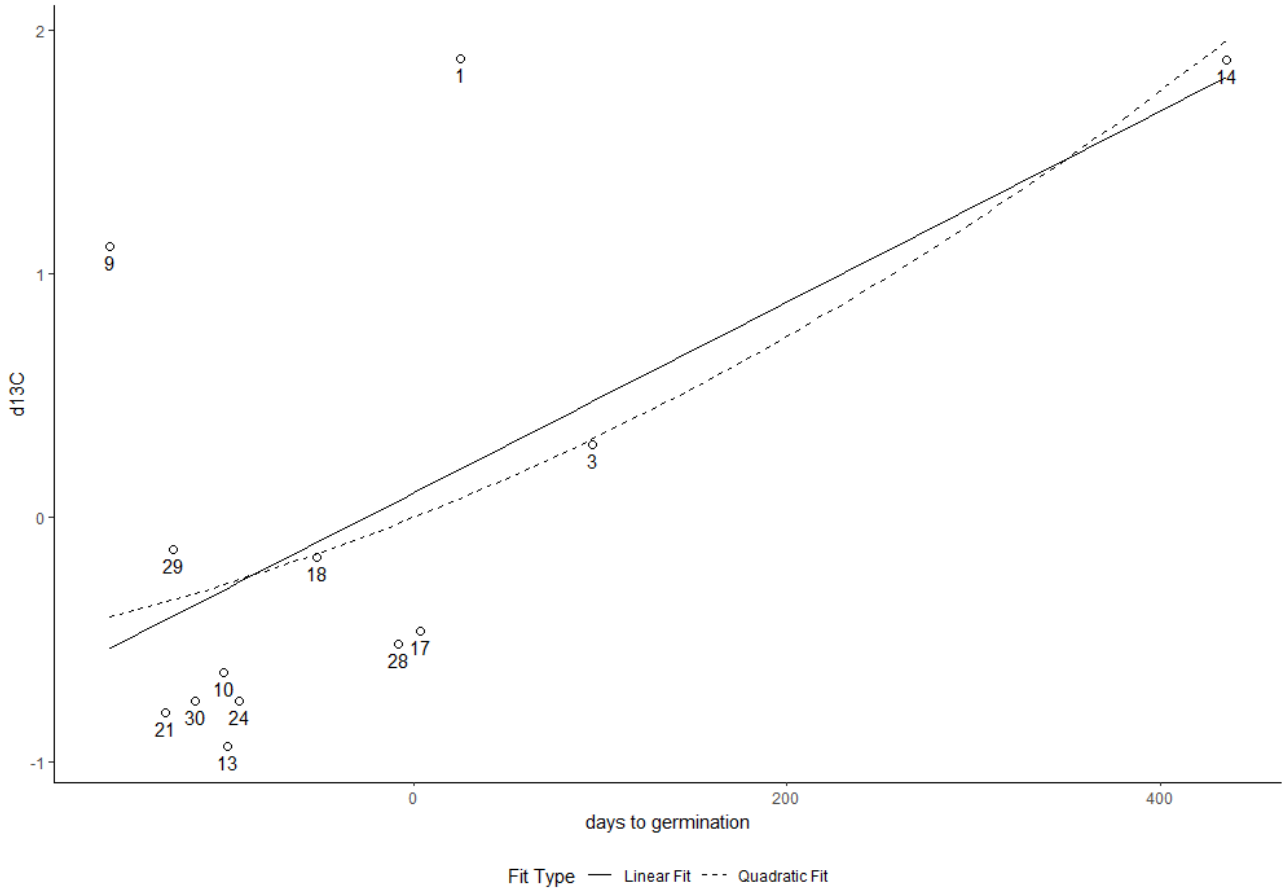


Figure S3: Relationship between δ13C and days to germination for the French population in the Spanish *P. pinaster* experiment. This selection gradient is an example of how, when sample size is low, the values of a single mother tree (# 14 in this case) may result in a significant model. However, the tendency of the distribution shows still a positive correlation, once the outlier is removed, meaning this selection gradient, though not fully reliable, cannot be discarded.

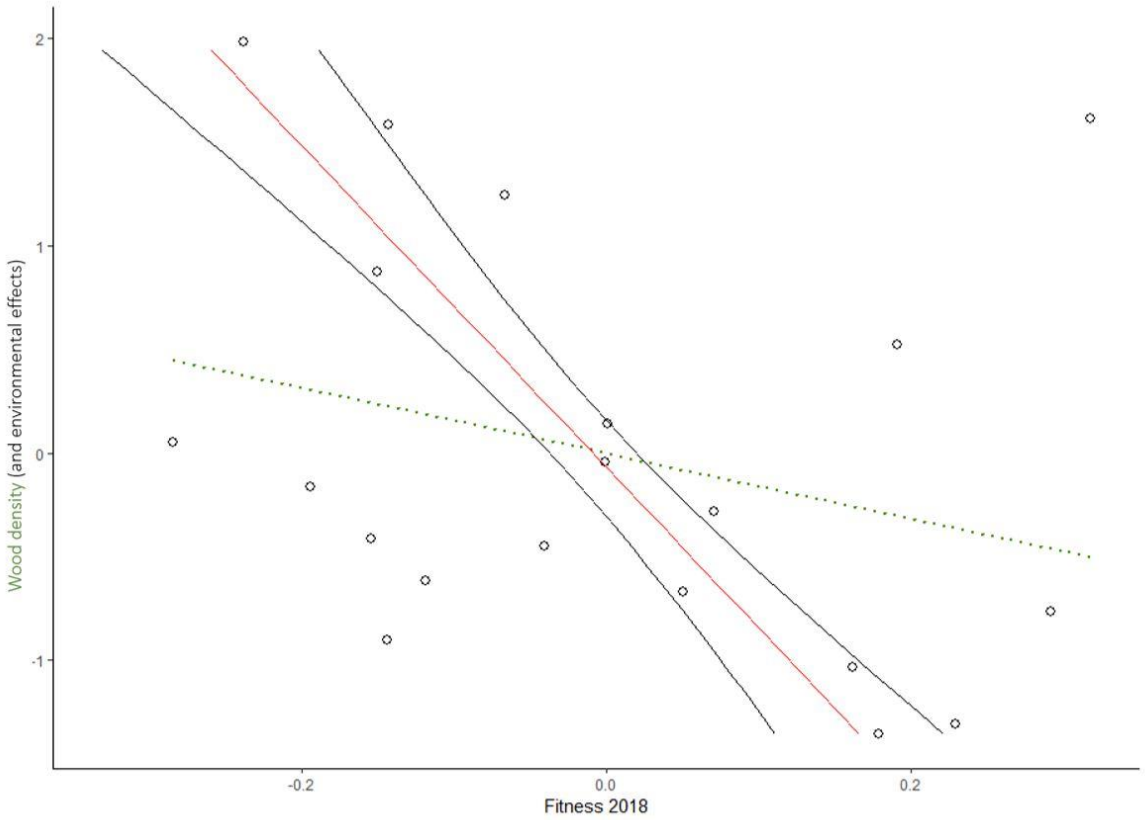


Figure S4: Comparison between model 1 (red line) and model 2 (green dotted line), as described below.

The two regressions represented here are expressed as follows:

$FIT18 \sim WD + CI + Age + latitude + longitude$ (1)

$FIT18 \sim WD$ (2)

This model comparison shows the importance of considering environmental effects, as fitness decreases more drastically when all variable considered (equation 1) than when only a simple model without covariates is taken into account (equation 2).

Chapter 3: The genetics of height and water use efficiency within the Corsican gene pool

Introduction

One of the insights Charles Darwin brought back from his travels was the importance of islands in the study of evolution and adaptation (Darwin, 1845). Ever since, evolutionists have taken a particular interest in islands and the particular opportunities they offer to study evolution (Losos & Ricklefs, 2009). By their limited size and absence of contact with continents, islands often exhibit simpler demography and genomic signatures of adaptive processes that are not confounded by population structure (Fulgione *et al.*, 2018). Insular populations are generally the product of ancient bottlenecks, limiting genetic diversity, and reduced effective population size (Nei *et al.*, 1975), related to a lesser carrying capacity than that of the mainlands. However, though limited in surface, islands can offer a great variety of local environments, forcing populations to adapt to contrasted conditions (Losos & Ricklefs, 2009). Such is the case in the Mediterranean islands, and particularly in Corsica. This 8,700 km^2 French island situated 80 km West off Tuscany (Italy) and 170 km South off the Côte-d'Azur (France) detached itself from the continent at the end of the Miocene era. Its last contact with the mainland took place during the Messinian salinity crisis, 5.3 million years ago (Mouillot *et al.*, 2008). Currently known as "the Mountain of the Mediterranean", Corsica possesses a wide range of altitudes (from sea level to over 2,700 m a.s.l), resulting in a multitude of different micro-climates and vegetation types, from typical Mediterranean on low coastal altitudes to alpine habitats above 1,500 m, as well as annual precipitations varying from 600 to 2,000 mm (Mouillot *et al.*, 2008). Although snow is typical during winter at the highest altitudes, the island is generally dry, hot, and extremely windy, making it particularly susceptible to fire risks (Mouillot *et al.*, 2002, 2008). The severe climatic conditions and frequent fire occurrences over large areas coupled with mountainous topography mean that gene flow between populations could be rather limited, resulting in genetic depletion and low genetic diversity (as illustrated for *Pinus pinaste*r by Mariette *et al.*, 2001), but still making local adaptation possible for some populations.

Pinus pinaster is a long-lived conifer with a discontinuous repartition range across the southwestern area of the Mediterranean basin and the southern part of the European Atlantic coast. Molecular studies have shown that this pine is genetically structured in different gene pools (Jaramillo-Correa *et al.*, 2015), probably the result of survival within several glacial refugia and limited gene flow across the later (Bucci *et al.*, 2007; Naydenov *et al.*, 2014). Evidence of adaptation of each gene pool to its local environment is abundant, particularly with respects to morphology and physiology traits. For instance, the Corsican *P. pinaster* is generally well adapted to drought and demonstrates remarkable trunk straightness (Durel & Bahrman,

1995). These features make the Corsican *P. pinaster* an exceptionally important resource for the extensive French breeding program, which started in 1960 to improve growth and straightness in this economically important forest tree. More specifically, hybrids between Corsican and Landes trees are now being proposed, as improved varieties to the private sector for reforestation in southwestern France. Another important point to consider regarding the Corsican *P. pinaster* is that it is represented by a single gene pool all across the island (Jaramillo-Correa *et al.*, 2015). This last point makes studying local adaptation of *P. pinaster* in Corsica particularly interesting from an evolutionary point of view, as the adaptation process signatures will be less, if not, confounded by genetic structure.

The present study focuses on two breeding-related traits with potential economic importance: total height (HT) and water use efficiency, WUE, as estimated by carbon isotope discrimination ($\delta^{13}C$). Carbon is naturally present in two isotopes in the air, as ^{12}C (98.9% of atmospheric carbon dioxide) and heavier ^{13}C (1.1%). These two isotopes are discriminated during photosynthesis, with $\delta^{13}C$ reflecting plant metabolism and environment and being related to Water Use Efficiency (O'Leary, 1981; Farquhar *et al.*, 1989). Our study focuses on a large common garden, "PINCORSE", made of half-sib families of 30 *P. pinaster* populations representing the Corsican local diversity. We were thus able to estimate quantitative genetics parameters, such as the narrow-sense heritability (h^2) and the quantitative genetic differentiation among populations (Q_{ST},), for both traits. Moreover, a sample of trees from the common garden was genotyped using *ca.* 100k SNPs. This dataset allowed to assess neutral population genetic structure precisely and to identify differentiated populations with conservation interest. This genotyping effort is remarkable considering the size and complexity of conifer genomes (McKay et al., 2012). Knowing the importance of the Corsican population of maritime pine there has been surprisingly few molecular studies in the last decade and the present study is the first since Mariette *et al.* (2001). They had detected genetic differences between populations from Corsica and Aquitaine (southwestern France), but lacked resolution to explore genetic variations within the island (this study used only three microsatellite markers).

The objectives of this work were two-fold: i/ study the genetic structure and differentiation between the Corsican *P. pinaster* populations in contrasted environments, using both molecular markers and relevant quantitative traits, and ii/ estimate the heritability for total height and WUE, two traits of great value for the maritime pine breeding programme.

Material and Methods

Common garden

The common garden PINCORSE is a combined provenance-progeny test structured into 30 populations and 20 half-sib families (~20 half-sibs in each family, see Durel & Bahrman, 1995). The Experimental Unit of INRA Cestas installed these 900 progenies on approximately 30 ha during three successive campaigns (2010, 2011, and 2012). During each campaign, a "plot" with different populations was planted, and families from these populations were randomised within blocks, with five complete blocks per plot. This resource was collected in Corsica during two surveys (1994 and 2000) by INRA staff (C-E. Durel, N. Bahrman, J-M. Louvet, E. Bertocchi, and J. Brach in 1994; C. Plomion and J. Brach in 2000). The whole natural range of maritime pine in Corsica was covered (altitude from sea level to more than 1,200 m a.s.l., rainfall from 400 mm to nearly 2,000 mm), including core and marginal populations.

Phenotyping

Total height (HT) of all surviving trees was measured at age 4 (N=14,386). A subset of populations and families (11 populations, 12 families per population, with 6-8 half-sibs per family) was also sampled (N=899) to evaluate Water Use Efficiency (WUE) based on carbon isotope discrimination ($\delta^{13}C$), following standard procedures. The more negative (further from zero) values of $\delta^{13}C$ represent lower water use efficiency WUE, and are considered to have lower drought tolerance.

Genotyping

Fifteen Corsican populations were selected for genotyping (36 samples per population in 12 populations, including 3 sibs per family, and 12 unrelated samples per population in 3 populations) (see Figure 1). Two individuals from Ospedale population in southern Corsica were also genotyped. Total sample size amounted to 470 individuals. DNA was extracted thanks to an improved protocol: sampled were kept at – 80°C for 24h hours before extracting. During the lysis step, grinding was improved by frozing the sample between each session in the grinder to avoid over-heating. The rest was done accordingly with the Qiagen kit protocol. SNP data was produced by sequence-targeted methods (3 Mbp) and externalised to an genomic facility (IGATs, Udine, Italy). A raw SNP file (VCF format) was obtained from this service, including several million SNPs in 465 samples (five samples produced few reads and were discarded prior to analysis).

This file was first ('soft') filtered by:

- minimum number of SNP: 1 (present in at least one sample)

- minimum coverage per sample: 8 reads

- minimum allele frequency : 20% (referred to single sample to call it heterozygous or not)

- minimum number of samples with informative data: 50%

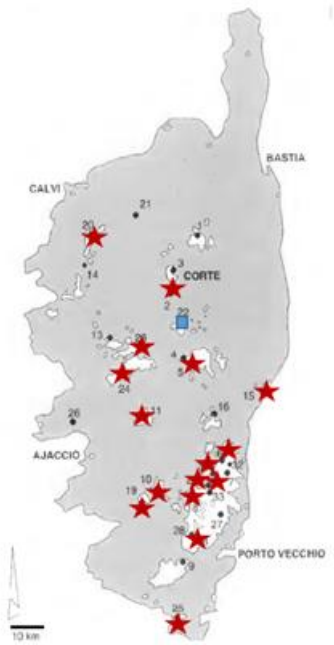


Figure 1. Fifteen populations sampled for genotyping (two additional Ospedale samples were also included). All samples were measured for total height at 4 years of age. Samples from Zonza, Vallemalla, Tova, Arza, Larone, and Ospedale were not evaluated for $\delta^{13}C$, while an additional population, Cervello (blue square) was added to the $\delta^{13}C$ phenotyping.

This produced a VCF file with 386,929 polymorphic and biallelic SNPs. This file was further ("hard") filtered using GATK (McKenna *et al.*, 2010) and the following filtering expression: "MQ < 30.0 || SOR > 3.0 || MQRankSum < -12.5 || ReadPosRankSum < -8.0 || DP > 35516". Finally, the VCF file was filtered by site quality (QUAL > 25) using vcftools (Danecek *et al.*, 2011) and to allow only a maximum of 20% missing data. In addition, 23 samples with more than 50% missing data were removed, resulting in a final dataset of 94,733 SNPs in 442 samples.

Data analysis

Quantitative genetics analyses

Total height (HT) and isotope carbon discrimination ($\delta^{13}C$) were analysed with the following mixed model in R (*lme4* package) (Bates *et al.*, 2015):

$$y \sim plot/block + (1|pop) + (1|pop/fam) \qquad (1)$$

where *y* is the target trait (either HT or δ13C), block within plot is specified as a fixed effect, and population and family nested within population as random effects.

Then, narrow-sense heritability (h^2) and genetic differentiation among populations (Q_{ST}) (Spitze, 1993) were computed as follows:

$$h^2 = \frac{\sigma_a}{\sigma_p} \qquad (2)$$

where σ_a is 4 times the family variance and σ_p is the total phenotypic variance, and

$$Q_{ST} = \frac{\sigma_{pop}}{2\,\sigma_a + \sigma_{pop}} \qquad (3)$$

where σ_{pop} is the among-population variance.

Finally, genetic correlations between HT and $\delta^{13}C$ were estimated, computing the Pearson correlation coefficient between the families Best Linear Unbiased Predictors (BLUPs) for these two variables (Henderson, 1973; Robinson, 1991).

Population genetic structure

Population genetic structure was studied with fastSTRUCTURE (Raj *et al.*, 2014) based on 180 unrelated individuals (one half-sib per family) from the 15 populations (12 individuals per population) and with 94,733 SNPs. In addition, based on the same data, we computed Weir and Cockerham (1984) estimator of F_{ST} using --weir-fst-pop instruction in vcftools. Finally, a Principal Component Analysis was applied using the glPca function in adegenet R package (Jombart & Ahmed, 2011), in order to visualize the genetic structure of the populations.

Results

Population genetic structure

FastSTRUCTURE runs with K = 1 to 15 did not find any population structure in Corsican maritime pine populations. Accordingly, F_{ST} was close to zero (0.0050) and not significant. However, a PCA based on the 94,733-SNP dataset allowed to identify one clearly differentiated population: Ventilegne, a marginal population in the southernmost part of the island (Figures 2a and b).

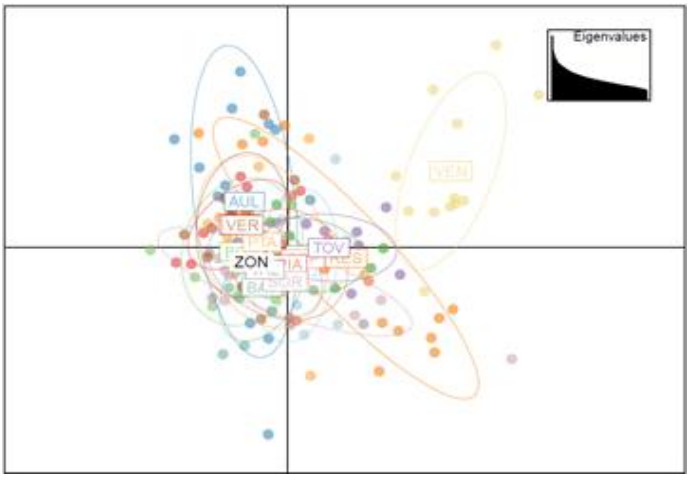


Figure 2a. PCA depicting PC 1 (1.63 %) vs. PC 2 (1.27 %) in Corsican maritime pine populations.

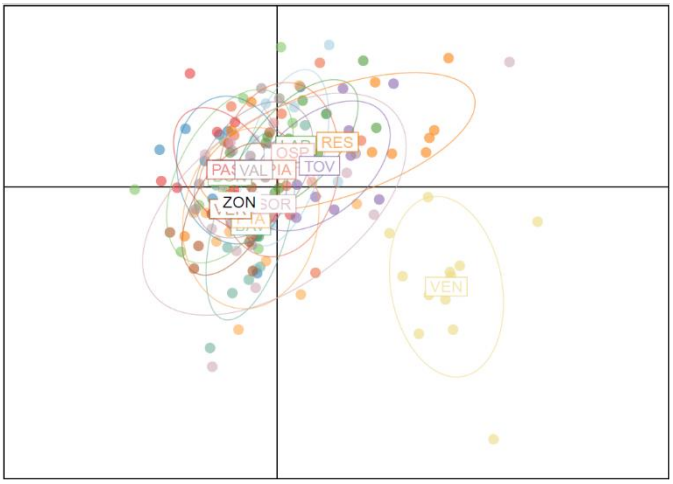


Figure 2b. PCA depicting PC 1 (1.63 %) vs. PC 3 (1.25 %) in Corsican maritime pine populations.

The Ventilegne population is remarkably differentiated on both PCs. As shown in the inset in Figure 2a, the eigenvalues decrease slowly, meaning each PC explains only a reduced amount of variability. Restonica, and to a lesser extent, Tova, are also marginally differentiated, when using other PCs for visual identification (data not shown).

Trait BLUPs, heritability and genetic correlations

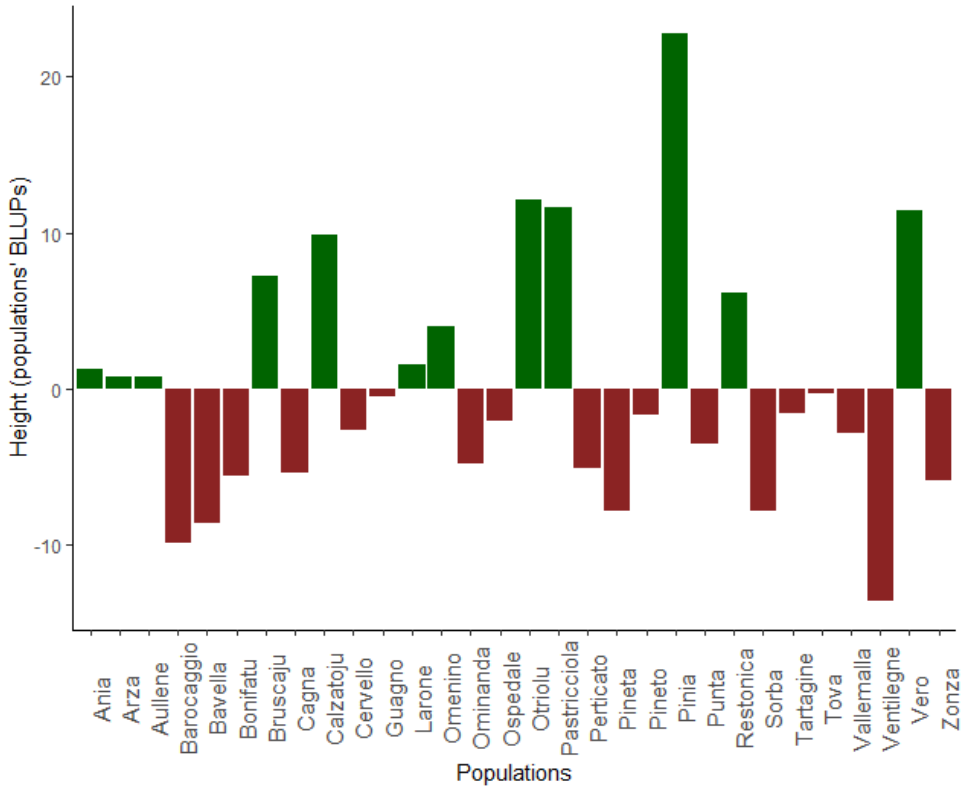


Figure 3. Population total height in PINCORSE, represented by population BLUPs. In green, population height BLUP > 0. In brown, population height BLUP < 0.

In contrast with population BLUPs for $\delta^{13}C$ that showed almost no variation (data not shown), population BLUP values for height vary from 22.72 (Pinia) to – 13.63 (Ventilegne). Interestingly, both populations are located at low altitude in their natural environment (50 m and 10 m a.s.l, respectively).

Correlations between population BLUPs for height and elevation of the sampled populations illustrate this peculiarity, as well as an overall negative correlation between these two variables (Figure 4).

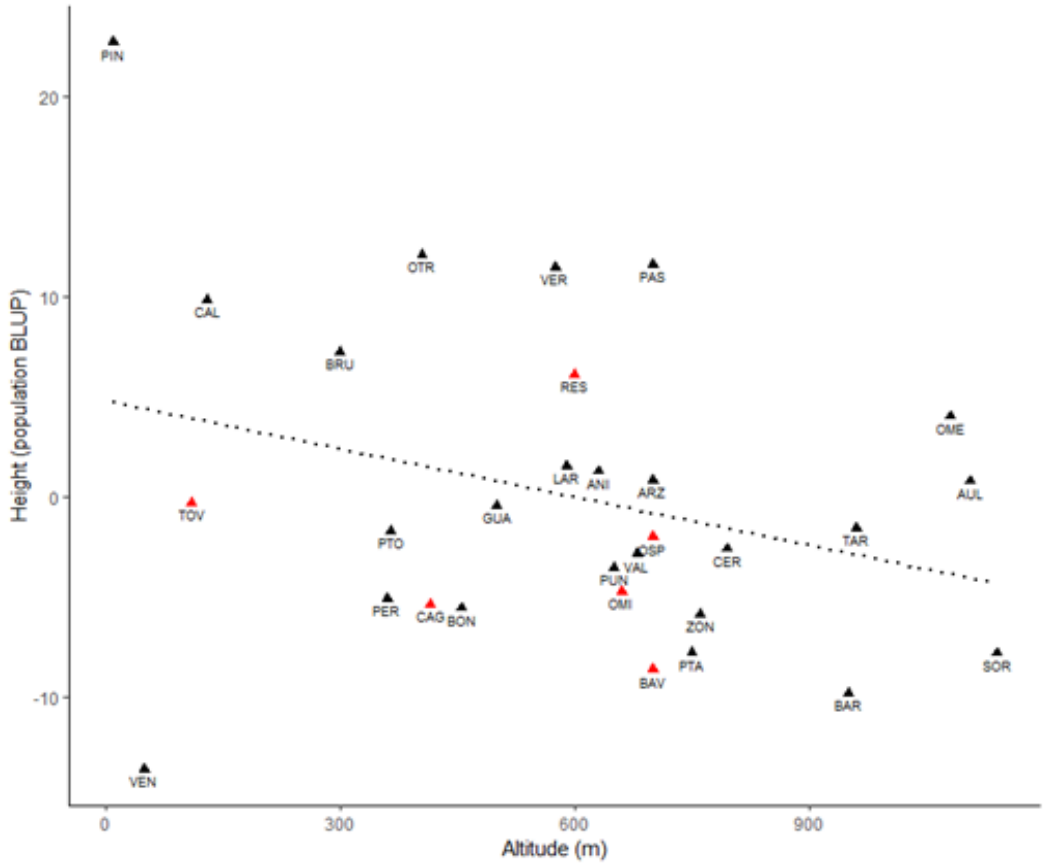


Figure 4: Correlation between altitude (in m a.s.l.) and population BLUP. The dotted line represents the correlation trend (Pearson's *r*: -0.39). Represented in red are the populations that were also studied by Durel & Bahrman (1995) (see *Discussion*).

Narrow-sense heritability h^2 was moderate for the two studied traits, $h^2 = 0.2906$ for total height (HT) and 0.4423 for $\delta^{13}C$. Genetic differentiation was low, with $Q_{ST} = 0.063$ for HT and $Q_{ST} = 0.000$ for $\delta^{13}C$. These results agree with small differences only in trait BLUPs across populations for $\delta^{13}C$ (see above) and the lack of population genetic structure found in fastSTRUCTURE runs. Genetic correlation between HT and $\delta^{13}C$ was slightly negative (-0.068) but not significant, suggesting the absence of trade-offs between these traits.

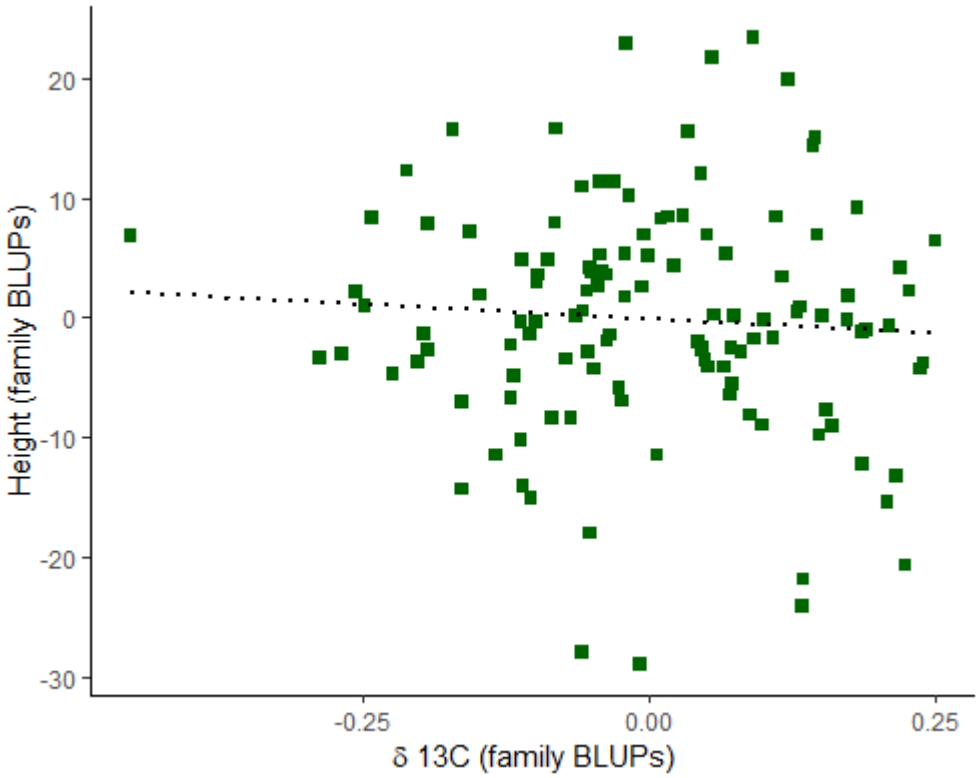


Figure 5. Genetic correlation between HT and δ13C based on family BLUPs. The black dotted line represents the correlation trend. As only the family BLUPs are used, the population effect is not taken into account in this correlation, as the use of BLUPS rescale data around zero, families with breeding potential are situated in the upper left quarter of the distribution, since they have higher water use efficiency WUE as well as better growth.

The data point placed in the furthest left part of the graph represents the family Restonica #14, characterized by very poor water use efficiency. Interestingly, though population effect is not taken into account here, the two families having the lowest height BLUPs are Ventilegne #12 and 13. On a population level, Ventilegne also showed the lowest values of height BLUPs.

Discussion

Our objective was to study several populations of *P. pinaster* from a single gene pool, in a region with high environmental variability. Gene flow between Corsican populations of *P. pinaster* seems high, as almost no structure was detected between them. Still, populations are differentiated in total height, probably in correlation with the altitude of their original stand. Moreover, we detected additive genetic effects for height and Water Use Efficiency as estimated by $\delta^{13}C$. These results have important implications for the conservation and breeding of *P. pinaster*.

Importance of environmental effects on trait expression

Although extremely useful for studying the genetic basis of quantitative traits in forest trees (Morgenstern, 2011), single common garden experimental settings present the main inconvenient of representing a single environment at a time, therefore limiting trait variability. For instance, water use efficiency (WUE) estimated by $\delta^{13}C$ is better expressed in the case of water limitation (Corcuera *et al.*, 2010). In their study, Marguerit *et al.* (2014) were able to detect substantial differences in $\delta^{13}C$ in *P. pinaster* between three sites with different mean precipitation. The same authors also found a difference in growth between the three sites, the wettest of the three displaying better growth. The Gironde region where the PINCORSE garden is planted has an oceanic climate, and therefore is one of the wettest in France (annual mean precipitation: ~ 1000 mm). Though informative for the intense breeding programme in the region, PINCORSE apparently does not allow to fully explore the genetic variability of certain traits. Interestingly, height in Corsican *P. pinaster* populations was also evaluated within the Gironde climate in 1995 by Durel & Bahrman (Figure 6).

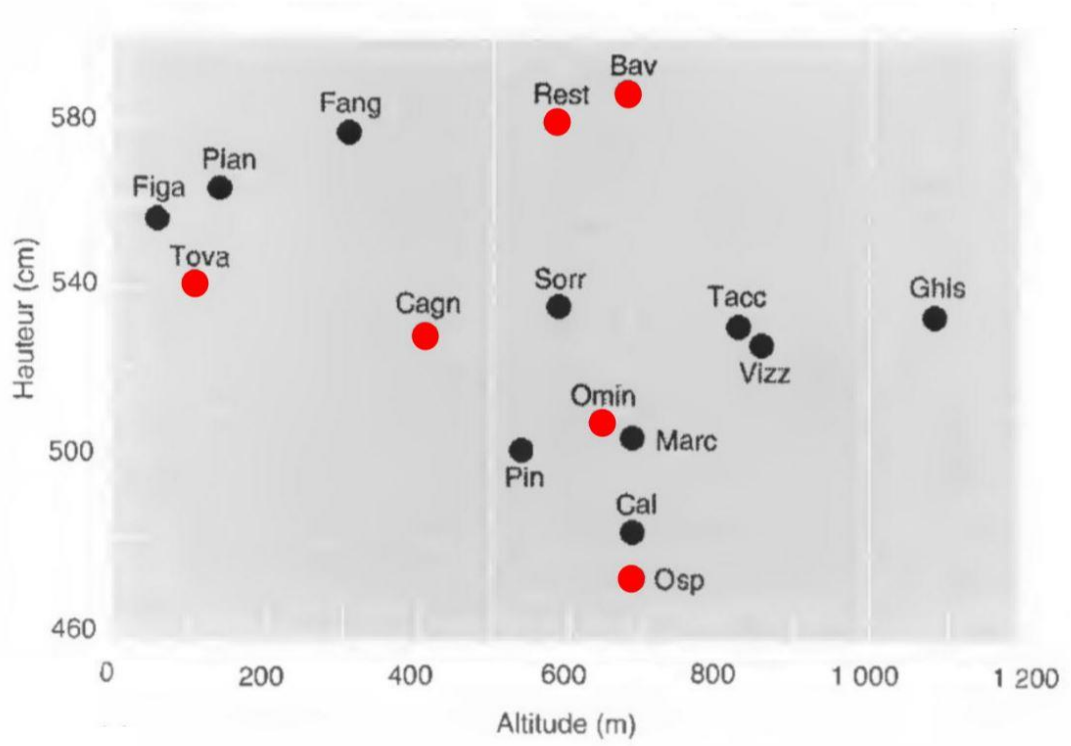


Figure 6. Correlation between height (cm) and altitude (m a.s.l.) for Corsican *P. pinaster* populations (Pearson's *r*: -0.36, non-significant), in the analyses by Durel & Bahrman (1995) in Le Bray common garden (Gironde). Individuals were measured at 9 years of age. Le Bray common garden is located close to PINCORSE, in the same geographic zone in Gironde. Red dots represent populations also measured in our study.

The two analyses are not directly comparable, as we represented the correlation between altitude (m a.s.l.) and the BLUPs of the populations, i.e. a measure of the genetic component for height with most environmental effects removed. Still, the general trend of the two correlations is similar (see Figures 4 and 6), and populations are placed comparably on the two graphs, with the exception of the Bavella population (Bav). This population was found among those with higher growth in Durel & Bahrman (1995), but performed poorly in our study. Some interpretation such as the role of tree age (9 years in Durel & Bahrman study and four years in ours) can be proposed regarding this discrepancy, but definitive conclusions on the influence of environment on genetic expression of height and WUE in Corsican *P. pinaster* populations could only be reached if tested in other, more contrasted environments.

Consequences for breeding and conservation

Accordingly to our expectations, the Corsican populations did not display any genetic population structure (F_{ST} not significantly different from zero), but against our expectations, nor did they show any differentiation for $\delta^{13}C$, and only moderate for total height (HT, $Q_{ST} = 0.063$). This indicates that there is no great variability to exploit at the population level. However, relatively high heritability (see below) and the absence of significant genetic correlations between $\delta^{13}C$ and height are relevant for breeding programs, since it means potential for selection on the family level for the two traits, with selection for one trait not countering a selection for the other. Similarly, Marguerit *et al.* (2014) did not find any trade-off between these two traits in the Landes provenance of *P. pinaster*. Moreover, though gene flow seems important enough to prevent differentiation between Corsica's *P. pinaster* populations, some marginal populations were identified and considered differentiated enough to be further investigated with conservation goals. Ventilegne in particular showed the lowest BLUP for height on population level (Figure 3), stands as an outlier in the correlation between height population BLUPs and altitude (Figure 4), and stands out on the PCAs (Figures 2a and b). On the family level, the two with the lowest BLUPs for height also originated from Ventilegne (namely, families #12 and #13). Furthermore, it is the southernmost population of the Corsican distribution of the species and it is geographically distant from the core populations (see map in Figure 1). Though not as striking, Pinia has extremely high population BLUP for height and has the lowest elevation (sea level) of the distribution (Figures 2 and 3). Besides, on the population level, Restonica stands out on the PCA. On the family level, Restonica #14 is the less advisable for breeding, as it shows an extremely low BLUP for WUE estimated by $\delta^{13}C$, associated with intermediate BLUPs for height.

Heritability and selection

For a trait to be successfully used in a breeding program or to be able to evolve in natural populations, the selected traits must be heritable. We therefore estimated narrow sense heritability, h^2, for our two traits of interests. Narrow-sense heritability for $\delta^{13}C$ was relatively high ($h^2 = 0.44$) and higher than previously reported. Indeed, Marguerit *et al.* (2014) estimated the narrow sense heritability of $\delta^{13}C$ at 0.29 ± 0.07. This difference, however, can be explained by the origins of the tested populations. In their trial, Marguerit *et al*. used populations from Aquitaine, a gene pool known to have different WUE than Corsican provenance. Similarly, in 2011, Lamy *et al.* estimated a $h^2 = 0.21 \pm 0.10$ for δ13C in western French, Spanish and

Moroccan *P. pinaster* populations, in a common garden located in Cestas (Gironde). These values highlight the well-known fact that heritability is both environment- and population-specific (ref).

Narrow-sense heritability for height was moderate: 0.2906. Unlike $\delta^{13}C$, genetic differentiation for this trait was low but significant, with Q_{ST}=0.063. The extremely low value of the F_{ST} (0.0050) would indicate a degree of differentiation for height exceeding that reached by drift alone, indicating the action of past selection for adaptive divergence in *P. pinaster* for this trait.

Further work

Though conifer genomes are remarkably complex (Chagné *et al.*, 2002; Neale & Savolainen, 2004; Mackay *et al.*, 2012), great progress has been made in the last decade to produce genomic resources (Chancerel *et al.*, 2011; Plomion *et al.*, 2016), allowing to develop association studies (Lepoittevin *et al.*, 2012; Budde *et al.*, 2014; Rodríguez-Quilón, 2017). In our case, sequencing efforts had led to the availability of 94,733 novel SNPs, exploitable for association studies with the two traits of interest. Since complex quantitative adaptive traits are often polygenic, single-locus allelic effects on one trait are mostly weak, hence the importance of developing SNP resources to account for a high part of the trait's variance. Other adaptive and/or commercial traits would be worth adding to our data set in order to study Corsican populations: if, as predicted, climate change causes a shift in seasonal rotations (Loustau *et al.*, 2005), understanding the genetic basis underlying phenology is crucial, even more so on an island, where no gene flow from the mainland may help adaptation. Moreover, Corsican *P. pinaster* have shown great susceptibility to *Matsucoccus feytaudi*, an invasive herbivore causing great damage (Jactel *et al.*, 1998, 2006). The development of quantitative genetics and association studies could help identify the populations that are the most resistant to the pest and the genetic architecture of such a trait (see Chapter 1).

Moreover, extra populations could be included in the analysis, including other marginal populations. As shown before, some marginal populations are differentiated from the common gene pool, and they may be of interest for conservation.

Conclusion

This study allowed to identify potential populations and families for breeding and conservation objectives. Although we are aware that quantitative genetics studies in a single common garden do not allow us to estimate genotype x environment interactions, we still think that this information is valuable, as the common garden is located in a region of intensive use and breeding of *P. pinaster*. The absence of higher genetic differentiation among populations for HT and WUE was unexpected, considering the topography, climate, and frequent fires in Corsica. This suggest a high level of gene flow. Narrow sense heritability for two traits of breeding and conservation interest were estimated for the first time in the Corsican gene pool, and considerable genotyping efforts offer great prospects for association studies involving the Corsican *P. pinaster* populations.

References

Bates D, Mächler M, Bolker B, Walker S. **2015**. Fitting Linear Mixed-Effects Models Using *lme4*. *Journal of Statistical Software* **67**: 1–48.

Bucci G, González-Martínez SC, Le Provost G, Plomion C, Ribeiro MM, Sebastiani F, Alía R, Vendramin GG. **2007**. Range-wide phylogeography and gene zones in *Pinus pinaster* Ait. revealed by chloroplast microsatellite markers. *Molecular Ecology* **16**: 2137–2153.

Budde KB, Heuertz M, Hernández-Serrano A, Pausas JG, Vendramin GG, Verdú M, González-Martínez SC. **2014**. In situ genetic association for serotiny, a fire-related trait, in Mediterranean maritime pine (*Pinus pinaster*). *New Phytologist* **201**: 230–241.

Chagné D, Lalanne C, Madur D, Kumar S, Frigério J-M, Krier C, Decroocq S, Savouré A, Bou-Dagher-Kharrat M, Bertocchi E, *et al.* 2002. A high density genetic map of maritime pine based on AFLPs. *Annals of Forest Science* **59**: 627–636.

Chancerel E, Lepoittevin C, Le Provost G, Lin Y-C, Jaramillo-Correa JP, Eckert AJ, Wegrzyn JL, Zelenika D, Boland A, Frigerio J-M, *et al.* 2011. Development and implementation of a highly-multiplexed SNP array for genetic mapping in maritime pine and comparative mapping with loblolly pine. *BMC Genomics* **12**: 368.

Corcuera L, Gil-Pelegrin E, Notivol E. **2010**. Phenotypic plasticity in *Pinus pinaster* δ13C: environment modulates genetic variation. *Annals of Forest Science* **67**: 812.

Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST, *et al.* 2011. The variant call format and VCFtools. *Bioinformatics* **27**: 2156–2158.

Darwin C. **1845**. *Journal of researches into the natural history and geology of the countries visited during the voyage round the world of H.M.S. 'Beagle' under the command of Captain Fitz Roy, R.N. /* (Ward and Lock, Eds.). London: J. Murray.

Durel C-E, Bahrman N. **1995**. Analyse de la diversité génétique des peuplements de pin maritime de Corse. Gestion et exploitation de la ressource génétique. *Revue Forestière Française* **5**: 509–522.

Farquhar GD, Ehleringer JR, Hubick KT. **1989**. Carbon Isotope Discrimination and Photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology* **40**: 503–537.

Fulgione A, Koornneef M, Roux F, Hermisson J, Hancock AM. **2018**. Madeiran Arabidopsis thaliana Reveals Ancient Long-Range Colonization and Clarifies Demography in

Eurasia. *Molecular Biology and Evolution* **35**: 564–574.

G.K. Robinson. **1991**. That BLUP is a Good Thing: The Estimation of Random Effects. *Statistical Science* **6**: 15–32.

Henderson CR. **1973**. Sire evaluation and genetic trends. *Journal of Animal Science* **1973**: 10–41.

Jactel H, Menassieu P, Ceria A, Burban C, Regad J, Normand S, Carcreff E. **1998**. Une pullulation de la cochenille *Matsucoccus feytaudi* provoque un début de dépérissement du Pin maritime en Corse. *Revue Forestière Française* **50**: 33.

Jactel H, Menassieu P, Vetillard F, Gaulier A, Samalens JC, Brockerhoff EG. **2006**. Tree species diversity reduces the invasibility of maritime pine stands by the bast scale, *Matsucoccus feytaudi* (Homoptera: Margarodidae). *Canadian Journal of Forest Research* **36**: 314–323.

Jaramillo-Correa JP, Rodríguez-Quilón I, Grivet D, Lepoittevin C, Sebastiani F, Heuertz M, Garnier-Géré P, Alía R, Plomion C, Vendramin GG, *et al.* 2015. Molecular proxies for climate maladaptation in a long-lived tree (*Pinus pinaster* Aiton, Pinaceae). *Genetics* **199**: 793–807.

Jombart T, Ahmed I. **2011**. adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. *Bioinformatics* **27**: 3070–3071.

Lamy J-B, Bouffier L, Burlett R, Plomion C, Cochard H, Delzon S. **2011**. Uniform Selection as a Primary Force Reducing Population Genetic Differentiation of Cavitation Resistance across a Species Range (PK Ingvarsson, Ed.). *PLoS ONE* **6**: e23476.

Lepoittevin C, Harvengt L, Plomion C, Garnier-Géré P. **2012**. Association mapping for growth, straightness and wood chemistry traits in the *Pinus pinaster* Aquitaine breeding population. *Tree Genetics and Genomes* **8**: 113–126.

Losos JB, Ricklefs RE. **2009**. Adaptation and diversification on islands. *Nature* **457**: 830836.

Loustau D, Bosc A, Colin A, Ogee J, Davi H, Francois C, Dufrene E, Deque M, Cloppet E, Arrouays D, *et al.* 2005. Modeling climate change effects on the potential production of French plains forests at the sub-regional level. *Tree Physiology* **25**: 813–823.

Mackay J, Dean JFD, Plomion C, Peterson DG, Cánovas FM, Pavy N, Ingvarsson PK, Savolainen O, Guevara MÁ, Fluch S, *et al.* 2012. Towards decoding the conifer giga-genome. *Plant Molecular Biology* **80**: 555–569.

Marguerit E, Bouffier L, Chancerel E, Costa P, Lagane F, Guehl J-M, Plomion C, Brendel O. **2014**. The genetics of water-use efficiency and its relation to growth in maritime pine. *Journal of Experimental Botany* **65**: 4757–4768.

Mariette S, Chagné D, Lézier C, Pastuszka P, Raffin A, Plomion C, Kremer A. **2001**. Genetic diversity within and among *Pinus pinaster* populations: comparison between AFLP and microsatellite markers. *Heredity* **86**: 469–479.

McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, *et al.* 2010. The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research* **20**: 1297–1303.

Morgenstern M. **2011**. *Geographic Variation in Forest Trees: Genetic Basis and Application of Knowledge in Silviculture*. Vancouver: University of British Columbia Press.

Mouillot F, Paradis G, Andrei-Ruiz M-C, Quilichini A. **2008**. Corsica. In: Mediterranean Island Landscapes. Landscape Series, vol 9. Springer, Dordrecht, 220–244.

Mouillot F, Rambal S, Joffre R. **2002**. Simulating climate change impacts on fire frequency and vegetation dynamics in a Mediterranean-type ecosystem. *Global Change Biology* **8**: 423–437.

Naydenov KD, Alexandrov A, Matevski V, Vasilevski K, Naydenov MK, Gyuleva V, Carcaillet C, Wahid N, Kamary S. **2014**. Range-wide genetic structure of maritime pine predates the last glacial maximum: evidence from nuclear DNA. *Hereditas* **151**: 1–13.

Neale DB, Savolainen O. **2004**. Association genetics of complex traits in conifers. *Trends in Plant Science* **9**: 325–330.

Nei M, Maruyama T, Chakraborty R. **1975**. The Bottleneck Effect and Genetic Variability in Populations. *Evolution* **29**: 1.

O'Leary MH. **1981**. Carbon isotope fractionation in plants. *Phytochemistry* **20**: 553–567.

Plomion C, Bartholomé J, Lesur I, Boury C, Rodríguez-Quilón I, Lagraulet H, Ehrenmann F, Bouffier L, Gion J-M, Grivet D, *et al.* 2016. High-density SNP assay development for genetic analysis in maritime pine (*Pinus pinaster*). *Molecular Ecology Resources* **16**: 574–587.

Raj A, Stephens M, Pritchard JK. **2014**. fastSTRUCTURE: Variational Inference of Population Structure in Large SNP Data Sets. *Genetics* **197**: 573–589.

Rodríguez-Quilón I. **2017**. *Ecological and association genetics in two Mediterranean pine species*. Universidad Politécnica de Madrid, Spain.

Spitze K. **1993**. Population structure in *Daphnia obtusa*: quantitative genetic and allozymic variation. *Genetics* **135**: 367–74.

Weir BS, Cockerham CC. **1984**. Estimating F-Statistics for the Analysis of Population Structure. *Evolution* **38**: 1358.

General discussion

In the ongoing environmental crisis, preservation of ecosystems is of paramount importance. Forests in particular are in the centre of concerns, as the various services they provide (ecological, economical, as well as social) are greatly threatened. To play an active part in their conservation, we must understand the genetic basis underlying adaptive traits of these long-lived and complex organisms. We chose *Pinus pinaster* as a model species for this PhD because of its importance in industrial and cultural tapestry of Southern Europe. As a result of its demographic history, *P. pinaster* is genetically highly structured, which can hinder the detection of adaptive signals. We beneficiated from two large common gardens (PINCORSE and CLONAPIN) as well as several regeneration experiments established across Europe in the framework of the GenTree H2020 European Project (http://www.gentree-h2020.eu/). Thanks to these experiments, we were able to study key points on local adaptation, and a consequent genotyping effort allowed us to conduct genotype/phenotype association studies as well.

Risks of counter selection

As I considered several adaptive traits in my PhD project, I realized how difficult it could be to integrate all of them in selection and breeding programs. Indeed, they are complex traits with complex relationships among each other and the environment. In *Chapter 1*, I found a negative correlation between the susceptibility of *P. pinaster* to the two studied pathogens, *A. ostoyae* and *D. sapinea,* probably because they respond to different defence mechanisms. Total height of the inoculated individuals was also correlated with susceptibility to *A. ostoyae* and *D. sapinea* (negatively and positively, respectively). In *Chapter 3*, I could not detect any significant correlation between total height and carbon isotope discrimination ($\delta^{13}C$) in PINCORSE, confirming the absence of trade-off between these two traits as described by Marguerit *et al.* (2014). Such results are good examples of the risks of counter selecting traits: when selecting individuals for height in the studied populations, there would be unintended counter selection for susceptibility to *D. sapinea*. Moreover, as susceptibility to this pathogen is correlated to maximum temperature in summer of the population of origin, water use efficiency (WUE) would be another trait to add in the selection process. These few but relevant examples highlight the importance of deep investigation of adaptive traits and their correlation before selection for breeding and conservation. Understanding their genetic basis is another important asset, as adaptive traits are complex and mostly polygenic, meaning the expression of several genes can be involved in multiple traits. Knowing them and understanding their balance could help avoiding involuntary counter selection. Additionally, the environment plays a major part in observed phenotypes in field studies. Considering as many environmental variables

(temperature, elevation, edaphic nature) as possible when measuring traits in the field and using these environmental characteristics as co-variables in analysis of the traits allows avoiding confounding effects and misinterpretation.

Traits measured across common gardens

One of the many great advantages of my PhD was the availability of large common gardens and regeneration experiments for *Pinus pinaster*. Although they do not include the exact same populations and genetic material, I used BLUPs for height estimated at the population level for both CLONAPIN (*Chapter 1*) and PINCORSE (*Chapter 3*). In addition, two population from Corsica, Pinia and Pineta, have been measured in both gardens. While Pinia is situated almost at sea level (10 m a.s.l.), Pineta is set at 750 m a.s.l., at intermediate elevation. Both CLONAPIN and PINCORSE common gardens are set in Gironde, in very similar climate. Height was measured at approximately the same age in both collections (5 years-old in CLONAPIN and 4 years-old in PINCORSE). The population BLUP estimates for height in these two populations shows the same trend in both common gardens: Pinia is a fast growing population, with height BLUP of +25.911 in CLONAPIN and +22.726 in PINCORSE, being an outlier for Corsican provenances, while Pineta has negative height BLUPs in both experiments (-11.612 and -7.991, respectively). This suggest that results of the two common gardens are comparable. Interestingly, the negative correlation between elevation and population BLUP for height (see also Durel & Bahrman 1995) in Corsica (*Chapter 3*, Figure 4) seems also to stand at the wide range scale, based on the correlations found with climate in *Chapter 1* (higher maximum temperature in July corresponds to lower altitudes in *Pinus pinaster*; see also Figure 1). This is interesting because the role of elevation at the wide range scale could have been confounded by the strong population genetic structure, but this is not the case in Corsica, where populations are not genetically differentiated (i.e. they constitute a single gene pool) and, thus, a significant correlation at both geographical scales seems plausible.

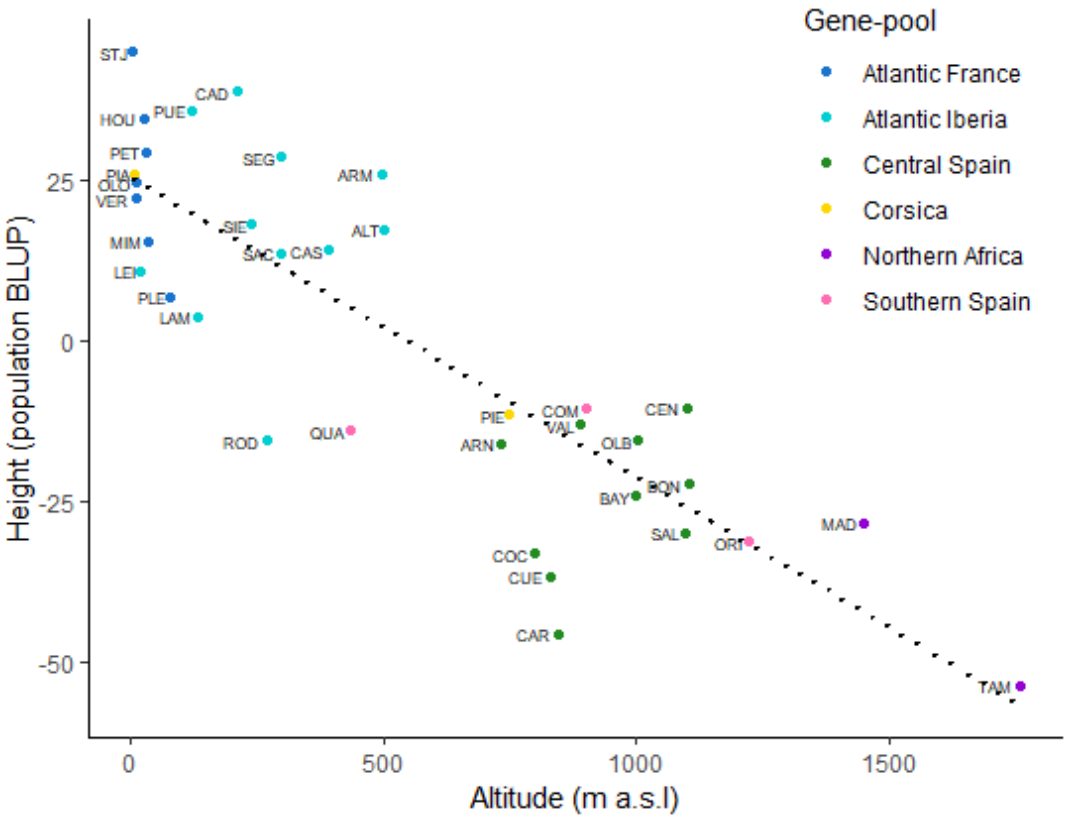


Figure 1. Correlation between height (expressed as population BLUP) and altitude in the CLONAPIN common garden. The dotted line represents the trend of the correlation (Pearson's *r*: -0.84). Data points are coloured according to the gene-pool of origin, and labelled with population of origin

The role of elevation in adaptation

As discussed in the paragraph above, from both *Chapter 1* and *Chapter 3*, a negative correlation between elevation and tree height BLUP (i.e. the genetic component) seems relevant at both local and wide range geographical scales. At higher elevations, environmental conditions change: compared to lowlands, solar radiation is stronger, daily thermal amplitudes are wider, atmospheric pressure is lower. Coping strategies can be observed in plants at high altitude: photosynthetic activity varies during the day according to the more favourable temperature and pressure conditions, leaf structure change (leaves tend to be thicker) and stomatal conductance increases. Forest trees at high altitude have adopted these strategies, and are generally shorter and physiologically distinct (Körner *et al.*, 1991; Streb *et al.*, 1998; Hultine & Marshall, 2000; Coomes & Allen, 2007; García-Plazaola *et al.*, 2015). In *Chapter 2*, we found that selection gradients involving needle traits, such as $\delta^{13}C$, were of great importance for *Pinus pinaster*

(though not as much for *Pinus sylvestris*). $\delta^{13}C$ is known to vary along altitudinal gradients (Körner *et al.*, 1988, 1991; Lajtha & Getz, 1993; Hultine & Marshall, 2000): carbon isotope discrimination generally increases with altitude. As $\delta^{13}C$ is correlated with many other traits such air temperature, soil moisture, atmospheric pressure and (other) leaf traits, the origin of this variation is difficult to pinpoint (Hultine & Marshall, 2000). This highlights the overall importance of adaptation along altitudinal gradients in *Pinus pinaster* and open several new questions. What is the correlation between altitude and pathogen resistance? Are they directly intertwined? Or is there an indirect correlation? What are the potential metabolic mechanisms of adaptation to altitude? All these also highlight the importance of confounding factors and the difficulty of dealing with them. As shown here, there is an overall confounding effect between climate and altitude in *Pinus pinaster* at the wide range scale, and both climate and altitude were relevant for *Chapters 1* and *3*. I chose climate in *Chapter 1* because it was a more explanatory variable for the phenotype differences across gene-pools, while elevation seemed more explanatory for *Chapter 3*, as elevation differences appears to be a strong driver of adaptation in Corsica.

Evolutionary potential

Some of the studied adaptive traits were analysed in more than one chapter of this PhD. In *Chapter 2*, I computed selection gradients for multiple mother traits measured in the field. Among the most significative ones were the following: in *P. sylvestris*, the Spanish population sowed in the Spanish common garden, GER18 ~ Height² (for germination in 2018, Adj-R^2: -0.29**); and in *P. pinaster*, the Spanish population sowed in the French experiment, HW18 ~ $\delta^{13}C^2$ (for height in winter 2018, Adj-R^2: 0.55**). To translate into evolutionary change, mother traits in significant selection gradients have to be heritable. Narrow-sense heritability for height and $\delta^{13}C$ were computed in *Chapter 3* for Corsican populations, and were estimated in h^2_{Height}: 0.2906 and $h^2_{\delta 13C}$: 0.4423, which would suggest substantial evolutionary potential. However, heritability for these traits are extremely variable across environments, as reviewed by Lind *et al.* (2018): h^2_{Height} ranges from 0.08 (Danjon, 1994) to 1.14 (Corcuera *et al.*, 2010). Although not as strikingly, $h^2_{\delta 13C}$ is also variable, from 0.17 (Brendel *et al.*, 2002) to 0.66 (Corcuera *et al.*, 2010). This, together with the remarkable phenotypic plasticity of $\delta^{13}C$ highlighted by Corcuera *et al.* (2010), part of which could also be heritable (i.e. genes for plasticity), shows that evolutionary potential cannot be inferred from one study site to another, or even from one growing season to another. Then evolutionary potential of a given population can only be

confirmed under the environment of the study, despite our results pointing to substantial capacity for evolution in several traits.

Life stages in forest trees

Another important point to consider for studying local adaptation in forest trees is that they are long-lived organisms, and the selective pressure they suffer can vary from one generation to another, even in the same population. The events that were monitored in the common gardens of *Chapter 2* revealed different early-life strategies in distinct populations (see *Perspectives* below), and highlighted the high mortality and selection pressure suffered by seedlings. As it happens, *P. pinaster* is not only highly affected by abiotic stress in early-life stages, but it is also more susceptible to biotic stresses: seeds and seedlings not only are easier prey for herbivores, but susceptibility to various pathogens, *A. ostoyae* for instance, is known to be age-dependant (Lung-Escarmant & Guyon, 2004). In addition, under increased stress, for example at the extreme environmental conditions of populations at high altitude, recruitment can be prevented, as demonstrated by the existence of treelines: beyond certain environmental thresholds, regeneration is impossible (Piotti *et al.*, 2009). The increased biotic and abiotic pressures caused by climate change emphasizes the importance of genetic variation in the early-life stages of forest trees (Lande & Shannon, 1996). It is therefore necessary to complement standard studies based on common gardens, which are normally based on adult trees, with sowing experiments (as the one I developed in *Chapter 2*), in order to have comprehensive views on local adaptation of this keystone group of long-lived organisms.

Experiments and protocol optimization

During my PhD project, I spent a lot of time on research for optimizing existing protocols and creating new ones. For the experiments in *Chapter 1*, the existing protocol for *A. ostoyae* inoculation was not designed for handling such a high number of individuals as intended by the project. Moreover, *A. ostoyae* is remarkably difficult to work with in an automated fashion. With extensive help of the team's laboratory technicians (Olivier Fabreguettes, Xavier Capdevielle, and Martine Martin-Clotté), we improved the protocol to allow: 1) inoculation on excised branches and 2) higher number of inoculated samples than ever before. This improved protocol was also long to implement because of the slow growing rate of the pathogen, but gave satisfactory results (details in *Chapter 1*, Supplementary Material).

The inoculation protocol of *D. sapinea* had also to be adapted to our experimental setting. Elaboration and testing of the protocol was done collaboratively with my interns and with the

help from members of the forest pathology team of BioGeCo. Together, we estimated that the use of non-lignified excised branches would yield the best results. Maximum duration of the entire cycle of inoculation had to be within 6 days to distinguish the symptoms caused by the pathogen from drying symptoms in the cut branch. Inoculation itself had to be quick and easy to allow significant sampling size. The novel protocol (see *Chapter 1*, Supplementary Information) exceeded expectations and will be used for *D. sapinea* inoculation in *P. pinaster* populations selected in the B4EST H2020 European Project (http://b4est.eu/).

Perspectives and forthcoming research

The paradoxical beauty of research is ending up always wanting to go further when something is apparently finished. As it is, each of my chapters could benefit from more research.

Chapter 1: One of the studied pathogens, *D. sapinea* was not initially included in my PhD project. I wanted to compare a root pathogen, *A. ostoyae*, with a branch one: *Melampsora pinitorqua* (see *General Introduction*). Since the pathogen's life cycle involves another tree species (*Populus tremula*) and cannot be grown in Petri dishes, *P. tremula* leaves were collected in late autumn 2016 (for a pilot study) and 2017 (for actual experimentation). Leaves were left outside, and kept in natural conditions for several months. However, the pathogen did not sporulate in laboratory conditions. This being the key step of the inoculation, I had to give up on *M. pinitorqua* for the time being and chose to work with *D. sapinea* instead. The interest in *M. pinitorqua* resided in three points: 1) investigating the response of *P. pinaster* to two pathogens affecting different organs, 2) further investigating among-populations susceptibility of *P. pinaster* and 3) correlating this susceptibility with variation in bud-burst phenology, as *M. pinitorqua* affects elongating branches (Desprez-Loustau & Baradat, 1991; Desprez-Loustau & Dupuis, 1994). Thus, conducting new experiments on *M. pinitorqua* would add complementary value to *Chapter 1*, as we have already studied a root pathogen, *A. ostoyae*, and an endophyte, *D. sapinea*, with negatively correlated effects on *Pinus pinaster*.

The nest count of *T. pityocampa* did not yield any significant results, but *P. pinaster* susceptibility to this pest is worth further investigation, especially in the context of climate change (Battisti *et al.*, 2006). Moreover, Meijón *et al.* (2016) found significant variations in metabolites, including phenols, in different origins of *P. pinaster*. Such metabolites are also investigated for their role in herbivory performance in another Thaumetopoeidae, the oak processionary moth *T. processionea* (Damestoy *et al.*, 2019). Therefore, it would be informative

to investigate the relation between presence or absence of *T. pityocampa* in *P. pinaster* stands and phenolic compounds.

Interestingly, the pine nematode *Bursaphelenchus xylophilus* seems to favour wood infected by *D. sapinea* (Futai *et al.*, 2007). Further knowledge on this correlation and the variation of *P. pinaster*'s susceptibility to *D. sapinea* could lead to valuable information for selection and breeding programmes, in order to fight this very dangerous pest.

Chapter 2: As mentioned in the chapter, the Italian *P. pinaster* experiment was only sowed in March 2019, and Italian populations in the French and Spanish experiments behaved differently than the other populations. Putting all results from the different experiments together might allow understanding the reasons underlying this difference, or at least to have enough information to hypothesize on them. Including the Italian regeneration common garden in our analyses would also provide a full reciprocal design, allowing to test for local adaptation (*sensu* Kawecki & Ebert, 2004).

While monitoring the seedlings in the regeneration experiments, the different ontogenic stages, as described by Chambel *et al.* (2007), were noted. Interestingly, the height of the seedlings in winter was not a good estimator of ontogenic stage: seedlings growing taller did not necessarily develop faster. Attribution of resources to stem elongation or root development reflects distinct adaptive strategies that would be interesting to evaluate. Moreover, stage 6 in our protocol notes the apparition of adult needles. As it has been shown that juvenile and adult needles have different function, studies on heteroblasty and the time to apparition of brachyblasts (adult needles) would also inform on different adaptive population strategies in early-stages of development in *Pinus pinaster* (Zotz *et al.*, 2011; Kuusk *et al.*, 2018).

Chapter 3: As mentioned in *Chapter 1*, bud-burst phenology is an important trait to study in *P. pinaster*, as it varies across populations from the known gene-pools and it can be correlated with pathogen infection. It can play an key role in climate change if, as predicted by Loustau *et al.* (2005), there is a shift in seasonality. Phenology data for all the populations in the Corsican common garden, PINECORSE, are already available, and can be used to estimate Q_{ST} and h^2, as well as in large scale phenotype/genotype association studies. Indeed, the genotyping power deployed for this chapter leaves ~100,000 SNPs ready to use, when the previous standard for genotyping in *P. pinaster* was set at ~6,000 SNPs (Plomion *et al.*, 2016). Moreover, all the other populations not genotyped in *Chapter 3* but that were also measured for height and phenology has also been sampled and their DNA extracted. With a small additional genotyping effort, we

could hence perform a phenotype/genotype association analysis for height/phenology of unprecedented scale in *Pinus pinaster*, involving the ~ 100,000 SNPs and 900 families from 30 populations belonging to the same gene pool (i.e. without the confounding effects of population structure).

Conclusion

With this PhD, I addressed local adaptation in *P. pinaster* from multiple perspectives: across the whole distribution, within a single gene pool and at different life-stages. I also produced an original framework for genomic studies of local adaptation in the species by adding biotic responses in a genotype/phenotype association. Moreover, the common gardens I benefited from allowed me to apprehend local adaptation from different levels, namely families, populations and ecotype (i.e. gene pool). Another advantage was having my PhD (partially) included in a large-scale European project, GenTree, which permitted me to use important amounts of data collected over several years. In that sense, big steps forward have been made towards the understanding of the complex *Pinus pinaster* genome with the genotyping of ~100,000 SNPs, meaning the possibility of conducting genotype/phenotype association studies of unprecedented scale in the species, and the production of valuable genomic information. As demonstrated with this PhD, local adaptation and evolutionary potential are highly variable across traits, populations and environments, highlighting the necessity of thorough investigation of adaptive traits in their natural context, and of considering them as the complex polygenic traits they are. Not only do we need to understand the genetic basis underlying each trait, but also the genetic basis of the genetic network underlying their interactions. The added value of each chapter points to the importance of using integrated methods to design and establish conservation and breeding programmes.

References

Battisti A, Stastny M, Buffo E, Larsson S. **2006**. A rapid altitudinal range expansion in the pine processionary moth produced by the 2003 climatic anomaly. *Global Change Biology* **12**: 662–671.

Brendel O, Pot D, Plomion C, Rozenberg P, Guehl J-M. **2002**. Genetic parameters and QTL analysis of delta13C and ring width in maritime pine. *Plant, Cell and Environment* **25**: 945–953.

Chambel MR, Climent J, Alía R. **2007**. Divergence among species and populations of Mediterranean pines in biomass allocation of seedlings grown under two watering regimes. *Annals of Forest Science* **64**: 87–97.

Coomes DA, Allen RB. **2007**. Effects of size, competition and altitude on tree growth. *Journal of Ecology* **95**: 1084–1097.

Corcuera L, Gil-Pelegrin E, Notivol E. **2010**. Phenotypic plasticity in *Pinus pinaster* δ13C: environment modulates genetic variation. *Annals of Forest Science* **67**: 812.

Damestoy T, Brachi B, Moreira X, Jactel H, Plomion C, Castagneyrol B. **2019**. Oak genotype and phenolic compounds differently affect the performance of two insect herbivores with contrasting diet breadth (J-P Schnitzler, Ed.). *Tree Physiology* **39**: 615–627.

Danjon F. **1994**. Heritabilities and genetic correlations for estimated growth curve parameters in maritime pine. *Theoretical and Applied Genetics* **89**–**89**: 911–921.

Desprez-Loustau M-L, Baradat PH. **1991**. Variation in susceptibility to twisting rust of maritime pine. *Annals of Forest Science* **48**: 497–511.

Desprez-Loustau M, Dupuis F. **1994**. Variation in the phenology of shoot elongation between geographic provenances of maritime pine (Pinus pinaster) - implications for the synchrony with the phenology of the twisting rust fungus, Melampsora pinitorqua. *Annales des Sciences Forestières* **51**: 553–568.

Durel C-E, Bahrman N. **1995**. Analyse de la diversité génétique des peuplements de pin maritime de Corse. Gestion et exploitation de la ressource génétique. *Revue Forestière Française* **5**: 509–522.

Futai K, Sriwati R, Takemoto S. **2007**. Cohabitation of the pine wood nematode, *Bursaphelenchus xylophilus*, and fungal species in pine trees inoculated with *B. xylophilus*. *Nematology* **9**: 77–86.

García-Plazaola JI, Rojas R, Christie DA, Coopman RE. **2015**. Photosynthetic responses of trees in high-elevation forests: comparing evergreen species along an elevation gradient in

the Central Andes. *AoB Plants* **7**: plv058.

Hultine KR, Marshall JD. **2000**. Altitude Trends in Conifer Leaf Morphology and Stable Carbon Isotope Composition. *Oecologia* **123**: 32–40.

Kawecki TJ, Ebert D. **2004**. Conceptual issues in local adaptation. *Ecology Letters* **7**: 1225–1241.

Körner C, Farquhar GD, Roksandic Z. **1988**. A global survey of carbon isotope discrimination in plants from high altitude. *Oecologia* **74**: 623–632.

Körner C, Farquhar GD, Wong SC. **1991**. Carbon isotope discrimination by plants follows latitudinal and altitudinal trends. *Oecologia* **88**: 30–40.

Kuusk V, Niinemets Ü, Valladares F. **2018**. A major trade-off between structural and photosynthetic investments operative across plant and needle ages in three Mediterranean pines. *Tree Physiology* **38**: 543–557.

Lajtha K, Getz J. **1993**. Photosynthesis and water-use efficiency in pinyon-juniper communities along an elevation gradient in northern New Mexico. *Oecologia* **94**: 95–101.

Lande R, Shannon S. **1996**. The Role of Genetic Variation in Adaptation and Population Persistence in a Changing Environment. *Evolution* **50**: 434.

Lind BM, Menon M, Bolte CE, Faske TM, Eckert AJ. **2018**. The genomics of local adaptation in trees: are we out of the woods yet? *Tree Genetics & Genomes* **14**: 29.

Loustau D, Bosc A, Colin A, Ogee J, Davi H, Francois C, Dufrene E, Deque M, Cloppet E, Arrouays D, *et al.* 2005. Modeling climate change effects on the potential production of French plains forests at the sub-regional level. *Tree Physiology* **25**: 813–823.

Lung-Escarmant B, Guyon D. **2004**. Temporal and Spatial Dynamics of Primary and Secondary Infection by *Armillaria ostoyae* in a *Pinus pinaster* Plantation. *Phytopathology* **94**: 125–131.

Marguerit E, Bouffier L, Chancerel E, Costa P, Lagane F, Guehl J-M, Plomion C, Brendel O. **2014**. The genetics of water-use efficiency and its relation to growth in maritime pine. *Journal of Experimental Botany* **65**: 4757–4768.

Meijón M, Feito I, Oravec M, Delatorre C, Weckwerth W, Majada J, Valledor L. **2016**. Exploring natural variation of *Pinus pinaster* Aiton using metabolomics: Is it possible to identify the region of origin of a pine from its metabolites? *Molecular Ecology* **25**: 959–976.

Piotti A, Leonardi S, Piovani P, Scalfi M, Menozzi P. **2009**. Spruce colonization at treeline: where do those seeds come from? *Heredity* **103**: 136–145.

Plomion C, Bartholomé J, Lesur I, Boury C, Rodríguez-Quilón I, Lagraulet H, Ehrenmann F, Bouffier L, Gion J-M, Grivet D, *et al.* 2016. High-density SNP assay

development for genetic analysis in maritime pine (*Pinus pinaster*). *Molecular Ecology Resources* **16**: 574–587.

Streb P, Shang W, Feierabend J, Bligny R. **1998**. Divergent strategies of photoprotection in high-mountain plants. *Planta* **207**: 313–324.

Zotz G, Wilhelm K, Becker A. **2011**. Heteroblasty—A Review. *The Botanical Review* **77**: 109–151.