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DOCTORAL THESIS / TESIS DOCTORAL:

Evolutionary ecology of fire-adaptive traits in a Mediterranean pine species

Ecología evolutiva de caracteres de adaptación al fuego en una especie de pino mediterráneo

Presentada por **Ruth C. Martín Sanz** para optar
al grado de Doctora por la Universidad de Valladolid

Dirigida por:
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… Que no son, aunque sean.

Que no hablan idiomas, sino dialectos.

Que no profesan religiones, sino supersticiones.

Que no hacen arte, sino artesanía.

Que no practican cultura, sino folklore.

Que no son seres humanos, sino recursos humanos.

Que no tienen cara, sino brazos.

Que no tienen nombre, sino número.

Que no figuran en la historia universal,

sino en la crónica roja de la prensa local.

Los nadie, que cuestan menos que la bala que los mata.

Eduardo Galeano (1940 – 2015)

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Structure of the Thesis

This thesis is based on two original works published in different international journals and two other manuscripts under preparation. The text is written in English and includes a thesis overview, an abstract, an introduction with the hypothesis and objectives of the thesis, a materials and methods section, describing in detail the different sampling sites and phenotyping procedures used to obtain pine phenotypes of different life-history traits in *Pinus halepensis*, a results section, a general discussion and conclusions. In addition, we have included a final section with future perspectives and gaps of knowledge regarding the topics studied in this thesis. The abstract and the conclusions are written both in English and in Spanish language. Finally, supplementary information with additional tables and figures for the thesis are included.

The thesis is framed within the study of fire adaptive traits, focusing on the complex interaction among genotypes, phenotypes and the environment.

Abstract

Forests have high ecological, economic and social value, besides playing a key role in the maintenance of biodiversity and as carbon sinks, but the current global change can cause adaptation problems of forest species as well as modify forests distribution and functioning. Mediterranean environments are especially sensitive to climate change, where predictions suggest that temperature increase and rainfall decrease will be especially drastic, together with a higher frequency and intensity of disturbances (forest fires and epidemic outbreaks of pests and diseases). The scarce availability of water is the most evident resource limitation in these Mediterranean environments and may increase the evolutionary trade-offs among vital functions predicted by the life-history theory. This theory is based on the idea that forest trees, like other living beings, must optimize the amount of energy and resources they dedicate to each of their vital functions since the available resources are limited.

Pine trees are long-life large organisms with short age at first reproduction and several advantages for the study of adaptive traits in trees from an ecological-evolutionary approach. Mediterranean pine forests constitute reservoirs of adaptive genetic diversity of great value in the face of environmental change. The ability of those populations to persist in the medium term will depend to a large extent on the existence of sufficient genetic variation in relevant traits, on the exchange of genetic information among populations (genetic flow) and on their adaptive phenotypic plasticity. Local adaptation is expected to arise from genetic variability within and between populations, but phenotypic plasticity also plays a major role in the ability of species to cope with environmental changes and may allow the appearance of adapted phenotypes without the existence of an underlying genetic change. However, knowledge about the adaptive role and the plasticity of key life-history traits is still very limited in forest species, especially in Mediterranean environments. Among the life-history traits stand out the reproductive ones, such as the threshold size of reproduction or fecundity, but serotiny degree or bark thickness are other fundamental traits related to adaptation to fire that have received so far less attention.

This thesis, framed in the field of evolutionary ecology, includes four works that correspond to scientific articles already published or manuscripts in preparation. It also includes various annexes with additional information. The main objective of this work was to compare how different environments, more or less favorable, condition the

compromises among adaptive traits (life-history traits) in a typical Mediterranean pine (*Pinus halepensis* Mill., Aleppo pine), which can be considered a model of maximum resilience in Mediterranean ecosystems. Specifically, we focused on two key resilience traits: serotiny of female cones to build an aerial seedbank that ensures regeneration after intense crown fires, and bark thickness that allows survival of adult trees in front of less severe fires until reaching a sufficient aerial seedbank, without forgetting its interrelation with other traits such as reproduction (female and male) or growth. Both traits are complementary but not mutually exclusive. Understanding their genetic and environmental variation patterns, unraveling the complex interaction between genotype, phenotype and environment, together with the allometric effects (ontogenetic or developmental), constitutes a fundamental challenge to be able to foresee the response of these forests under the new environmental scenarios, and for the management and conservation of forest resources under the current global change. The use of provenance trials in contrasted common environments allowed us to separate the genetic effects from environmental effects and interacting developmental differences. In addition, climatic and fire information from the populations' origin areas was used to identify ecotypic patterns of variation.

Throughout the different studies involved in this thesis, we found clear evidence of intraspecific genetic variation and high phenotypic plasticity, as well as genotype-by-environment interaction and signs of local adaptation in the different studied traits. This suggests the existence of potential evolutionary change to face new selective pressures, variable within the species distribution range. The quantitative genetic differentiation between populations was higher than the differentiation found with molecular markers for fire-adaptive traits under contrasting environments. The growth-limiting environments for *P. halepensis*, mainly continental conditions with high annual and/or daily thermal oscillation that reduce the vegetative period, and the shortage of precipitations in spring and summer, accelerated the early release of seeds and decreased the allocation to bark.

The detailed study of serotiny degree in *P. halepensis* pursued on the one hand to determine the phenotypic plasticity of this trait taking into account the allometric and genetic effects, and on the other hand, to examine whether or not there are endogenous factors that can affect the opening of serotinous cones. For this, measurements in the provenance trials were completed with field and laboratory manipulative experiments. We found that unfavorable environments for growth caused the early release of seeds and that the duration of serotiny in *P. halepensis* implies the supply of water to the cones through its peduncles by the bearing plant, which suggests the existence of maintenance costs of serotiny.

We verified the existence of phenotypic plasticity and allometric plasticity in bark thickness, a fire-adaptive trait poorly studied in conifer species. Confirming our hypothesis environments with lower resource availability limited both the relative allocation to the bark and absolute bark thickness. Importantly, this can increase immaturity risk in *P. halepensis* populations (death by moderately intense fires before reaching an aerial bank of seeds that ensures regeneration) under the dryer environments caused by climate change.

We also studied the relationship between ecogeographic variables and different adaptive phenotypes in *P. halepensis* -including growth, female and male reproduction and fire-adaptive traits-, as well as the correlations among traits. In general, we found evidence of local intraspecific adaptation. We also confirmed that trade-offs in terms of allocation of resources to adaptive traits related to growth, reproduction and defense against fire matched the predictions of life-history theory and differential allocation. Finally, further confirming the hypothesis of local adaptation, we verified that the quantitative genetic differentiation among populations was greater than the neutral genetic differentiation for serotiny and bark thickness.

All the results obtained confirm that adaptive traits must be taken into consideration within the new paradigm of adaptive forest management under global change. This means considering not only traits related to growth or reproduction, but also those directly related to fire resilience, in order to implement better conservation and management practices maintaining the adaptive potential of forest populations facing the multiple challenges expected in the near future.

Resumen

Los bosques tienen un alto valor ecológico, económico y social, además de desempeñar un papel clave en el mantenimiento de la biodiversidad y como sumideros de carbono, pero el cambio global actual puede causar problemas de adaptación de las especies forestales al igual que modificar la distribución y el funcionamiento de los bosques. Los ambientes mediterráneos son especialmente sensibles al cambio climático, donde las predicciones sugieren que el aumento de la temperatura y la disminución de las lluvias serán especialmente drásticos, junto con una mayor frecuencia e intensidad de las perturbaciones (incendios forestales, y epidemias de plagas y enfermedades). La escasa disponibilidad de agua es la limitación de recursos más evidente en estos entornos mediterráneos, pudiendo acrecentar los compromisos evolutivos entre funciones vitales predichos por la teoría de historia vital. Esta teoría se basa en que los árboles forestales, al igual que el resto de seres vivos, deben optimizar la cantidad de energía y recursos que dedican a cada una de sus funciones vitales, ya que los recursos disponibles son limitados.

Los pinos son organismos de gran tamaño, vida larga y edades tempranas de primera reproducción con diversas ventajas para el estudio de caracteres adaptativos en árboles desde un enfoque ecológico-evolutivo. Los pinares mediterráneos constituyen reservorios de diversidad genética adaptativa de gran valor frente al cambio ambiental. La capacidad de esas poblaciones para persistir en el medio plazo dependerá en gran medida de la existencia de variación genética suficiente en caracteres relevantes, del intercambio de información genética entre poblaciones (flujo genético) y de su plasticidad fenotípica adaptativa. Se espera que la adaptación local surja de la variabilidad genética dentro y entre poblaciones, pero la plasticidad fenotípica también juega un papel fundamental en la capacidad de las especies para hacer frente a los cambios ambientales, pudiendo permitir la aparición de fenotipos adaptados sin que exista un cambio genético subyacente. Sin embargo, el conocimiento sobre el papel adaptativo y la plasticidad de caracteres clave de historia vital todavía es muy limitado en especies forestales, especialmente en ambientes mediterráneos. Entre los caracteres de historia vital destacan los reproductivos, como el tamaño umbral de reproducción o la fecundidad, pero la serotinia o el espesor de corteza son otros caracteres fundamentales relacionados con la adaptación al fuego que han recibido hasta ahora menor atención.

Esta tesis se enmarca en el campo de la ecología evolutiva e incluye cuatro estudios que corresponden a artículos científicos ya publicados o manuscritos en preparación. Incluye también diversos anexos con información adicional. El objetivo principal de este trabajo fue comparar cómo distintos ambientes más o menos favorables condicionan los compromisos entre caracteres adaptativos (rasgo del ciclo de vida) en un típico pino mediterráneo (*Pinus halepensis* Mill., pino carrasco), que puede considerarse un modelo de máxima resiliencia en los ecosistemas mediterráneos. En concreto, nos hemos centrado en dos caracteres de resiliencia claves: la serotinia de los conos femeninos para construir un banco aéreo de semillas que asegure la regeneración tras fuegos de copas intensos, y el espesor de corteza que permite la supervivencia de los árboles adultos frente a incendios menos severos hasta alcanzar un banco aéreo de semillas suficiente, sin olvidar su interrelación con otros caracteres como la reproducción (femenina y masculina) o el crecimiento. Ambos caracteres son complementarios pero no excluyentes. Comprender sus patrones de variación genética y ambiental, desentrañando la compleja interacción entre genotipo, fenotipo y ambiente, junto con los efectos alométricos (ontogénicos o de desarrollo), constituye un reto fundamental para poder prever la respuesta de estos bosques bajo los nuevos escenarios ambientales, y para la gestión y conservación de los recursos forestales bajo el cambio global actual. El uso de ensayos de procedencias en ambiente común (*common gardens*) y contrastados entre sí, nos ha permitido separar los efectos genéticos de los ambientales y de los puramente debidos al desarrollo. Además, la información climática y de incendios de las áreas de origen de las poblaciones se utilizó para identificar patrones de variación ecotípicos.

A lo largo de los diferentes estudios de esta tesis, encontramos evidencias claras de variación genética intraespecífica y elevada plasticidad fenotípica, así como interacción genotipo-ambiente e indicios de adaptación local en los diferentes caracteres estudiados. Esto sugiere la existencia de potencial de cambio evolutivo para hacer frente a nuevas presiones selectivas, variable dentro del rango de distribución de la especie. La diferenciación genética cuantitativa entre poblaciones fue superior a la diferenciación encontrada con marcadores moleculares para los caracteres de adaptación al fuego bajo ambientes contrastantes. Los ambientes limitantes para el crecimiento de *P. halepensis*, fundamentalmente condiciones continentales con gran oscilación térmica anual y/o diaria que reducen el periodo vegetativo, y con escasez de precipitaciones en primavera y verano, aceleraron la liberación precoz de semillas y disminuyeron la asignación a la corteza.

El estudio pormenorizado del grado de serotinia de *P. halepensis* persiguió por un lado, determinar la plasticidad fenotípica de la serotinia teniendo en cuenta los efectos alométricos y genéticos, y por otro lado, examinar si existen o no factores endógenos que puedan afectar a la apertura de los conos serótinos. Para ello, las mediciones en los ensayos de procedencias se completaron con experimentos manipulativos en laboratorio y campo. Encontramos que los ambientes desfavorables para el crecimiento causaron la liberación precoz de semillas y que la duración de la serotinia en *P. halepensis* implica el suministro de agua a los conos a través de sus pedúnculos por parte de la planta, lo que sugiere la existencia de costes de mantenimiento de la serotinia.

Verificamos la existencia de plasticidad fenotípica y plasticidad alométrica en el espesor de corteza, un rasgo de adaptación al fuego poco estudiado en especies de coníferas. Confirmando nuestra hipótesis, los entornos con menos disponibilidad de recursos limitaron tanto la asignación relativa a la corteza como el grosor absoluto de corteza. Es importante destacar que esto puede aumentar el riesgo de inmadurez en las poblaciones de *P. halepensis* (muerte por incendios moderadamente intensos antes de llegar a un banco aéreo de semillas que garantice la regeneración) en lo ambientes más secos causados por el cambio climático.

También estudiamos la relación entre variables ecogeográficas y diferentes fenotipos adaptativos en *P. halepensis* -incluyendo crecimiento, reproducción femenina y masculina y caracteres de adaptación al fuego-, así como las correlaciones entre caracteres. En general, encontramos evidencia de adaptación local intraespecífica. También confirmamos que las compensaciones en términos de asignación de recursos a los caracteres adaptativos relacionados con crecimiento, reproducción y defensa contra el fuego coincidían con las predicciones de la teoría de historia vital y la asignación diferencial. Finalmente, confirmando de nuevo la hipótesis de adaptación local, verificamos que la diferenciación genética cuantitativa entre poblaciones fue mayor que la diferenciación genética neutra para la serotinia y el espesor de corteza.

Todos los resultados obtenidos confirman que los rasgos de adaptación deben ser tomados en consideración dentro del nuevo paradigma del manejo forestal adaptativo bajo el cambio global. Esto significa considerar, no solo los rasgos relacionados con el crecimiento o la reproducción, sino también aquellos directamente relacionados con la resiliencia al fuego, para implementar mejores prácticas de conservación y gestión manteniendo el potencial adaptativo de las poblaciones forestales que se enfrentan a los múltiples desafíos esperados en el futuro cercano.

1. Introduction

1.1. Forest ecosystems and climate change

Forests occupy 30.6 % of the Earth's surface (FAO 2015; Figure 1) and are critical points of biodiversity (Myers *et al*., 2000). Forests provide fundamental ecosystem services such as water regulation and supply, generation, renewal and maintenance of soil, purification of air, mitigation of the effects of droughts and floods, pollution control, etc. (Daily, 1997; FAO, 2015). In addition, they are a source of oxygen fundamental for life on Earth, as well as important carbon sinks, becoming key elements for the mitigation of anthropogenic climate change (Canadel and Raupach, 2008). Forest ecosystems are also useful for the economy and for the subsistence of millions of people, since they are the source of many highly demanded products such as food, medicines, wood, resin or cork. Although the rate of net forest loss has decreased lately, there is still an annual net reduction in the forest area of 3.3 million hectares per year (period 2010-2015; FAO, 2015), with the greatest losses in the tropics. In contrast, in other regions such as Europe, deforestation rates are decreasing and forest area is even increasing. Currently, forests cover nearly 40% of the European Union surface (Figure 2).

Mediterranean forests stand out for their high biodiversity, as a result of a noteworthy variety of habitats and of the historical and paleogeographic episodes mainly occurring during the last glaciation. Consequently, the Mediterranean basin that hosts most of the Mediterranean forests in the world, has been identified as a biodiversity hotspot (Myers *et al.*, 2000; Mittermeier *et al*., 2011; Figure 3).

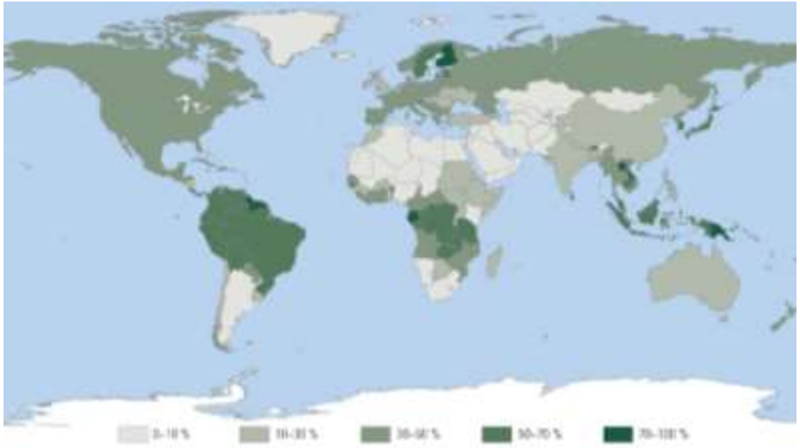

FIGURE 1 | Forest area as a percentage of total land area in 2015. From FAO (2015).

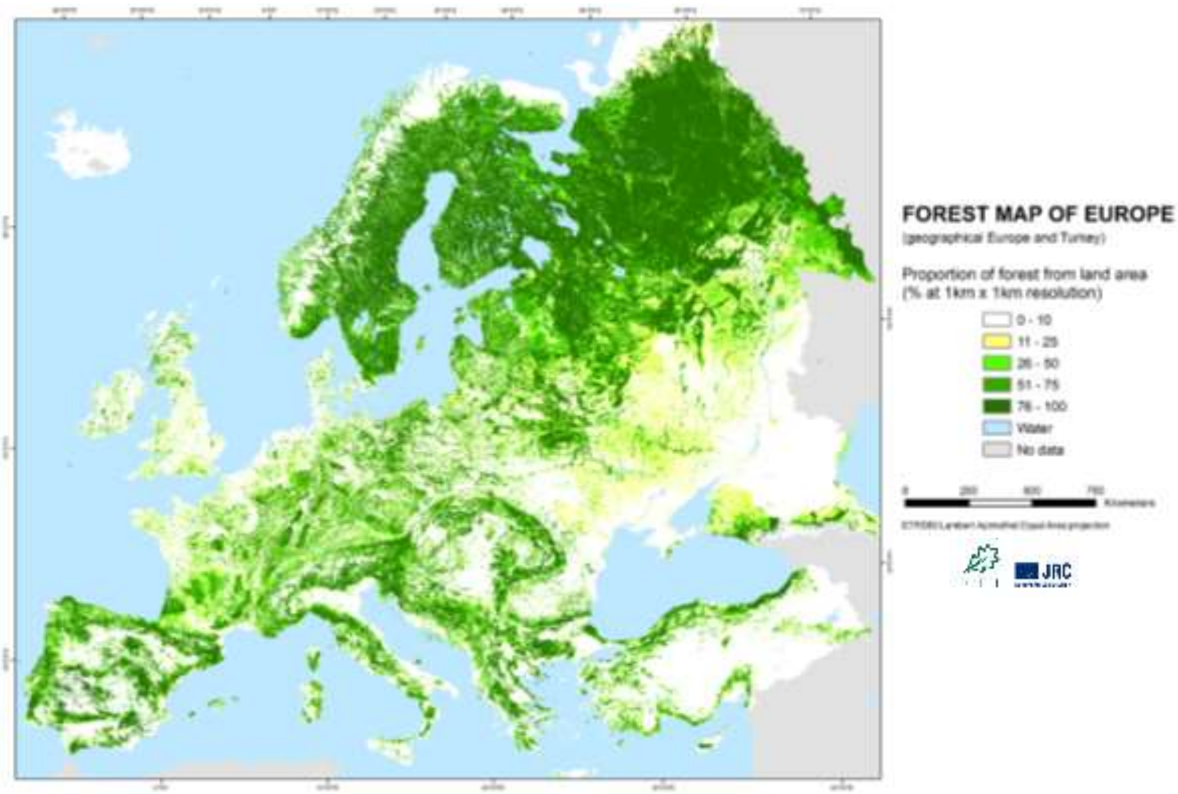

FIGURE 2 | Surface covered by forests in Europe in 2011. Information based on remote sensing technologies and forest inventory statistics. From European Forest Institute (http://www.efi.int/) and Kempeneers *et al.* (2011).

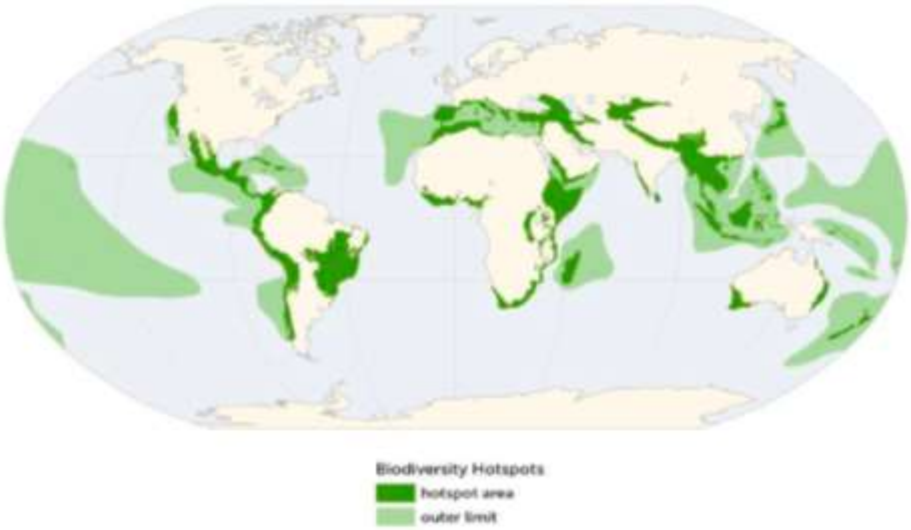

FIGURE 3 | World biodiversity hotspots. From Mittermeier *et al.* (2011).

Climate change is mainly characterized by increasing air temperature and changing precipitation regimes (IPCC, 2013) and is becoming one of the most important challenges faced globally by ecosystems and societies (see for example, Thomas *et al*., 2004; Thuiller, 2007; Bernier and Schöne, 2009). Moreover, climate change potential consequences could be worsened by already existing human-induced threats such as the introduction of exotic pests and pathogens, habitat degradation and fragmentation, land-use changes or modifications in wildfire regimes (Blondel *et al.,* 2010; Keenan, 2015). In this context, forest ecosystems seem to be particularly vulnerable as the long life-span of trees can limit rapid adaptation to environmental changes and their sessile nature restricts natural migration (Lindner *et al.,* 2010). This can put at stake forest resilience, i.e. the capacity to resist disturbances recovering after them and maintaining its structure and function (sensu Lloret *et al*., 2011). Specifically, Mediterranean forest ecosystems are generally expected to be particularly vulnerable to climate change (IPCC, 2007; Lindner *et al*., 2010) due to the expected increase in the frequency of extreme events such as droughts and fires, which are likely to be more severe in dry, high-temperature regions (IPCC, 2007).

In the face of all these numerous drivers of global change, species can persist, either migrating to new ecological niches or adapting to new conditions in current locations or, on the contrary, become extinct locally (Aitken et al., 2008). Forests are resilient, their distribution ranges are highly dynamic and many tree populations maintain high genetic diversity, so they are expected to adapt rapidly. However, environmental variations under current climate change are of such magnitude or will occur at speeds much higher than the natural adaptive capacity of forest species, possibly jeopardizing their persistence (Petit and Hampe, 2006; Petit et al., 2008; Milad et al., 2011). The persistence of tree species under forecasted climate change scenarios will depend on their genetic diversity and their phenotypic plasticity, which plays a major role in the response of plant populations to environmental changes (Matesanz et al., 2010; Valladares et al., 2014). Therefore, understanding the past adaptive processes in forest tree species, and how forests can resist and recover after extreme climatic events or intense perturbations across different regions is key to predict the future responses of forests to climate change (see for example, Savolainen et al., 2007; Petit et al., 2008).

In particular, Mediterranean forests are subject to strong threats due to both climate change and disturbances, such as drastic changes in fire regimes. In order to predict whether species or populations may or may not adapt to these rapid and severe changes, it is essential to understand the causes of variation (genetics, plasticity, allometry-ontogeny...) affecting to species' life-history traits, and particularly, to fire-adaptive traits. Likewise, it is key to consider the possible existence of ecotypic patterns, local adaptation or trade-offs among fire-adaptive traits and other characters related to other vital functions such as reproduction or growth. In this thesis, we have tried to solve the gaps of knowledge related to these aspects, taking as study species a Mediterranean pine with high resilience.

1.2. Genetic variation

Genetic variation is a fundamental requirement for the variability of species, populations and ecosystems (Allendorf and Luikart, 2007), and for the existence of evolutionary adaptation enabling species adaptation to environmental changes (Le Corre and Kremer, 2003), in addition to being considered the most basic level of biological diversity. While different processes (mutation, genetic drift and migration) generate or deplete genetic variation stochastically, speeding up or constraining adaptation, natural selection is the only evolutionary force that is considered to lead to adaptation due to its directional nature −even taking into account that it can also limit genetic variation−. For phenotypes to evolve, it is essential that the particular trait shows heritable variation. Natural selection acts on this variation, selecting phenotypes with higher fitness. Genetic changes might modify the average phenotype in the population across generations, possibly improving the population fitness and thus, leading to adaptation (Le Corre and Kremer, 2003). However, there is increasing evidence that adaptation can be also shaped by phenotypic changes that do not imply changes in the genotype, but in environmentally driven modifications of gene expression (epigenetics), since epigenetic changes can be transmitted among generations (Duncan et al., 2014).

There are two types of traits based on their effects on an organism's phenotype: qualitative and quantitative. Qualitative traits fit into discrete categories, are controlled by a single gene or a small group of genes and are less affected by the environment (Miglani, 2010; seed characteristics such as seed coat color, 'yes or no' traits and many more). Quantitative traits occur as a continuous range of variation, are controlled by a large number of genes with small additive effect (i.e. they are polygenic traits) and are more affected by the environment, such as height. Within evolutionary genetics, quantitative genetics would be an extension of population genetics, with both fields focusing on the genetic basis of phenotypic variation among individuals in a population. This genetic variation can be detected at molecular or phenotypic level. Population genetics deals with the dynamics of allele frequencies both within and among populations under the influence of evolutionary forces such as drift, mutation, selection or migration. However, genes underlying many phenotypic traits are still unknown, especially in forest species, so this approach often fails to consider phenotypes. Lately, advances in genotyping have improved the characterization of genomes reducing costs and time needed, but in contrast accurate phenotyping is still costly in money and time (Ingvarsson and Street, 2011). Quantitative genetics concentrates on how individual variation in genotype and environment contribute to the variance in phenotype. Most key adaptive plant traits are quantitative and polygenic (Mackay *et al*., 2009, Pritchard *et al.,* 2010). The statistical techniques normally used to analyze quantitative genetic data assume that traits follow a Gaussian distribution, which is generally true for growth-related traits, but not true for other life-history traits such as reproduction or serotiny. This hinders the analysis of the data. However, in recent years, new statistical techniques have been implemented in quantitative genetics improving parameters estimation (see Nakagawa and Schielzeth, 2010, Holand *et al.,* 2013, Appendix VI in Santos-del-Blanco, 2013).

Genetic adaptive variation patterns could be confounded with variation trends due to demo-stochastic processes. These processes, together with natural selection, model the species population genetic structure (Box 1; Freedman *et al.,* 2004). This could render the study of polygenic traits in natural populations more difficult. Recently, statistical techniques have been developed that take population genetic structure and inter-individual relatedness into account (Yu *et al*., 2006, Eckert *et al.,* 2010).

Box 1. Spatial Genetic Structure

Spatial genetic structure, i.e. the non-random distribution of alleles or genotypes in space is driven by mutation, migration, selection and drift (Wright, 1943, 1951) and is influenced by neutral and selective genetic processes, such as dispersal, inbreeding, bottlenecks, colonization events or environmental adaption. Local adaptation to distinct environments can configure genetic structure. Knowledge about the spatial genetic structure at different spatial scales is important for species conservation management.

1.3. Phenotypic plasticity

Environmental differences cause phenotypic variation (growth rates, shape, morphology, etc.) among neighboring individuals (caused by differences in microclimate, microsite, competition and exposure to insects and diseases) and also in populations of the same species growing in different environments (caused by differences in elevation, precipitation, temperature regimes, soil type, etc.; White *et al.*, 2007). Leaving aside the non-additive genetic effects, the observed phenotypic value of a quantitative (i.e. polygenic) trait results from the sum of three components: genotype (G), environment (plasticity, E) and their interaction (P = G + E + G x E). Phenotypic plasticity is ultimately an individual property (Stearns, 1989a; West-Eberhard, 2003 and many more). Therefore, it is considered that for phenotypic plasticity to be adequately addressed, it must be studied with replicated genotypes, that is, with experiments that use the same genotype (clones) or individuals with a known genetic relationship (genetic families) (Såstad *et al.*, 1999; Richards *et al.*, 2006; Herrera, 2009). However, populations or even species can also be used as experimental subjects for research on phenotypic plasticity (Pigliucci 2001; Valladares *et al.*, 2006; Richards *et al.*, 2006; and Gianoli and Valladares 2012 for a review) when the objective seeks to find differences in plasticity along an environmental gradient (see, for example Gianoli and González-Teuber, 2005; Bell and Galloway, 2008). In addition, this broad approach to plasticity allows the inclusion of more study units.

In recent years, phenotypic plasticity and its adaptive role for a plethora of traits have received huge interest by the scientific community, mainly due to its critical role in the response of plant populations to climate change, putatively allowing adaptation without genetic changes (Aitken *et al.,* 2008; Matesanz *et al.*, 2010; Chevin *et al.*; 2012, Valladares *et al.*, 2014). Phenotypic plasticity can be defined as the environmentally induced variation in growth or development of an organism and is an important strategy by which plants can ideally maximize fitness in fluctuating environments (Bradshaw, 1965; Scheiner, 1993). Adaptive plasticity, together with genetic change and migration, may allow populations to avoid local extinction under global change (see, for example Nicotra *et al.,* 2010). Moreover, even when phenotypic plasticity of plants is often described as adaptive (Bradshaw, 1965; Dudley and Schmitt, 1996), neutral or maladaptive plasticity do also exist (Alpert and Simms, 2002; Ghalambor *et al*., 2007; Sánchez-Gómez *et al*., 2008, Godoy *et al.*, 2012). Importantly, the mechanisms underlying plastic responses of reproductive traits —directly related to fitness— and whether these plastic responses could be adaptive under current or future conditions remain unknown (Nicotra *et al.*, 2010). In population genetic studies, counter-gradient variation patterns, i.e. phenotypic and genetic clines exhibiting opposing directions (unlike the most common co-gradient variation patterns where phenotypic and genetic variation show parallel responses to environmental gradients) can interfere with the processes of adaptive differentiation (Conover and Schultz, 1995; Kremer *et al.*, 2014). Despite the importance of phenotypic plasticity for plant adaptive traits, many studies continue to mix genetic variation with phenotypic plasticity (Chambel *et al.*, 2007). Besides, the use of experimental approaches as common gardens can avoid phenotypic differences due to genotype x environment interactions.

Genotype-by-environment interaction (G × E), i.e. genetic variation in phenotypic plasticity among populations (Schlichting, 1986), is a central concept in ecology and evolutionary biology because it has wide-ranging implications for trait development and for understanding how organisms will respond to environmental change. Genotypes performance commonly varies across environments leading to variance differences and rank changes among genotypes (Cooper and DeLacy 1994). Importantly, we should distinguish between two types of G x E (El-Soda *et al.,* 2014; Heslot *et al.*, 2014; Roles *et al.*, 2016; Saltz *et al.*, 2018). On the one hand, the biologically more relevant cross-over interaction in which reaction norms cross with each other (rank differences), that is, the fittest genotype in one environment is not necessarily the fittest in another environment. And on the other hand, a scale-effect interaction (non-cross-over G x E or variance differences), where no intersection between reactions norms is observed within the range of environments, i.e. a change is produced in the amount of genetic variance expressed in different environments, but without changes in rank order. Although G × E has been extensively documented and its estimation in quantitative genetic models is useful for comparing across diverse environments, species or traits, we still know little about the underlying biological causes of variation of G × E (Bell and Dochtermann, 2015). Furthermore, differences in methodologies across studies may also produce different G × E estimates (Saltz *et al*., 2018). Therefore, a better understanding of genotype-by-environment interaction at the organismal, functional, and molecular levels would help explain variation across traits in functional similarities between genetic and environmental perturbations, genetic architecture, and what allelic effects may be exposed in new environmental conditions (Saltz *et al.,* 2018).

It is well established that many phenotypic traits in plants vary as a function of growth and development, which are also highly plastic. So conclusions regarding phenotypic plasticity can dramatically change if developmental, i.e. ontogenetic differences are taken into account (Wright and McConnaughay, 2002; Valladares *et al.*, 2006). Assessing the covariation of a given trait with body size (allometry) is routinely used to unveil the ontogenetic component of plasticity (Wright and McConnaughay, 2002; Weiner, 2004). Meaningfully, there is evidence that several traits variation have an allometric component in different plant species, although the direction of this trend could vary among genera (Cowling and Lamont, 1985; Sultan, 2000; Thanos and Daskalakou, 2000; Niklas and Enquist, 2003; Weiner *et al.*, 2009, Bonser *et al.*, 2010, Anderson *et al.,* 2012, Tonnabel *et al.,* 2012, Santos-del-Blanco *et al.*, 2013 and many others). Resource allocation in plants changes along ontogenetic trajectories; therefore, distinguishing between environmental effects from purely developmental differences is critical when studying plasticity in allocation (Poorter and Nagel, 2000; Wright and McConnaughay, 2002; Weiner, 2004). While accounting for ontogenetic changes in such complex plants as adult trees is elusive, the concepts and theory of allometry are probably the best available tools. There are different strategies to study plant allometric patterns, all of them with advantages and disadvantages (Poorter and Sack, 2012). However, allometric equations is the most common method. Usually, these equations are in the form of a logarithmically-transformed power law (Niklas, 1994, Ter-Mikaelian and Korzukhin, 1997).

1.4. Local adaptation

In evolutionary terms, a population is locally adapted when its fitness (i.e. the number of copies of genes in the next generation made by individuals of the specified genotype or phenotype) is higher than average in its local environment (in its home) compared to other non-local populations (Kawecki and Ebert, 2004). The distribution of genetic and phenotypic variation along environmental clines or divergence among populations from contrasting environments might also indicate local adaptation (Conover *et al.*, 2009). Although identifying signals of local adaptation in trees could help us predict the future of forests due to its important role in species persistence facing climate change (see Alberto *et al.,* 2013 for a review), the genetic basis of local adaptation remains poorly understood (Savolainen *et al.,* 2013). Local adaptation usually occurs when a phenotype conferring high fitness in one environment is costly in another environment (Kawecki and Ebert, 2004), and is produced by a local allelic shift that maximizes fitness in a specific environment. This usually occurred during the expansion of the species since by expanding their geographical range, species faced new selective pressures that act on phenotypic variation and thus, on genetic variation. However, human-driven climate change will impose stronger and faster selection pressures (Davis and Shaw, 2001), challenging local adaptation processes in tree species (reviewed in Savolainen *et al.,* 2013).

Long-lived tree species are often found in large natural populations (connected by extensive gene flow) and occupy large geographical distributions covering different environmental gradients, enabling the study of local adaptation processes, the possibilities of tree populations to adapt to changing environments and disentangling selective from stochastic evolutionary forces (Petit and Hampe 2006; Neale and Kremer 2011). Forest tree populations generally show high levels of genetic variation, phenotypic plasticity and genotype-by-environment interaction for various adaptive traits, which increases their capacity for adaptation and resilience (Sgrò *et al.*, 2011; Fady *et al.*, 2015) and is usually interpreted as indicative of local adaptation (Howe and Aitken, 2003; Petit and Hampe, 2006; Bucci *et al.,* 2007; Savolainen *et al.,* 2007; Petit *et al.,* 2008; Grivet *et al.,* 2011; Santos-del-Blanco *et al.,* 2012; Alberto *et al.,* 2013). Tree species ability to adapt depends on their genetic diversity, phenotypic plasticity, selection pressure, fecundity, interspecific competition and biotic interactions (Aitken *et al*., 2008). Local adaptation could be confounded with neutral genetic effects, plasticity or maternal effects (Savolainen *et al.,* 2013). Discriminating between phenotypic plasticity and genetic divergence requires the measurement of traits in individuals from different populations under comparable (and different) environmental conditions (i.e. tested in common garden or reciprocal transplant experiments). Different tools are available to overcome the limitations of specific test environments for the study of genetic variation in adaptive traits. Using a standardized environmental distance among populations' origin and test site environments is a sound method to check whether the results from a provenance common garden experiment are generalizable to other putative provenance x site combinations based in the climatic information (Climent *et al.*, 2008; Santos-del-Blanco *et al.*, 2012, 2013; Hernández-Serrano *et al.,* 2014; Voltas *et al.,* 2015).

Distinguishing to what extent population differentiation is caused by selective (adaptive) or neutral (stochastic) processes (Leinonen *et al.,* 2013) is particularly relevant. For this, population genetics approaches include scanning for F_{ST} outliers (there are many methods; see for example Luikart *et al.*, 2003; De Mita *et al.,* 2013; Ruiz-Daniels *et al., in press*), correlation of allele frequencies with environmental variables (more recent and powerful methods; Jaramillo-Correa *et al.,* 2015), QTL and association mapping which rely on correlating phenotypes with genotypes and finally, Q_{ST}-F_{ST} comparisons (Savolainen *et al*., 2013). All methods search for signals of natural selection by looking for departures from a standard neutral model. In Q_{ST}-F_{ST} comparisons, the variation between population at marker loci (populations' neutral genetic differentiation: F_{ST}; Wright, 1951) is compared to populations' quantitative differentiation (Q_{ST}, Spitze, 1993). Both statistics quantify the proportion of total variation that occurs among populations and any significant difference between them (assuming that populations are in drift-migration equilibrium) is held to be evidence for natural selection and local adaptation for a given trait (Merilä and Crnokrak, 2001; McKay and Latta, 2002). If phenotypic (polygenic) traits show higher differentiation than neutral markers ($Q_{ST} > F_{ST}$), it is assumed that divergent selection is responsible for local adaptation (McKay and Latta, 2002; Savolainen *et al.,* 2007; Leinonen *et al.,* 2013).

1.5. Life-History theory

Life history theory (LHT; Stearns, 1989b) is a part of evolutionary ecology and biology, which refers to the series of fitness-related events in the life of an organism that are modeled by natural selection to produce the maximum number of viable offspring (Roff, 1992; Yampolsky, 2003). All living beings must optimize the amount of energy and resources devoted to each vital function (maintenance, growth, and reproduction) in the face of different environmental constraints, both biotic and abiotic. As resources are limited, any investment in a function occurs at the expense of a reduction in available resources to other functions. Moreover, some physical or developmental constraints could prevent a particular combination of life-history traits. Thus, LHT predicts that the distribution of resources between vital functions may be subject to physiological and evolutionary trade-offs regulated by the balance between the costs and benefits in terms of fitness of each of these functions (Koricheva, 2002), thus preventing the simultaneous optimization of all functions. One prediction of the LHT is that in unpredictable environments with intense and recurrent perturbations and variable resources, early reproduction is favored due to the uncertainty of delaying reproduction (Stearns, 1977; Stearns, 1992). In this sense, the age of first reproduction would be inversely related to life expectancy or survival. Therefore, a genetic trade-off between investment in growth and reproduction is a general expectation derived from LHT (Roff, 1992), and has been experimentally confirmed in various pine species associated with geographic and environmental gradients (see, for example, Climent *et al.,* 2008; Santos-del-Blanco *et al.,* 2010). Other well-established negative correlations are produced between growth and defensive strategies against biotic and abiotic stress (Zas *et al.,* 2005; Miki *et al.,* 2006, McDowell *et al.,* 2008, Sampedro *et al.,* 2011; Moreira *et al.,* 2012, and many more).

Considering the selective effects of the environment on trade-offs between life-history traits, differences could be expected in the strategies adopted by species of the same genus, populations of the same species, or even among genotypes within a population (see, Keeley and Zedler, 1998; Howe *et al*., 2003), although at the individual level, trade-offs might not be evident given that not all trees have access to the same level of resources (Reznick *et al.,* 2000). In fact, it is expected that spatial environmental heterogeneity at the regional scale increases both the genetic divergence among populations and the intrapopulation genetic variability (Savolainen *et al.,* 2007). On the other hand, small-scale spatial heterogeneity and environment temporal fluctuation would increase intrapopulation genetic variability (Campbell, 1979; Yeaman and Jarvis, 2006). If trade-offs among traits or among groups of traits are generalizable, it is possible that they have conditioned the evolution of the different populations or species, modulating their divergence to the different biotic and abiotic environmental conditions to which they have been subjected. Most of the works that analyze in an integrating way this type of compromises among life-history traits are focused on short-lived species (Koricheva, 2002).

The comparison of the genetic architecture of traits closely linked to fitness with that of other traits is scientifically challenging, since it deals with the interplay between past directional selection (reducing within-population variation and promoting differentiation between populations) and the availability of enough additive genetic variation that enables future evolutionary processes (Merilä and Sheldon, 1999). Furthermore, there is growing evidence showing the importance of plasticity in trees affecting the estimation of genetic parameters and the necessity to conduct experiments with genetic entries replicated in a range of environmental conditions, i.e. common garden or reciprocal transplant experiments (Sgrò and Hoffmann, 2004). Therefore, exploring patterns of phenotypic integration among a multitude of characters, that is, the pattern of covariation or correlation among traits or groups of traits that are related functionally, ontogenetically or genetically in a given organism (Pigliucci 2003; Schlichting 1989) will provide a more realistic knowledge about species adaptation strategies (Pigliucci, 2005).

Many studies have described high genetic variation in life-history traits in plants and specifically in forest tree species, likely reflecting local adaptation to varying environmental conditions (Andrew *et al.,* 2007; Alberto *et al.*, 2013), as well as trade-offs in populations of the same species that have evolved under a span of environments (Loehle, 1988; Obeso, 2002; Santos-del-Blanco *et al.*, 2012; Grivet *et al.*, 2013). Clinal variation, i.e. continuous genetically-based phenotypic variation along environmental gradients, have also been described for different life-history traits in forest trees (Santos-del-Blanco *et al.*, 2013; de la Mata *et al.,* 2014; Gauli *et al.*, 2015).

A wide range of measurable characters have been proposed as life-history traits in trees and other woody plants: size and age at first reproduction, sexual allocation, maximum size at maturity, maximum longevity, lifetime offspring number, mode of pollination and seed dispersal, or size of the seeds (Stearns, 1977). Knowledge about these traits in forest trees has increased in recent years, but there are other life-history traits in trees related to adaptations to disturbances (like severe drought or fire) still poorly known in this context.

Allocation is the central concept in LHT because resources allocated to one function are not available to other functions, so allocation implies trade-offs. Differences in allocation to functions appear to be more important for many ecological questions than differences in physiological mechanisms at the cellular or molecular levels (Schwinning and Weiner, 1998). Moreover, a basic assumption on resource allocation is that investments in some functions such as reproduction involve costs or efforts (see, for example, Obeso, 2002). Thus, minimizing or balancing such costs and benefits against each other are key aspects behind the evolution of life histories. However, when comparing the literature on relative allocation to different traits, in addition to finding some methodological problems related to the concept of allometry, we also encountered some confusion among three fundamental concepts: allocation, costs and effort (see for example Lawes *et al.,* 2013). Unfortunately, there is still a considerably heterogeneous use of these terms (see Karlsson and Mendez, 2005 for clarification). The existence of somatic costs, consequence of the distribution of resources for the different functions or traits, and the usual shortage of resources in Mediterranean environments, leads to think that the existence of compensations between and within the three groups of vital functions (growth, reproduction, and maintenance or defense) predicted by the LHT might reduce the resilience capacity of Mediterranean forest species (Koricheva, 2002; Obeso, 2002; Zas *et al.*, 2005).

1.6. Fire-adaptive traits

Fire is one of the main natural pre-human disturbance factors that occurs with sufficient frequency and intensity to be a strong selective pressure on plants, favouring fire-adaptive traits (Pausas *et al*., 2004a; Pausas and Keeley, 2009; Keeley *et al.,* 2011). In fact, in recent years we have seen an explosion of the number of papers dealing with the role of wildfires shaping plants (i.e. fire ecology studies, Bellingham, 2000; Schwilk and Ackerly, 2001; Pausas and Keeley, 2009; He *et al.,* 2011, 2012, 2016; Keeley *et al.,* 2011; Lamont and He 2016). Divergence in fire-adaptive trait and local adaptation within species from different environments have been extensively studied. Additionally, the adaptive value of different fire-adaptive traits under other non-fire stressors has been a focus of discussions, even to consider some of those traits as exaptations rather than adaptations to fire (Bradshaw *et al.,* 2011, but see Keeley *et al*., 2011 and many others). There is a current large agreement among ecologists and evolutionary biologists about the fact that many fire response traits are adaptations to specific fire regimes (Keeley *et al.*, 2011). These are called fire-adaptive traits and provide a fitness advantage under particular fire regimes. For example, the absence of self-pruning of dead branches, early female flowering, serotiny, thick bark or resprouting are typical fire adaptations in tree species (Schwilk and Ackerly, 2001; Ne'eman *et al.*, 2004). Different studies have shown that these traits vary within species under different fire regimes (Pausas *et al.,* 2012, Hernández-Serrano *et al.,* 2013). Fire regime includes diverse fire characteristics such as frequency -fire return interval regarding plant longevity-, intensity -flame height regarding canopy fuels height-, seasonality and type of fuels consumed in a given area (Keeley *et al*., 2012). Importantly, fire intensity defines two contrasted fire regimes: surface or understory fires, when the flame height is lower than the canopy and crowns are not burned, and crown fires, when crowns are burned. This distinction is key because adaptations to fire vary between ecosystems under one type of fire or the other. However, during the last decades, dramatic changes have occurred in fires intensity and frequency, as well as in burned area, with large fires becoming increasingly common (Piñol *et al*., 1998; Lloret and Marí, 2001). These changes in fire regime, as well as the new environmental scenarios posed by climate change, may challenge the adaptive strategies of many plant species, even jeopardizing their persistence.

There are different classifications for plants based on their post-fire strategy, although they are usually divided into obligate resprouters, obligate seeders, facultative species and fire avoiders (see for example Pausas *et al.,* 2004a; Pausas and Keeley, 2014; Pausas, 2015a). Each of these strategies involves specific combinations of traits to improve fitness under different fire regimes (Keeley and Zedler, 1998). Within this previous broad classification, three fire syndromes have recently been defined for pine species in particular (Pausas, 2015a; Table 1).

TABLE 1 **|** Main fire traits for each fire syndrome in pines.

Fire trait	Fire-tolerator	Fire-embracer (obligate seeder)	Fire-avoider
Resprouting	No	No	No
Thick bark	Yes	No (moderately)	No
Serotiny	No / Yes	Yes	No
Early reproduction	No	Yes	No
Self-pruning	Yes	No	No
Example species	*Pinus pinaster*	*Pinus halepensis*	*Pinus cembra*

Notes: Modified from Pausas (2015).

In this thesis we will focus on two adaptations to fire: serotiny and bark thickness. These traits seem to have evolved several times through gymnosperms and angiosperms phylogeny (Schwilk and Ackerly, 2001; Grotkopp *et al.*, 2004; He *et al.,* 2012). This could explain its high variability among closely related plant species and between populations of the same species (Richardson *et al.,* 1990; Tapias *et al.*, 2004; Cramer and Midgley, 2009; Lawes *et al.*, 2011; He *et al.*, 2012; Tonnabel *et al.*, 2012; Hernández-Serrano *et al.*, 2013; Rosell *et al.*, 2014; Castellanos *et al.*, 2015; and many more). Here we show an overview of both traits, going into more detail of each one of them in the studied species section of this thesis (see below).

1.6.1. Serotiny

The term *serotiny* usually refers to the retention of seeds within close fruits or cones for several years after ripening until an environmental driver causes dehiscence and seeds release. Fire is the most known factor that causes the opening of serotinous fruits or cones (what is more properly called *pyriscence*, Lamont, 1991), but there are also other causes of cone or fruit opening (see Box 2). Although the correct term is *pyriscence*, it is not commonly used, being much more frequent the term *serotiny*. Therefore, throughout this thesis we will always use this last term.

Box 2. Terminology of different cues for delayed seed release

- Badryspory: delayed seed dispersal without reference to the cue for release (Lamont *et al.,* 1991).
- Pyriscence: seed release caused by the occurrence of fire (Lamont, 1991).
- Xeriscence: seed release caused by dry atmospheric conditions (Nathan *et al.,* 1999).
- Necriscence: seed release caused by the death of the supporting plant or branch (Lamont, 1991).
- Soliscence: seed released when enclosure is heated by the sun (Lamont, 1991).
- Hygriscence: seed release when the enclosure is wetted (Ihlenfeldt, 1971).
- Pyrohygriscence: seed release caused by the alteration of wet-dry periods after fire (Cowling and Lamont, 1985).

Serotinous species typically occur in areas characterized by high diversity and diversification rates, as well as severe threats to natural ecosystems (Myers *et al.*, 2000; Sauquet *et al.*, 2009). Specifically, serotiny is typical of plants from Mediterranean-climate characterized by natural crown fire regimes (Keeley *et al.,* 2012). Among Mediterranean-type climate ecosystems, serotiny is more common in Australia and South Africa than in the Mediterranean Basin, California or Chile (Gómez-González *et al*., 2011; Keeley *et al.,* 2012). Researchers have suggested different reasons to explain the scarcity of serotinous species in the Northern hemisphere, where just *Cupressaceae* and *Pinus* are serotinous. Some of these suggested reasons have been (1) the existence of phylogenetic constraints, although the fact that African *Ericaceae* species show serotiny contradicts this hypothesis, (2) more reliable winter rainfall in the Southern hemisphere could make serotiny less risky than in a climate with more variable precipitation (Cowling *et al.,* 2005), or (3) where soil nutrients are limited and the cost of seeds is high, serotiny could be favored over soil stored seedbanks (Keeley and Zedler, 1998). In addition, phylogenetic reconstructions have revealed that serotiny expresses a phylogenetic signal, given that it is distributed in a considerable number of plant families, including several angiosperms and two gymnosperms (Figure 4; Keeley *et al.,* 2012). Other studies have also disclosed several historical transitions from soil seedbanks to aerial seedbanks within genera (e.g. *Banksia*: He *et al.,* 2011; *Pinus*: Schwilk and Ackerly, 2001; He *et al.,* 2012; *Leucadendron*: Tonnabel *et al.,* 2014), as well as the opposite pattern with evident losses in serotiny, rather than gains (non-serotiny becomes fixed; Lamont *et al.*, 2013). This seems to indicate that the loss of serotiny is the derived evolutionary condition. Meaningfully, these studies denote high lability in seedbank types during plant evolutionary history, including evidence that serotiny and soil-stored seedbanks are alternative strategies (Schwilk and Ackerly, 2001; He *et al*., 2011; Keeley *et al.,* 2012; He *et al.,* 2012; Tonnabel *et al.*, 2014). However, predicting when serotiny would be favored over soil seedbanks is a harder problem. Serotiny implies higher costs per seed (carbon, water, and canopy growth constraints) in return for potentially decreased risk of seed predation or losses to pathogens and decay (Lamont and Enright, 2000), and seed release into an unsuitable environment due to plant senescence could lead to seed mortality (Bond, 1980; Lamont, 1991). Moreover, a key problem that obligate seeder species confront is immaturity risk, i.e. death by moderately intense fires before achieving sufficient recruitment (Lamont *et al.,* 1991, Keeley *et al.,* 1999), if recurring fires occur before plant maturity or before sufficient seedbanks develop.

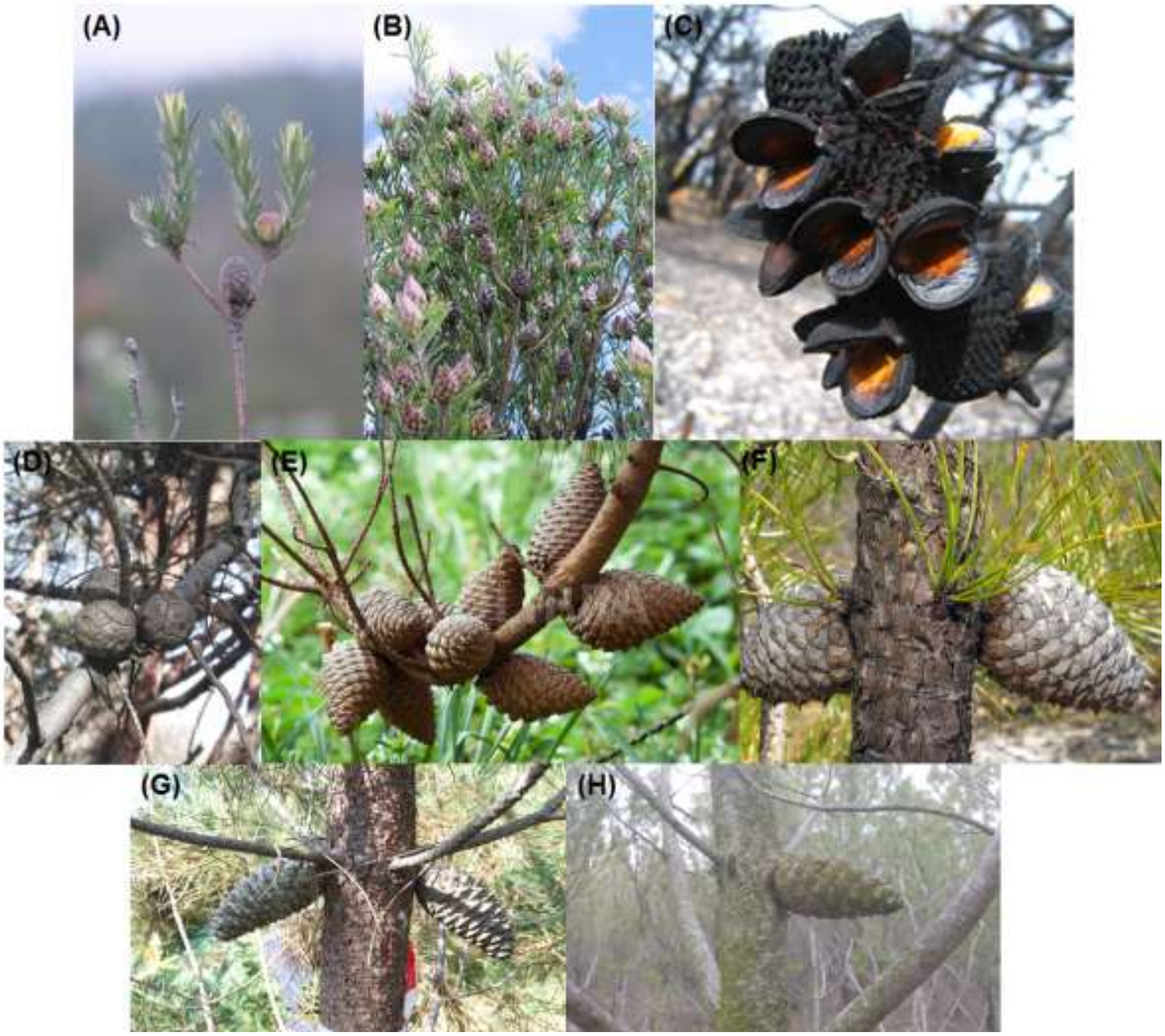

FIGURE 4 | Serotinous structures of different genera and species: (A) *Leucadendron salicifolium* (Proteaceae), (B) *Leucadendron rubrum* (*Proteaceae*), (C) open burned serotinous cone of *Banksia sp.* (*Proteaceae*), (D) *Cupressus macrocarpa* (*Cupressaceae*), (E) *Pinus radiata* (*Pinaceae*), (F) *Pinus rigida* (*Pineaceae*), (G) *Pinus pinaster* (*Pinaceae*) and (H) *Pinus halepensis* (*Pinaceae*). Photos (A, B, D and G) from Climent *et al.* (*in press*), photo (C) from j.g.pausas blog (http://jgpausas.blogs.uv.es/2011/03/18/australia-born-to-burn/), photo (E) from National Geographic Education blog (https://blog.education.nationalgeographic.org/2014/03/21/pining-away/), photo (F) by ©S. Richard (https://www.flickr.com/photos/9428166@N03/3483475825/) and photo (H) by ©R.C. Martín-Sanz.

Serotinous species need to allocate a large amount of resources for the maintenance of the seedbank, so their allocation to the female function has been even compared with cases of maternal care in animals (Harris and Pannell, 2010; Tonnabel *et al.*, 2012). Degree of serotiny, quantified as the expected cone longevity, maximum cone age or proportion of closed cones (Lamont, 1991) shows high variation both between and within species (Cowling and Lamont, 1987; Enright and Lamont, 1989; Lamont *et al.*, 1991; Groom and Lamont, 1997; Midgley, 2000; Tapias *et al.*, 2001, 2004; Cramer and Midgley, 2009; Crawford *et al.*, 2011; Ledig *et al.*, 2013; Hernández-Serrano *et al*., 2013, 2014 and others). This observed variation in serotiny degree between and within species seems to depend on resources availability and fire regimes. Therefore, when resources are scarce, allocation to serotiny should be reduced in favor of allocation to plants survival and to the annual production of seeds (Tonnabel *et al.*, 2012). In fact, for a serotinous tree, it is fundamental to maximize its survival before the fire until achieving a sufficient aerial seedbank. Predictability in fire frequency leads also to a decrease in allocation to serotiny for the benefit of higher allocation to plant growth, survival and seed production (Enright *et al*., 1998; Tonnabel *et al.*, 2012).

The genetic architecture of serotiny has been almost exclusively studied in conifer trees. Early studies on serotinous *Pinus* species supported a high genetic control of serotiny (Rudolph *et al.*, 1959; Teich, 1970; Perry and Lotan, 1979), interpreted as a single locus trait (Teich, 1970). More recent works, also in pines, have proposed a polygenic genetic base of serotiny (Parchman *et al.,* 2012, Budde *et al.,* 2014) and have firstly quantitative evaluated its narrow-sense heritability (Hernández-Serrano *et al.*, 2014). A moderate heritability of serotiny implies high evolvability within populations. Lately, results obtained in common garden experiments (increasingly used to study serotiny) confirmed the high differentiation observed among natural populations, and has been interpreted as ecotypic variation due to local adaptation (Hernández Serrano *et al.,* 2014). A direct evolutionary relationship to different fire regimes is still missing because historical fire records are generally too short-term and influenced by human factors (Archibald *et al.,* 2013).

A consequence of the quantitative (polygenic) genetic control of serotiny is that it implies that part of the observed phenotypic variation is necessarily due to environmental effects. Other handicaps when studying phenotypic plasticity of serotiny is that this trait, as many other phenotypic traits in plants, vary with growth and development (Cowling and Lamont, 1985; Tonnabel *et al.,* 2012) being necessary to take into account allometric and ontogenetic differences (Wright and McConnaughay, 2002; Weiner, 2004). Despite its key importance, allometric plasticity in serotiny had not been directly quantified until the elaboration of this thesis.

As previously stated, serotiny implies higher seed production costs than soil seedbanks (reviewed in Climent *et al., in press*), but other possible costs of serotiny, such as architectural and biomechanical costs (closely related to total reproductive costs) have not yet been investigated. Another factor that may influence the plastic phenotypic expression of serotiny is whether producing and maintaining serotinous fruits comes with a cost. Although difficult to quantify, maintenance costs may be highly relevant to understand the allometric and plastic effects on cone serotiny. It is well established that serotinous cones of many angiosperms such as *Banksia* or *Leucadendron* depend on the water supply of the bearing plant to open or remain closed (Midgley and Enright, 2000; Cramer and Midgley, 2009; Harris and Pannell, 2010; Tonnabel *et al.*, 2012). But, the environmental effects on serotiny in gymnosperms is considered to be dependent solely on external factors (see, for example, Nathan *et al.*, 1999). However, the apparently dead cones of some *Pinus* and *Hakea* species share common features, a green layer below the surface of the cone and a peduncle with sapwood that allows a vascular interaction between the tree and the cones, preventing them from drying out and open (Midgley and Enright, 2000; Cramer and Midgley, 2009; Martín-Sanz *et al.,* 2017). Despite its importance, maintenance costs of serotinous cones have been little studied. The first work where these costs were addressed was carried out in a conifer, *Cupressus sempervirens* (Lev-Yadun, 1995). This study confirmed that the sealing of the cones was controlled by the water content of their scales so that the cones opened when they dried even at very low temperatures. Some studies have indirectly investigated the existence of these costs, correlating the retention time of leaves with that of serotinous cones (Midgley, 2000), analyzing the effect of drought and competition among cones (Espelta *et al.*, 2011) or by studying the loss of water and carbon by the cones (Cramer and Midgley, 2009). Although maintenance cost of the cones could be considered small compared with that of the leaves, water and carbohydrate costs would increase as the number of cones in the plant increases (Cramer and Midgley, 2009; Espelta *et al.*, 2011). These maintenance costs would imply that there is not only a direct environmental effect on the opening of serotinous cones without fire (xeriscence), but also an indirect effect due to changes in the physiological status of the tree. In addition, due to the consumption of water by female cones, serotiny should increase the already high cost in water of female reproduction compared to male reproduction (Obeso, 2002). In fact, higher levels of sexual dimorphism have been linked with higher serotiny degree (Harris and Pannell, 2010). Furthermore, physical cone damage (e.g. due to squirrels or crossbills) can also provoke precocious cone opening while insects or herbivory can impede cone dehiscence.

Finally, aerial seed banks are ecologically paramount, since they allow populations persistence when survival of adult trees is low in fire-prone environments at intermediate fire recurrences (Enright *et al.*, 1998; Pausas *et al.,* 2004b). In those habitats, even low levels of aerial seedbanks increase species fitness (Lamont and Enright; 2000). In obligate-seeder species, aerial seed banks might be affected by a joint complex variation of total reproduction and serotiny degree. However, literature analyzing the variation causes of aerial seedbanks is extremely scarce.

1.6.2. Bark thickness

Bark characteristics are important in structuring plant communities. Especially the correlation between bark thickness and fire regime has recently attracted increasing interest by plant scientists (Pausas, 2015b; Rosell, 2016; Pausas, 2017). Bark is the outermost part of stems in woody plants and includes all tissues outside the vascular cambium. It has different functions which are reflected in its highly diverse colours, textures, smells, densities, anatomy, chemistry and thicknesses (Figure 5). Bark is formed by two main components with different origins and structure, and also contrasting functions: the living inner bark (mainly the secondary phloem), which is produced by the vascular cambium and by the phelloderm (or by the apical meristem if there is no cortex), and is located between the vascular and the cork cambiums; and the dead outer bark (formed by dead cells) that is originated by the phelloderm when there is only one periderm or, when there are more than one periderm, by all the meristems (including, for example, the secondary phloem), being therefore an indirect result of the vascular cambium, and its placed outside the cork cambium (Figure 6; see details in Evert and Eichhorn, 2006 and Romero, 2014). The inner bark is related to transport and storage of water, photosynthates and secondary compounds (Srivastava, 1964; Roth, 1981; Scholz *et al*., 2007), being usually thicker in drier areas probably due to selection favoring its water storage capacity (Rosell and Olson, 2014; Rosell, 2016). The outer bark protects the tree from fires, adverse climatic conditions such as cold or drought or pathogens and herbivores, reduces water loss and/or provides structural support (Vines, 1968; Niklas, 1999; Pfanz *et al*., 2002; Romero and Bolker, 2008;

Paine *et al*., 2010; Hoffman *et al.*, 2012; Graves *et al*., 2014; Romero, 2014; Rosell and Olson, 2014; Schafer *et al*., 2015; Rosell, 2016). However, despite its functional relevance, the role of bark in plant ecological strategies and the causes of its variation remain poorly understood, although it is well established that stem size explains a large percentage of its variation (c.a. 72% of total bark thickness; Rosell, 2016).

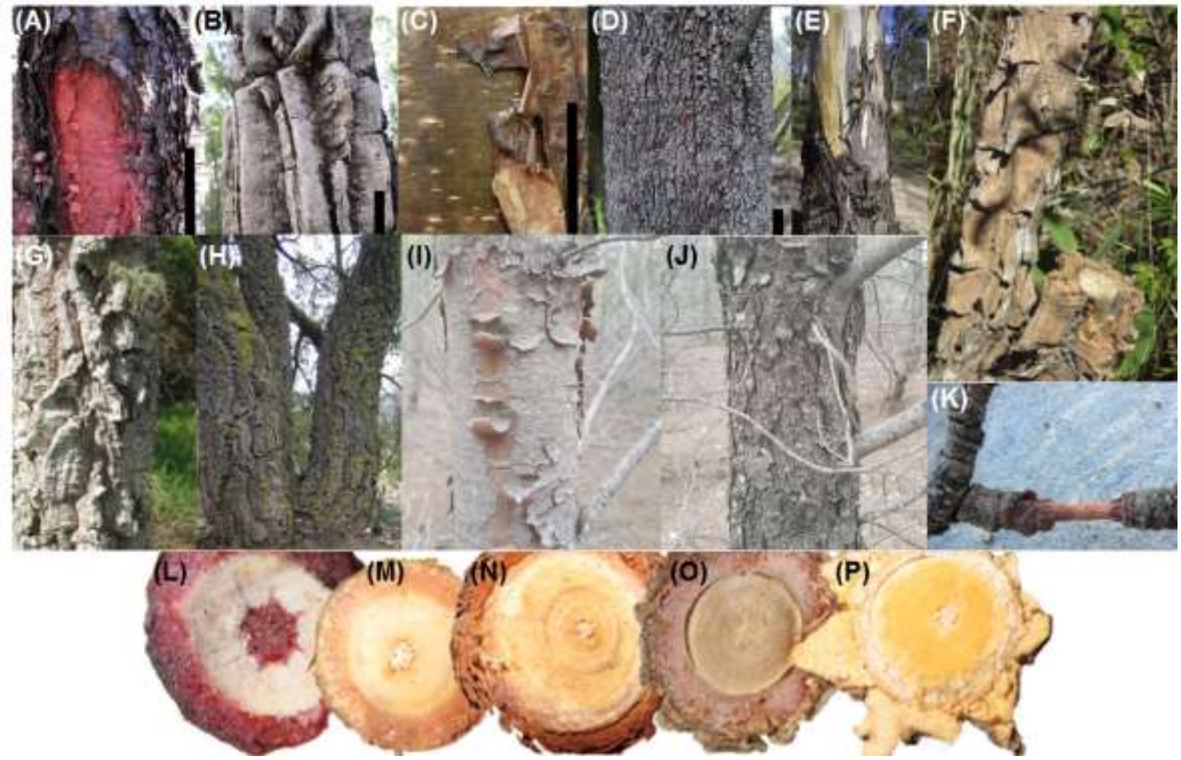

FIGURE 5 **|** Diversity of bark among species: (A) *Persoonia linearis* (*Proteaceae*), (B) *Alstonia actinophylla* (*Apocynaceae*), (C) *Bursera instabilis* (*Burseraceae*), (D) *Exocarpus cupressiformis* (*Santalaceae*), (E) *Eucalyptus tenuiramis* (*Myrtaceae*), (F) *Eremanthus seidelii* (*Asteraceae*), (G) *Myrcia bella* (*Myrtaceae*), (H) *Quercus suber* (*Fagaceae*), (I-J) are different populations of *Pinus halepensis* (*Pinaceae*) and (K) *Byrsonima verbascifolia* (*Malpighiaceae*). (L-P) Cross-sectional variation in bark thickness: (L) *Buchanania obovata* (*Anacardiaceae*), (M) *Brachychiton paradoxus* (*Malvaceae*), (N) *Lophostemon lactifluus* (*Myrtaceae*), (O) *Planchonia careya* (*Lecythidaceae*), (P) *Alstonia actinophylla* (*Apocynaceae*). Photos (A-E) from Rosell *et al.* (2015), photos (F-H) and (K) from Pausas (2015b), (I-J) by ©R.C. Martín-Sanz and (L-P) from Rosell *et al.* (2014).

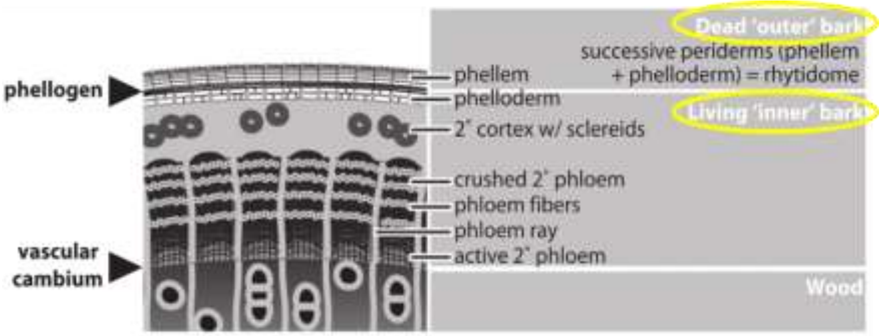

FIGURE 6 | Bark structure in cross-section. Modified from Rosell (2016).

Bark thickness is the most studied bark property so far, due to its correlation with cambial insulation and protection, especially against fires (Jackson *et al.*, 1999; van Mantgem and Schwartz, 2003; Bauer *et al.*, 2010; Hoffmann *et al*., 2012; Lawes *et al*., 2013; Pausas, 2015b). The degree of heat insulation by bark increases with the square of its thickness (Hare, 1965; Vines, 1968; Peterson and Ryan, 1986). Therefore, bark thickness strongly decreases the thermal diffusivity –the most important bark protective property (Vines, 1968)–, which has been shown to be independent of bark structure, density or moisture content (Martin, 1963; Uhl and Kauffman, 1990; Pinard and Huffman, 1997). In fact, bark thermal diffusivity is approximately 20% of that of wood of the same density and about twice lower than that of air (Martin, 1963). Bark, besides protecting meristemic tissues (cambium or buds) and the phloem, also protects the xylem, since stem death could be also produced by hydraulic failure (Ducrey *et al.*, 1996, Midgley *et al.*, 2011; Michaletz *et al.*, 2012). However, despite the multiple functions of bark, there is increasing evidence that the high variability in bark thickness among species in fire-prone ecosystems is due to the variability of fire regimes, since having a thick bark increases fitness in many fire-prone environments (‘fire hypothesis’; different fire regimes select for different bark thicknesses, reviewed on Pausas, 2015b, but see Rosell *et al.,* 2017 for a different perspective). Therefore, regarding vital strategies against fires I mentioned above, thick bark is usually related to fire tolerator species (whose vital strategy is adult survival) such as *Pinus pinaster*, *Pinus ponderosa, Pinus palustris, Araucaria araucana, Fitzroya cupressoides* or *Sequoiadendron giganteum* among others (Keeley and Zedler, 1998; Jackson *et al.* 1999; Lara *et al.,* 1999; Beaty and Taylor, 2007; He *et al*., 2012, for example). Other hypotheses to explain the development of thick bark have also been proposed (see review in Pausas, 2015b). These are the biotic hypothesis (bark protects the stem against harmful organisms such as pathogens, pests or herbivores), the climate hypothesis (bark protects against extreme climates such as cold or drought) and the biomechanical hypothesis (bark is a mechanism to increase mechanical stability). However, there is no clear evidence that the variability in bark thickness on a global scale has been produced by selective forces related to these three hypotheses. But neither is the fire hypothesis completely applicable to explain bark thickness variability on a global scale. Meaningfully, not all ecosystem types are burned naturally and those that burn as often as necessary (compared to plants life cycle) to be relevant at an evolutionary scale are not as abundant.

Importantly, bark is costly concerning resource allocation and also in opportunity costs caused because bark limits the diffusion of water, oxygen, CO_2 and light through the stem (Pfanz and Aschan, 2001; Teskey *et al*., 2008). As far as we know, the costs of bark and wood construction in terms of resource allocation have not been studied in detail. While bark and wood densities can be very similar in some species (like in conifer trees; Miles and Smith, 2009), wood and bark have different physical properties, chemical composition and biological functions. Wood is mainly formed by lignin and cellulose, but bark lignin is much more complex in structure than wood lignin (Romero, 2014). Bark has higher reserves concentration and contains other large chain compounds that are costly to produce (Barbaroux *et al.,* 2003; Larjavaara and Muller-Landau, 2010). For example, bark has ten times more minerals than wood (Jensen *et al.*, 1963), the proportions of galactose, mannose, and starch are higher in bark (Romero, 2014), and the presence of allelopathic substances, chlorophyll, bark extractives such as fatty acids, alcohols, resins, pigments, tannins and phenolic acids (Wainhouse *et al.*, 1997; Franceschi *et al*, 2005) make bark possibly more costly than wood. This putatively high costs imply that the relative resource allocation to bark must be subjected to strong evolutionary trade-offs between the fitness benefits of a thicker bark and its cost at different ontogenetic stages (Schwilk and Ackerly, 2001), i.e. thick bark would not have appeared in ecosystems where differences in thickness would not lead to increased fitness. This idea underlies several evolutionary studies on the protective role of bark thickness (most of them in tropical ecosystems), comparing from a few to many species in natural populations and different ecosystems, usually focusing on species which strategy against fire is adult survival (Richardson *et al.*, 1990; Keeley and Zedler, 1998; Paine *et al*., 2010; Lawes *et al.*, 2011a, 2011b, 2013; Dantas and Pausas, 2013; Poorter *et al*., 2014; Rosell *et al*., 2014, Rosell, 2016). However, few studies have addressed the intraspecific variation in bark thickness (Climent *et al*., 2004; Tapias *et al.*, 2004; Stephens and Libby, 2006; Briand *et al*., 2014), and even fewer have used common garden experiments where genetic and environmental effects can be properly separated (Matziris, 2000; Tapias *et al.,* 2004; Stephens and Libby, 2006; Kohnle *et al.*, 2012). The possible plasticity of bark thickness and allometry responding to contrasting environments has barely been investigated and bark variation has only been related to different fire regimes, mainly surface fires (Keeley and Zedler, 1998; Jackson *et al.,* 1999; Schwilk *et al.,* 2013; Graves *et al*., 2014; Pausas, 2015b; but see Richardson *et al.,* 2015 and Jager *et al.*, 2015 about the effects of soil and climate in temperate rainforests). Most of these works have found high variability in bark thickness among species and ecosystems (see Figure 5), but see Rosell *et al.* (2014) where differences found were higher within than among ecosystems.

Following the theory of allometry, residuals of the log-log bark thickness-tree diameter regression (see Paine *et al.,* 2010; Rosell, 2016) have been commonly used to study bark allometry and allocation to bark, due to the necessity of differentiating the ontogenetic (i.e. developmental) effects from the environmental effects. Other alternative approaches have also applied, such as the relative bark thickness (2 x absolute bark thickness divided by tree diameter; Midgley and Lawes, 2016; see Pausas, 2015b Supplementary Information for details on different procedures). While simple and straightforward, we think that the linear relationship between bark thickness and diameter does not represent accurately enough the real resource allocation to bark. Our point is that allometry of bark allocation should be rather assessed through variables that are as close-as-possible to the biomass of bark and sapwood (the functional part of the trunk). Therefore, in this thesis, when focusing in the allometry of bark allocation, we looked at the allometric relationship between bark and sapwood volumes, basing our analyses on the percentage of bark volume in the total stem volume from tree base to breast height (see page 92 in Materials and Methods section). Whether the bark thickness-sapwood diameter allometric relationship has an adaptive significance has been very recently studied (Rosell *et al.,* 2017).

Despite the relevance of differential allocation to bark, the survival of a plant facing a given fire depends on its absolute –not relative– bark thickness (Midgley *et al*., 2010; Lawes *et al.,* 2011a). In this sense, the critical absolute bark thickness above which a tree can survive different types of fire is a highly useful value (VanderWeide and Hartnett, 2011; Dehane *et al*., 2015), so for evaluating fire survival capacity of *P. halepensis* trees in this thesis, we focused on the absolute bark thickness. It is important to bear in mind that thermal diffusivity of bark is highly influenced by its thickness (see, Hare, 1965; Vines, 1968) but independent of bark structure, density or moisture content (Martin, 1963; Uhl and Kauffman, 1990; Pinard and Huffman, 1997).

Basal bark thickness is relevant since trees can die by the girdling of their basal stem in surface fires, even of low intensities (Jones *et al.*, 2004). Nonetheless, several experiments have also shown that trees with less than 85% of basal girdling can survive after a fire (Wagener, 1961; Ryan *et al.,* 1988; Ryan, 1993; Ducrey *et al*., 1996). Bark thickness decreases along the bole of the tree from the base, so that bark thickness at breast height (where it is usually measured; see, Harmon, 1984; Stephens and Libby, 2006; and Rosell *et al*., 2015 for exceptions) or along the bole is also essential considering the so-called chimney effect on trunks that occurs during fires. This effect can generate a warming at leeward in height in the trunk (on the opposite side to the fire spread direction) that could affect living tissues that are less protected by bark than the tree base; although this effect can also cause basal girdling, starting also at leeward (Figure 7; see Fuego_Lab blog, http://fuegolab.blogspot.com.es/2017/04/el-efecto-chimenea-en-los-troncos-de.html). These fire resistance studies are highly relevant nowadays, usually focusing on the height of scorch escape, the insulation capacity of the bark or the plant flammability among other tree fire resistance properties (see, for example van Mantgem and Schwartz, 2003; Dehane *et al.*, 2015; Madrigal *et al.*, 2017). In this kind of studies, the critical time for cambium kill is broadly used to compare the bark insulating ability among species or populations (Peterson and Ryan, 1986; Fernandes *et al*., 2008; Lawes *et al*., 2011a, 2011b; Schwilk *et al*., 2013).

FIGURE 7 | (A) Representation of the chimney effect in trunks, (B) photograph showing the chimney effect in a prescribed burn. Modified from ©Fuego_Lab blog (http://fuegolab.blogspot.com.es/2017/04/el-efecto-chimenea-en-los-troncos-de.html).

Although bark thickness is a fairly easy trait to measure in the field, depending on the question addressed, different types of measurements should be used: absolute values (thresholds) to know the plants survival ability against fire, relative values to be able to compare among species or ecosystems and to study the plasticity of resources allocation, and allometric coefficients that serve for the previous task and also to study bark allometry. In addition, differentiating between inner and outer bark, and between bark at the tree base, bark along the bole and bark at the branches would also be very useful (Van Mantgem and Schwartz, 2003).

1.7. Case study species: *Pinus halepensis* Mill.

Besides the fundamental role of forest trees in mitigating the impact of climate change, they are attractive model organisms for studying adaptation, evolution and the possible effects of climate change (Alberto *et al.*, 2013). Among forest tree species, conifers and especially pines show additional advantages as they are all monoecious and long-lived species with outcrossing mating systems, large natural populations, low levels of domestication, high levels of genetic variation and phenotypic plasticity, and there is an extensive network of common garden experiments already established for the accurate assessment of the phenotype (Neale and Savolainen, 2004).

Pinus halepensis Mill. (Aleppo pine) is an excellent model for studying past and future local adaptation processes (Lev-Yadun and Sederoff, 2000), as well as for investigating fire-adaptive traits. This is due to several characteristics of its life cycle (Ne'eman *et al.*, 2004, 2011; Tapias *et al.*, 2004). *P. halepensis* has an early age of first reproduction -permitting an early measurement of reproductive traits-, besides a high reproductive allocation that increases costs of reproduction. The interannual variability in reproduction is scarce, unlike typically masting species, facilitating recording long-term continuous data series. Female cones remain attached to the branch through its thick peduncle even after opening and releasing of seeds, which facilitates the retrospective record of female reproduction events even from its beginnings. Like all other pines, the segregation of male and female reproductive structures within the tree crown allows testing specific hypotheses about the role of each reproductive function. There is a wide range of sizes within the same population and between populations, which allows testing the allometry of sexual expression and allocation. Crown architecture in this species is relatively simple and has a relatively low lifespan compared to other forest trees, which improves the correlation between reproductive investment for life and that estimated in a shorter period.

1.7.1. Phylogeny

Pinus halepensis is a Mediterranean pine species belonging to section *Pinus*, subsection *Pinaster*, i.e. the Mediterranean pine group (Gernandt *et al.*, 2005, Figure 8). Phylogenetically *P. halepensis* is very close to *P. brutia*, in both phenotypic and molecular taxonomic traits (Figure 8; López *et al.*, 2002, Grivet *et al.*, 2013). However, both species seem to have evolved differently for several key adaptive traits (see Box 3 for main differences between both species). In fact, *P. halepensis* shows quite divergent life history traits within Mediterranean pines indicating that this species represents an extreme of adaptive evolution within this clade (Grivet *et al.*, 2013) in the direction of the fire-embracer strategy or obligate seeder species.

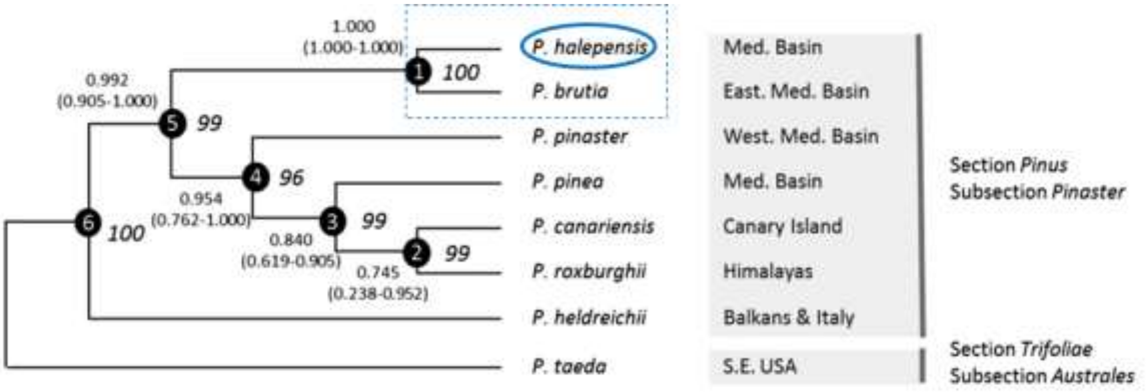

FIGURE 8 | Phylogenetic tree for the seven Mediterranean pine species and for an American outgroup, with the group *P. halepensis-P. brutia* identified. Modified from Grivet *et al.* (2013).

Box 3. Main differences between *P. halepensis* and *P. brutia*

- *P. brutia* is less tolerant to dry environments, but less frost sensitive than *P. halepensis* (Falusi *et al.*, 1983; Calamassi *et al.*, 1984; Voltas *et al.*, 2008).
- *P. brutia* is more precocious in forming secondary needles and first buds at early developmental stages (Climent *et al.*, 2011).
- *P. halepensis* has higher reproductive precocity, higher investment in reproduction and higher serotiny than *P. brutia* (Thanos and Daskalakou, 2000; Chambel *et al.*, 2013).
- *P. halepensis* female cones have thick long peduncles, whereas *P. brutia* cones are sessile (without peduncle; Chambel *et al.*, 2013).
- *P. brutia* has thick reddish bark, while *P. halepensis* generally shows thinner grey bark (Chambel *et al.*, 2013).

1.7.2. Distribution and ecology

This species has a wide geographic distribution, from Syria, Turkey and Israel in the east to the Iberian Peninsula and Morocco in the west (Figure 9). Populations in the east are scarce and isolated, being more frequent the above mentioned, closely related species, *P. brutia* Ten. Present-day distribution is derived from a range westward spread which explains the lower genetic diversity both in neutral markers and in some quantitative traits found in different parts of the distribution, mainly in Iberian populations (Bucci *et al.*, 1998; Tapias *et al.*, 2001; Gómez *et al.*, 2005; Grivet *et al.*, 2009).

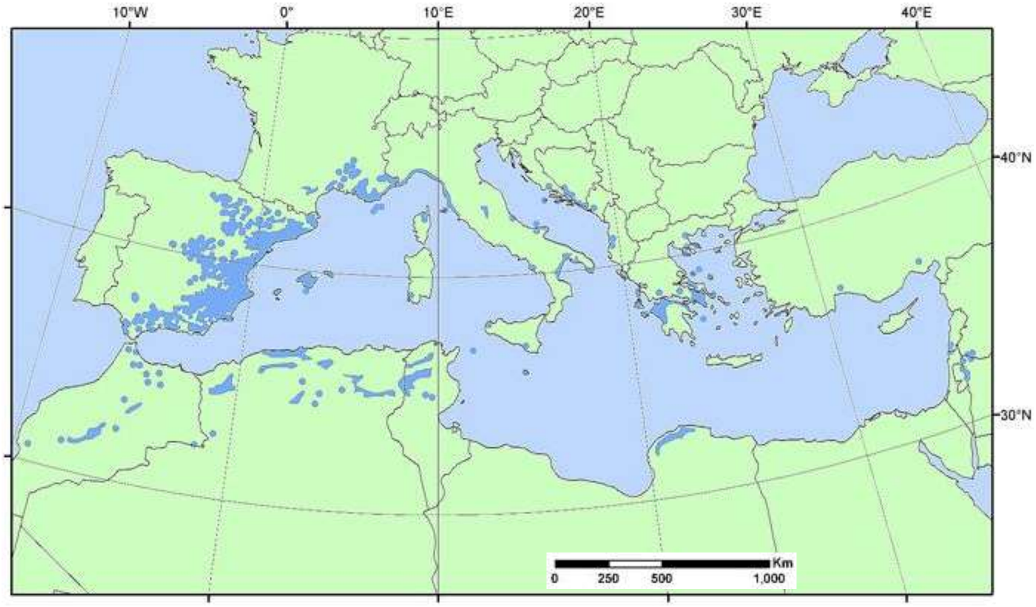

FIGURE 9 | *Pinus halepensis* distribution map. Blue shaded areas correspond to the species natural distribution. From www.euforgen.org.

The natural distribution of *P. halepensis* include a wide range of bioclimates in the Mediterranean region (Fady *et al.,* 2003), but due to its high tolerance to drought stress, is usually located in drier and warmer areas than other Mediterranean pine species. It grows on all substrates and at variable altitudes (0-2600 m a.s.l.).

Pinus halepensis has been extensively used in afforestation, therefore is also found in extensive planted stands across Mediterranean dry zones (Gil *et al*., 1996), targeting ecological restoration, or less frequently wood production. It is frequently used in low-demanding ornamental plantations under conditions unsuitable for other species (Chambel *et al*., 2013). Likewise, *P. halepensis* has been used to afforestate former agricultural lands, although it's great colonizing ability (based on its reproductive traits) allows it to expand also to these areas naturally (Vallejo *et al*., 2012). However, the extensive use of this species for afforestation and restoration has been largely debated for various reasons. Mainly, this species, like *P. pinaster*, has been blamed for increasing virulence and even causing forest fires (Bellot *et al.,* 2004; Maestre and Cortina, 2004). This negative view is also due to a wrong common belief that this species is not native to Spain (see Gil *et al.,* 1996 and Fuego_Lab blog http://fuegolab.blogspot.com.es/2017/09/el-mito-de-las-plantas-que-favorecen.html).

1.7.3. Reproductive biology and fire adaptation

Pedunculated female cones and male cones appear in spring, and female cones reach maturity in autumn two years after pollination. Multiple flowering in the same year leading to multiple yearly cone cohorts is not rare in this species (Pardos *et al.*, 2003; Climent *et al.*, 2008). The small-sized seeds are efficiently wind-dispersed thanks to a low wing load (Grivet *et al.*, 2013, Figure 10). *P. halepensis* is one of the most precocious and most reproductive pine species (Richardson, 2000; Tapias *et al.*, 2001; Climent *et al.*, 2008; Santos-del-Blanco *et al.*, 2010, 2013). Compared with pines typically seen as masting species, *P. halepensis* is considered not to be, but actually, this matter has not been studied in depth. Therefore, somatic costs of reproduction detected in this species (Santos-del-Blanco *et al.*, 2014) are highly relevant. Through a manipulative experiment, this study suggested that costs of reproduction in *P. halepensis* might be more relevant in terms of future reproduction, rather than just in vegetative growth. This species also has a variable and intermediate level of serotiny which has been extensively studied lately (Tapias *et al.*, 2001; Ne'eman *et al.*, 2004; Hernández-Serrano *et al.*, 2014 and others). It shows xeriscent cones which eventually open even in the absence of fire after a variable number of years or under particularly dry and warm conditions (see Box 2). *P. halepensis* is considered a fire-embracer or obligate-seeder since, lacking resprouting ability, its vital strategy is based on early building an aerial seed bank, rather than on adult survival (Tapias *et al.*, 2001; Ne'eman *et al.*, 2004; Pausas *et al.*, 2004; Pausas and Keeley, 2014; Pausas, 2015a). Therefore, fire usually kills adult trees and triggers seed release allowing regeneration after stand-replacing fires (Tapias *et al.*, 2004; Fernandes and Rigolot, 2007). However, there is evidence that under a too recurrent fire regime, this species may have difficulties in producing a sufficient amount of viable seeds in the inter-fire interval (Díaz-Delgado, 2003). Within this strategy of stand persistence, rather than individual survival, its fire adaptation is mostly based in early reproduction and early building a canopy (aerial) seed bank (Daskalakou and Thanos, 1996; Tapias *et al.*, 2001). Consequently with this obligate seeder strategy, *P. halepensis* is characterized by a low investment in a thick bark allowing adult endurance. However, this species is mentioned in the literature both as thin- and as moderately thick-barked (Fernandes *et al.*, 2008; Chambel *et al.*, 2013; Grivet *et al*., 2013). Actually, our field observations indicate wide differences in bark among populations. In addition, some works report variable survival to low or moderate intensity fires (Ducrey *et al.*, 1996; Trabaud and Valina, 1998; Rigolot, 2004; Fernandes *et al.*, 2008), also coinciding with our own field observations.

FIGURE 10 **|** (A) Needles, (B) immature male cones, (C) winged small seeds and (D) female cones of *Pinus halepensis*. Photos by ©R.C. Martín-Sanz.

1.7.4. Intraspecific variation

Previous studies have shown significant differences among *P. halepensis* populations for different adaptive traits, with eastern populations showing faster growth and lower investment in early reproduction (including low serotiny) and populations towards the southwest with the opposite trends (Climent *et al.*, 2008; Santos-del-Blanco *et al.*, 2013). Besides this variation among populations, high intra-population diversity has been also recorded for several traits (growth, Matziris, 2000; reproductive traits, Matziris, 1998; Santos-del-Blanco *et al.*, 2010; serotiny, Hernández-Serrano *et al.*, 2014), with higher heritability values for reproductive versus growth traits, which means that reproductive traits show higher possibility of evolution.

These evidences of genetically based variation of different traits might reflect local adaptation. Due to the recent migration history of the species (Grivet *et al.*, 2009), *P. halepensis* presents low genetic variation in the Iberian Peninsula, but with high level of differentiation among populations, as well as high phenotypic variation that favors different responses to variable environments. Growth traits in *P. halepensis* are highly plastic, while genetic effect and genotype by environment interaction, although significant, are not so important (Chambel *et al.*, 2007). The adaptive relevance of drought stress in shaping populations' genetic structure has been also disclosed in previous studies for traits such as biomass allocation (Chambel *et al.*, 2007; Climent *et al.*, 2008), intrinsic water use efficiency (Voltas *et al.*, 2008), wood anatomy (Esteban *et al.*, 2010), vulnerability to xylem embolism (Klein *et al.*, 2013) and water uptake patterns (Voltas *et al.,* 2015); with high plasticity in addition to ecotypic patterns. Moreover, a high ecotypic variation has been found for female reproduction in our model species, interpreted as strong genetic control of reproductive traits to cope with selection pressures, apart from plasticity and allometric effects (Tapias *et al.*, 2004; Climent *et al.*, 2008; Santos-del-Blanco *et al.*, 2013). Numerous studies have linked the reproductive onset to site differences, in particular, higher reproductive precocity has been found in more arid environments (González-Ochoa *et al*., 2004; Climent *et al*., 2008) or in areas with high fire recurrence (Thanos and Daskalakou, 2000). The fact that this species is more reproductively precocious in harsh environments implies a high resource investment at early ages (Alfaro-Sánchez *et al*., 2015), which under a scarcity of resources possibly caused by climate change in some areas of its distribution may endanger the persistence of some *P. halepensis* populations. Actually, a very recent study in which the infra-specific variation of *P. halepensis* was integrated into species distribution models, predicted that populations from southern Spain and North Africa are very exposed to future habitat losses under the ongoing climate change (Serra-Varela *et al.,* 2017). Regarding genotype by environment interaction, previous studies have shown that growth traits performed very differently among populations under different environments, while for reproductive traits, populations behave more similar (Santos-del-Blanco *et al*., 2010, 2013).

1.7.5. Serotiny

In *P. halepensis*, the narrow-sense heritability for serotiny (moderate: $h^2 = 0.20$) and its genetic differentiation among populations (Q_{st} moderately higher than F_{st}) have been quantified recently (Hernández-Serrano *et al.,* 2014), with ecotypic variation patterns that link higher degree of serotiny to higher frequency of crown fires (Tapias *et al.,* 2004; Hernández-Serrano *et al.*, 2013; Castellanos *et al.*, 2015). The negative allometric effect found for serotiny degree in this species (Thanos and Daskalakou, 2000; Tapias *et al*., 2001; Ne'eman *et al*., 2004), which is considered adaptive for plants confronting short fire-return intervals, allows the maintenance of a big enough aerial seedbank at early reproductive stages. However, there is no quantitative evidence of plasticity for this fire-adaptive trait. In addition, the causes that trigger cone opening in the absence of fire are still unclear and are a key point of debate. Moreover, some authors ponder that *P. halepensis* has a dual strategy based on two different types of cones in the same individual: serotinous and not serotinous cones with different physical structure and anatomy (Moya *et al.,* 2008; Salvatore *et al*., 2010). Several studies consider only mechanical changes in serotinous cones mediated by external weather conditions (Nathan *et al.,* 1999) as the main trigger of cone opening in the absence of fire in *P. halepensis*, but other studies consider possible endogenous causes, such as the age of the cones (Tapias *et al.,* 2001) or the internal conditions of the cones resulting from the balance of plant resources (water and perhaps other substances transferred through the peduncle; Espelta *et al.,* 2011). This would suggest a possible competition for water between older and younger cones. The hypothesis of the latter study is based on field observations (Alfaro-Sánchez *et al.,* 2015) and on parallelism with other species, both gymnosperms (Lev-Yadun, 1995) and angiosperms (see for example, Cramer and Midgley, 2009; Harris and Pannell, 2010). Our own field observations indicated that serotinous cones of broken or dry branches open distinctly before cones of healthy similar branches of the same tree, suggesting an endogenous control of cone opening. Given the limited knowledge about the possible maintenance costs of serotiny, it is not known if the possible effects related to the hydric condition of the tree and the cones could be exhibited only in a certain range of external conditions -with intermediate temperatures-, while at warmer and/or drier conditions cones might open irrespectively of their water status. Furthermore, despite its likely importance, it is not known whether a physical connection between the cone and the supporting branch is even possible, which would allow physiological exchanges between the tree and the cones. This was a key starting point for our work. Interestingly, the particular thick peduncle of *P. halepensis* could be related to a more long-lasting conducting capacity than in other close relative species as *P. brutia*. Moreover, thick peduncles are not displayed by other serotinous pines species related to *P. halepensis* within the *Pinaster* section (Gernandt *et al.,* 2005), such *as P. pinaster* Ait. and *P. canariensis* C. Sm.

Significant differences in the size of the aerial seedbank has been also found among populations of *P. halepensis* -probably due to the variation in serotiny degree and female reproduction, i.e., the total number of female cones produced (Andersen, 1989; Tapias *et* *al.*, 2001)- with a greater aerial seedbank in xeric areas for this species distribution (Tapias *et al.*, 2004).

1.7.6. Bark thickness

Bark thickness was observed to be moderately heritable in *P. halepensis* (h^2 = 0.15-0.24; Matziris, 2000) what implies the existence of genetic variability, but differentiation among populations and phenotypic plasticity in this trait is still unknown. Considering a likely trade-off between alternative life-history strategies (Keeley and Zedler, 1998), the genetic differentiation found in this species for female reproduction and serotiny (Climent *et al*., 2008; Budde *et al*., 2014; Hernández-Serrano *et al.*, 2014) would imply a negatively correlated variation in bark thickness. As mentioned, *P. halepensis* is capable of surviving fires of low or moderate intensity under certain conditions. This fact is of paramount importance, as it enables trees to achieve a sufficiently large aerial bank of serotinous cones before adults are killed by fire. The absolute bark thickness that a tree has at the base or at breast height would allow tree survival facing different fire types. The life expectancy of *P. halepensis* trees is usually between 20 and 50 years (Agee, 1998; Vázquez and Moreno, 1998), but can be as low as 16 years under short fire return intervals (Tessler *et al*., 2014). Moreover, *P. halepensis* needs between 15 and 20 years to achieve a sufficient aerial bank of mature seeds that allow the population persistence in case of fire. Therefore, the age of the trees with which we work in this thesis (18 years) is key given the life-history of the species. This thesis aims to increase our knowledge about bark thickness, a key fire-adaptive trait very poorly studied in conifers.

2. Objectives of the thesis

The main objective of this thesis is to improve the understanding of the effects of resources scarcity, mainly water shortage on key adaptive traits (life-history traits) in a Mediterranean pine (*Pinus halepensis*, Aleppo pine), which shows high resilience in Mediterranean ecosystems. I particularly focused on the genetic and environmental variation of two complementary fire-adaptive (resilience) traits: serotiny of female cones to constitute an aerial seedbank and bark thickness allowing individual survival. I also intended that the research questions posed here would improve the knowledge on the adaptive genetic variation of Mediterranean pines, with special emphasis on the reproductive aspects, having a key repercussion for the sustainable management of forests under the global change. The general hypothesis of this work is that environmental limitations, particularly the scarce availability of water typical of Mediterranean environments, reduce the resilience capacity with trade-offs between and within the three fundamental groups of life-history traits (growth, reproduction, and maintenance or defense).

The specific objectives and hypotheses of this thesis ─all focused in the studied species *Pinus halepensis*─ were (see also summary in Table 2):

- To determine the effect of different sites with distinctive rainfall, temperature and soil type on serotiny, considering also the interacting effect of plant size and population differentiation *(Study I)*.

- To test the hypothesis that increased aridity might decrease the aerial seedbank, taking into account both, female reproduction and serotiny degree, and the hypothesis that the aerial seedbank would be higher in populations from growth-limiting stressful environments of origin *(Study I)*.

- To determine whether older cones open at lower temperatures and whether we could distinguish two different types of cones in relation to their physical differences *(Study II)*.

- To test the hypothesis that the internal conditions of the cones resulting from the balance of plant resources (mainly water transferred through the peduncle) may induce a faster cone opening, including competition for water between cones. We expect that modifying the internal water status of the cones, would be more critical at moderate external conditions, while at very hot and dry conditions all cones will open irrespectively of their water supply *(Study II)*.

- To determine the effect of environmental constraints and its interplay with population differentiation on the absolute bark thickness (directly related to fire survival), testing the hypothesis that the chance of survival to the same fire regime would be higher in environments with higher resource availability *(Study III)*.

- To investigate the possible different patterns of allocation to bark among populations and under differently limiting environments, expecting a lower allocation to bark under the scarcity of resources. We also expected a clear ecotypic difference among populations related climatic conditions and/or historical fire frequency records at the place of origin *(Study III)*.

- To test the hypothesis that fitness components are related to and modulated by the environment, and that correlations and trade-offs among traits agree with predictions from life-history and allocation theories in pines *(Study IV)*.

- To check whether the observed differentiation among populations for different life-history traits could be attributed to local adaptation or merely to genetic drift or historical effects by Q_{ST}-F_{ST} comparisons *(Study IV)*.

TABLE 2 | Overview of the structure of this thesis, including objectives, materials and methods, and results in form of publications and manuscripts.

	Objectives	Materials and Methods			Results
		Sampling Site	Phenotypes	Methods	
Study 1	(1) Allometry of serotiny under contrasting environments (2) Phenotypic plasticity of serotiny (3) Aerial seed bank across sites and provenances	ADE (cold) CUC (mild) VED (dry)	Fire adaptive trait: *Serotiny* Canopy Cone Bank (CCB)	• Bayesian LMM* and GLMM** • REML*** LMM and GLMM • Pearson's correlations	Martín-Sanz *et al.,* 2016. American Journal of Botany
Study 2	(1) Are there serotinous and not serotinous cones? (2) Role of age on serotinous cone opening (3) Maintenance costs of serotiny	CUC OLM GUA‡ VAL‡	Fire adaptive trait: *Serotiny*	• 2 laboratory experiments • 1 field experiment • REML LMM and GLMM • McNemar's test	Martín-Sanz *et al.,* 2017. PLoS One
Study 3	(1) Critical absolute bark thickness under contrasting environments (2) Ecotypic patterns of bark thickness (3) Allocation to bark under contrasting environments	CUC (mild) VED (dry)	Fire adaptive trait: *Bark thickness*	• REML LMM • Principal Component Analysis • Spearman's correlations	Martín-Sanz *et al.,* in prep
Study 4	(1) Ecotypic trends and trait trade-offs (2) Intraspecific genetic differentiation and phenotypic variation	CUC (mild) VED (dry)	Growth Reproduction Fire adaptive traits: *Serotiny, Bark thickness*	• Bayesian LMM and GLMM • Pearson's correlations • Principal Component Analysis	Martín-Sanz *et al*., in prep

* Linear Mixed Models ** Generalized Linear Mixed Models *** Models adjusted using Restricted Maximum Likelihood

‡ Plant stands; the other sites are part of a provenance common garden experiment

3. Materials and Methods

3.1. Study sites

3.1.1. Provenance common garden experiment

Phenotypic data used in this study originate from a *P. halepensis* provenance common garden experiment installed in Spain, belonging to the Spanish National Forest Genetic Trial Network (www.genfored.es). The main objective of this network was to determine among-population ecotypic variation and to estimate quantitative genetic parameters for adaptive traits.

Common garden experiments or forest genetic trials with replicated individual genotypes (or broadly, genetic entries) across multiple environments allow a more precise phenotyping as the amount of environmentally induced variation can be estimated and partitioned out (Ingvarsson and Street, 2011), thus enabling to estimate phenotypic plasticity and genotype x environment interaction. Although common gardens allow the assessment of genetic divergence among many populations, they do not permit testing directly the hypothesis of local adaptation for all populations, unlike reciprocal transplants experiments (Conover and Schultz, 1995; Kawecki and Ebert, 2004; Rutter and Fenster, 2007; Merilä and Hendry, 2014). However, this drawback is solved by using ecological distances such as Gower's (Rutter and Fenster, 2007).

Depending on the genetic material used to establish the common garden experiments they can be divided into provenance, progeny or clonal trials. While provenance trials allow an overview of the broad adaptive variation of a given species, they do not allow the estimation of quantitative genetic parameters that describe the genetic architecture of the phenotypic traits studied, such as heritability, in contrast to progeny and clonal trials. Combined trials like provenance-progeny or provenance-clonal trials can fulfill both objectives, but in turn they pose the problem of huge sizes.

It is very important to understand the difference between provenances and sites in this type of experiments. The provenance is the location or environment of origin of the tested genetic materials, coming from the sampled natural populations. On the other hand, the test sites are the different places where the replicated sampled populations (provenances) have been planted under a common environment.

Our sampling for the common garden was done randomly in wild populations. Population seed lots were obtained by bulking open-pollinated seeds from a subsample of 20 to 30 trees spaced at least 100 m apart from each other in each population. Then, derived seedlings were produced under identical conditions in a nursery and planted into the final common garden sites after one or two years. For each site, 16 seedlings from each population were planted in a row–column design at 2.5 × 2.5 m spacing, with four blocks and four plants per population and block, assuring identical initial tree density in all sites. Mortality occurred mainly during the first 3 years of the establishment and affected mostly the harshest environments for *P. halepensis*. We confirmed the lack of correlation among population values of survival and the phenotypic variables used in this thesis, allowing us to discard biases due to differential mortality.

The provenance common garden was set up in 1997 and replicated at six sites in eastern and central Spain. The complete trial includes 52 *P. halepensis* populations from continental Spain, Balearic Islands (Spain), France, Italy, Greece and Tunisia, thus covering most of the species' range (see Climent *et al.*, 2008 for details). We chose a subset of 19 native populations (Figure 11, and Table S10.3.1 in Supplementary Information 10.3) representing the main geographic and environmental gradients of origin, and with a good balance between them. This selection of 19 populations was made mainly for practical reasons when field works.

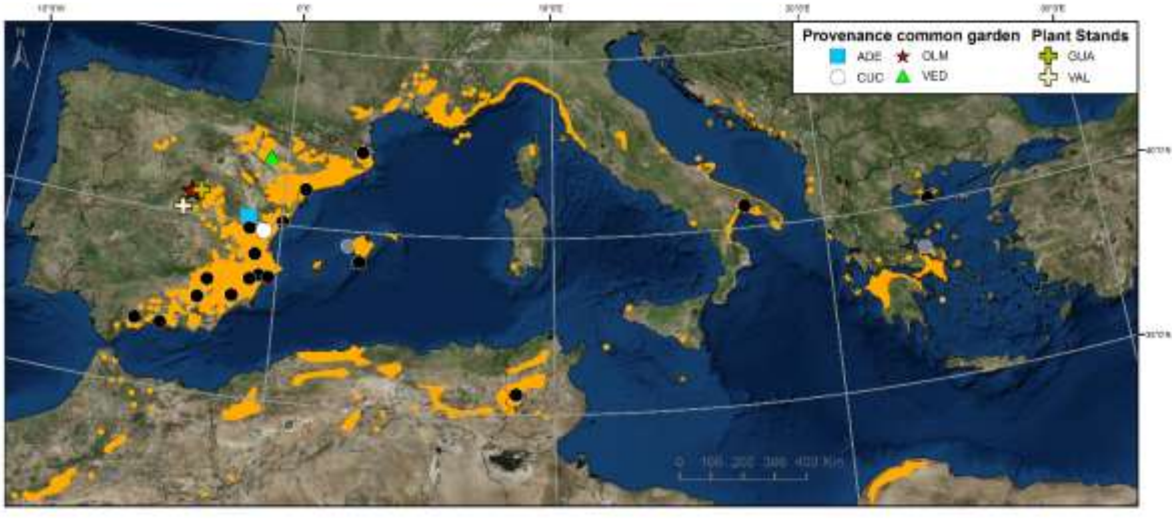

FIGURE 11 | Distribution map of *Pinus halepensis* source populations (black and grey points -grey points are two populations not used in Chapter 4-), and all sites used in this thesis. Four sites from a common garden experiment (ADE, CUC and VED were used in *Study I*, CUC and OLM in *Study II*, CUC and VED in *Studies III and IV*) and two plant stands used also in *Study II*. Orange areas indicate the species' natural distribution range.

In this thesis I used data from four out of the six originally installed sites (Figures 11 and 12; Supplementary Information 10.3, Figure S10.3.1), enabling precise and thorough evaluation of tree phenotypes for a long time (Neale and Savolainen, 2004). In addition, microenvironmental heterogeneity within each trial site produced differences in growth among trees of the same provenance, allowing us to include size effect as a proxy of ontogenetic age for the same chronological age of the trees, following the postulations by McConnaughay and Coleman (1999) and Poethig (2003).

FIGURE 12 | *Pinus halepensis* provenance common garden trials: (A) ADE located in Valencia province, (B) CUC located in Castellón province, (C) OLM situated in Madrid province and (D) VED located in Zaragoza province. All trials constitute replicated copies planted with the same provenance plant stock and following the same methods.

Three of the selected sites showed particularly contrasting environment (Table 3). The mild site with high resource availability (Altura, Castellón province, eastern Spain; hereafter CUC or high-resource site) has Mediterranean humid climate with a relatively warm winter and moderate summer drought. The other two sites have continental Mediterranean climate with low winter temperatures and high annual and daily temperature oscillation at the cold and dry site (Rincón de Ademuz, Valencia Province, eastern Spain; henceforth, ADE or cold site) and with warmer summer, more prolonged summer drought, and frequent and intense dry winds at the warm and dry site (Zuera, Zaragoza Province, northeastern Spain; henceforth, VED or low-resource site). The fourth trial site (hereafter, OLM) used in this thesis was used together with CUC site and two *P. halepensis* stands for *Study II*, aiming at detecting maintenance costs of serotiny. Beyond these climate differences, soil type is different among the sites (Table 3). Mortality occurred mainly during the first 3 years of the common garden experiment and affected mostly the cold (98 dead trees) and dry sites (52 dead trees, Supplementary Information 10.3, Table S10.3.2). We checked that we sufficiently accounted for putatively differential competition resulting from scarce mortality among sites and blocks by including tree size in the models. In addition, cone damage by squirrels or crossbills or insect pests (easily distinguishable) was also checked, being negligible among all trial sites, for studying serotiny.

We finally focused the main part of this thesis in two sites of the common garden (CUC and VED, favorable, and dry continental conditions for *P. halepensis*, respectively) due to the lack of measurements for some of the studied traits in the other trials. Due to the different climate and soil type at each of these two sites, and keeping in mind that trees were even-aged across sites –18 years, close to the species' life expectancy under short fire return intervals–, both growth and survival were much higher at CUC site compared to VED site (Martín-Sanz *et al.,* 2016). Moreover, site also affected significantly to reproduction, degree of serotiny (long-lasting closed cones in the crown), tree form or slenderness (Figure 13 and Table 4).

TABLE 3 **|** Description of the trial sites from the *Pinus halepensis* common garden experiment (P_{CG} Site) and the two stands sampled to study maintenance costs of serotiny *(Study II)*.

P_{CG} Site	**Latitude**	**Longitude**	**Altitude (m)**	**P (mm)**	**Ps (mm)**	**Psp (mm)**	**T (℃)**	**MTWM (℃)**	**MTCM (℃)**	**A (months)**	**K**	**WS (m/s)**	**WS_S (m/s)**	**Soil type**
ADE	40º6’38’’N	1º14’14’’W	844	364	94	99	13.3	22.9	5.5	2.80	0.46	4.70	3.89	Eutric Fluvisol (A)
CUC	39º49’29’’N	0º34’22’’W	605	509	79	129	14.4	22.9	7.8	2.51	0.22	5.44	3.94	Calcaric Cambisol (B)
OLM	40º38’42’’N	3º26’44’’W	731	475	62	129	13.6	24.3	4.9	3.50	0.34	5.22	4.78	Eutric Cambisol (N)
VED	41º52’22’’N	0º38’54’’W	423	402	83	117	14.1	24.1	5.5	3.14	0.45	5.60	4.90	Gypsic Xerosol (B)
Stand														
GUA	40º41’16’’N	3º6’38’’W	840	596	69	163	12.3	22.5	3.8	3.06	0.16	4.78	4.39	Calcaric Cambisol (B)
VAL	40º9’18’’N	3º41’8’’W	672	491	52	135	13.7	24.5	5.0	3.67	0.35	5.07	4.58	Calcaric Cambisol (B)

Notes: P, annual precipitation; Ps, summer precipitation (June, July and August); Psp, spring precipitation (March, April and May); T, mean annual temperature; MTWM, mean temperature of the warmest month; MTCM, mean temperature of the coldest month; A, drought duration parameter; K, drought intensity parameter (all previous variables from Gonzalo-Jiménez, 2010); WS, mean annual wind speed at 80 m above surface and WS_S, mean summer wind speed at 80 m above surface (from http://atlaseolico.idae.es/meteosim); soil type classification according to FAO (2015) guidelines, letters in parentheses indicate the soil pH: acid (A), basic (B) or neutral (N).

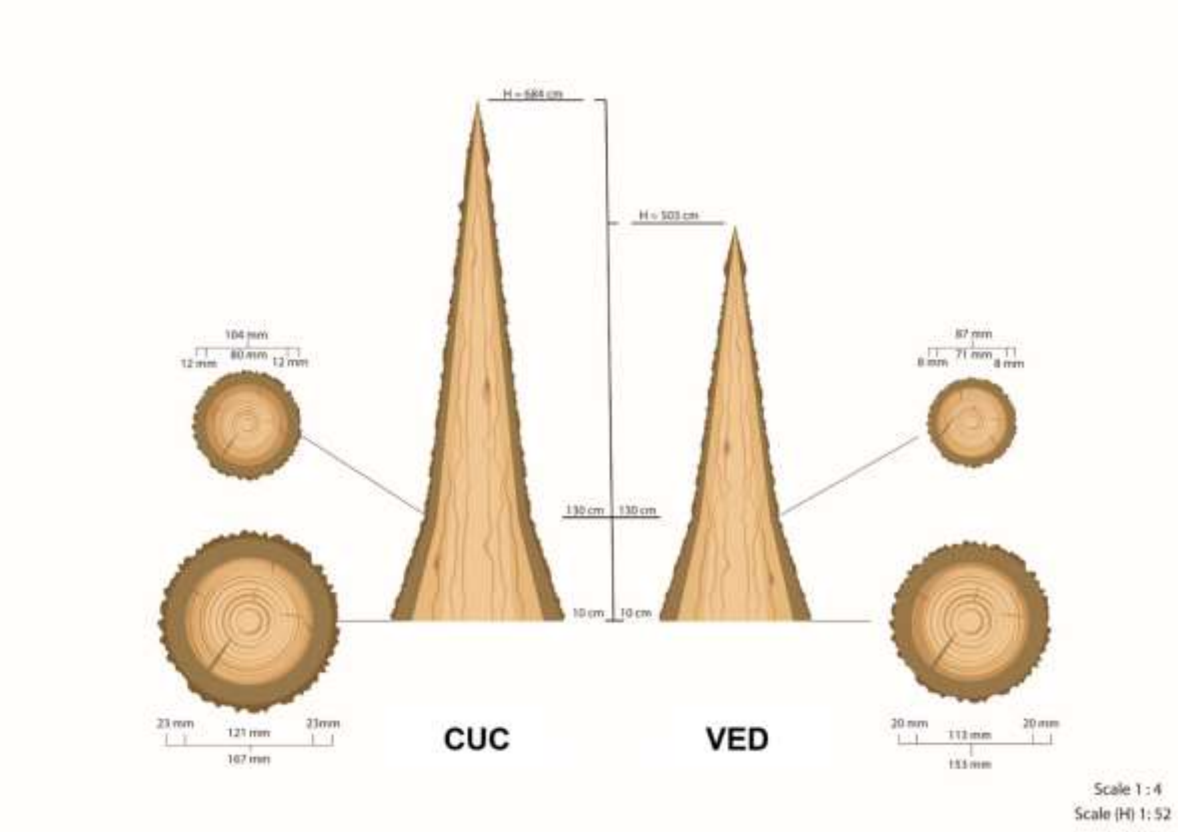

FIGURE 13 | Scheme of the average tree at each of the test sites. H is the total height of the tree. Diameters and bark thicknesses of the circular cross-sections were measured at 10 and at 130 cm from the ground. This scheme does not represent the real decrease in diameter and bark thickness along the trunk above 130 cm due to the lack of measurements at higher heights. This decline is neither constant nor homogeneous.

TABLE 4 | Mean values of different growth variables at both test sites. H: total tree height; D10: diameter at tree base (at 10 cm); D130: diameter at breast height (at 130 cm); BT10: bark thickness at tree base; BT130: bark thickness at breast height; Survival: percentage of tree survival; RA: reproductive allocation (10*number of female cones/tree height); HST: percentage of highly serotinous trees (those with > 80% of cones closed).

Site	**H (cm)**	**D10 (mm)**	**D130 (mm)**	**BT10 (mm)**	**BT130 (mm)**	**Survival (%)**	**RA**	**HST (%)**
CUC	684	167	104	23	12	95	0.4	57
VED	503	153	87	21	8	86	0.7	43

Notes: First four variables from year 2015. Last three variables from year 2012 and extracted from Martín-Sanz *et al.* (2016). All variables were significantly different between sites: $P < 0.0001$.

3.1.2. Other Pinus halepensis stands

The main objective of *Study II* was determining the maintenance costs of serotiny in *P. halepensis.* To do this, we established one manipulative field experiment at two sites of the already described common garden (CUC and OLM) and did two laboratory experiments, for which we collected serotinous cones from young trees (aged < 20 yr) in two different planted stands in Central Spain (Guadalajara -GUA- and Madrid -VAL-, Figure 11 and Table 3). These two sites are public lands not protected in any way. For the type of sampling performed no special permit was required, although we had verbal permission from forest managers anyway. The field study did not involve protected or endangered species. All cones were initially stored in a cold chamber with controlled temperature. We randomly selected 15 trees in each stand to assess tree age, by ring-counting in cores extracted at the tree base (height 0.1 m) with an increment borer (Figure 14). The two stands are even aged, estimation of age in 15 trees provides a reliable estimation of tree age at the stand level.

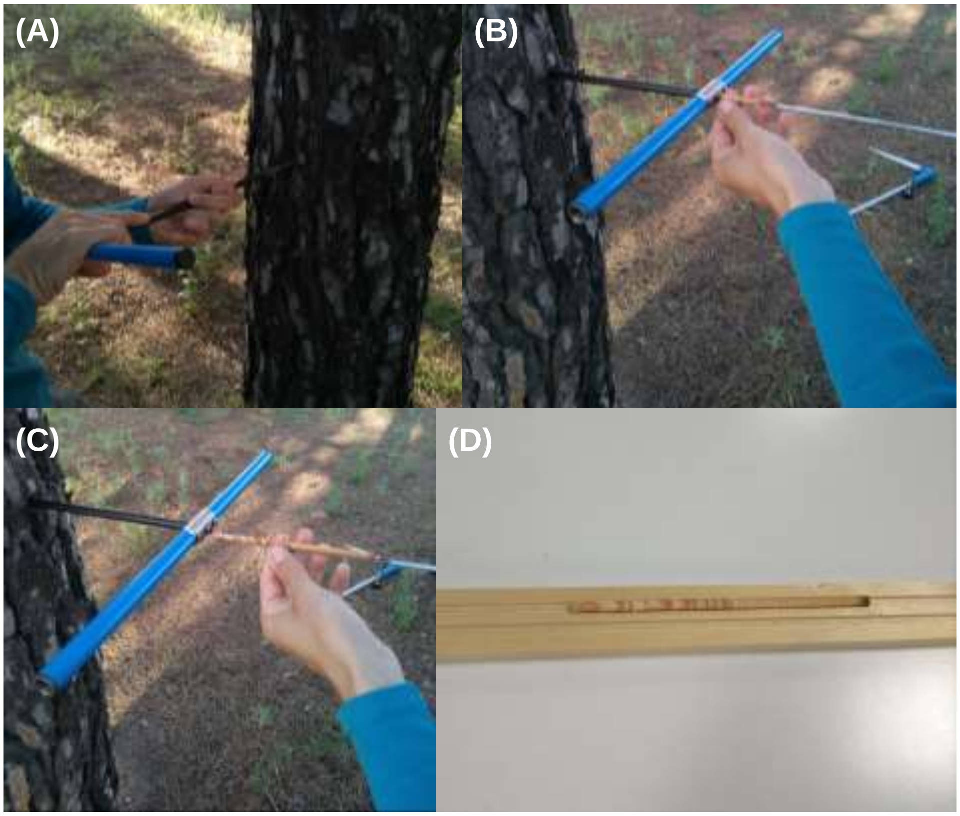

FIGURE 14 | Core extraction process to assess tree age by ring-counting in the plantations. (A) Pressler bit (increment borer), (B) detail of core extraction, (C) complete extraction of the core and (D) core placed for ring-counting in the laboratory. Photos by ©R.C. Martín-Sanz.

3.2. Studied phenotypic traits

All common garden sites were measured periodically since their establishment so that wide data series were already available prior to this work. In addition, I carried out two intense field campaigns in winter and spring 2013 and 2015 to evaluate new variables not previously measured. Recorded variables were related to vegetative growth, reproduction and adaptations to fire (Table 5).

TABLE 5 | Summary of reported *Pinus halepensis* traits recorded in three contrasted sites of the common garden experiment.

Trait	Trait description	Study	Tree age (years)	Year of measurement	Trial site
Aff	Age at first female flowering	IV	-	-	CUC
Hff	Height at first female flowering	IV			
QMR	Qualitative male reproduction *(qualitative variable)*	IV	13	2010	CUC
DBH10	Diameter at breast height	IV			
H	Total height	I, IV	15 / 16*	2013	ADE CUC VED
DBH12	Diameter at breast height	I, IV			
TFR	Total female reproduction	I, II, IV			
TMR	Total male reproduction**	IV			
SER	Degree of serotiny	I, II, IV			
CCB	Canopy cone bank	I, IV			
TSSL	Threshold size for serotiny loss *(derived variable)*	I			
fRA	Female reproductive allocation *(derived variable)***	IV			
mRA	Male reproductive allocation *(derived variable)***	IV			
SERa	Serotiny degree without allometric effects *(derived variable)***	IV			
CCBa	Canopy cone bank without allometric effects *(derived variable)***	IV			
H/DBH	Height-diameter allometry *(derived variable)***	IV			
DBH130	Diameter at breast height	III	17 / 18*	2015	CUC VED
DB10	Diameter at the tree base	III			
BT130	Bark thickness at breast height	III, IV			
BT10	Bark thickness at the tree base	III, IV			

*Tree age for growth traits, as well as for bark thickness, is the lowest (15 and 17 yrs, respectively) because these variables were measured before starting the growth period (in winter 2013 or 2015). ** These variables were only measured or derived for CUC and VED sites.

3.2.1. *Growth traits*

We measured total tree height at tree age 15 with a telescopic pole to the nearest cm (Figure 15A-B). Diameter at breast height was also measured with a caliper to the nearest mm (Figure 15C-D). This diameter was measured when trees were 15 and 17 years old during the two field work campaigns. During the second field campaign carried out in 2015, stem diameter at the tree base (10 cm above the ground) was also measured.

Height at age 15 was used as the main size variable at the three common garden sites used to disentangle plasticity of serotiny *(Study I)*. We preferred height as the best single proxy of tree size (hence ontogenetic stage) compared with breast height diameter, due to the high experimental error of girth measurement at this age in this species. Nevertheless, individual diameter and height were indeed highly correlated (Pearson r = 0.87, *P* = 0.0001).

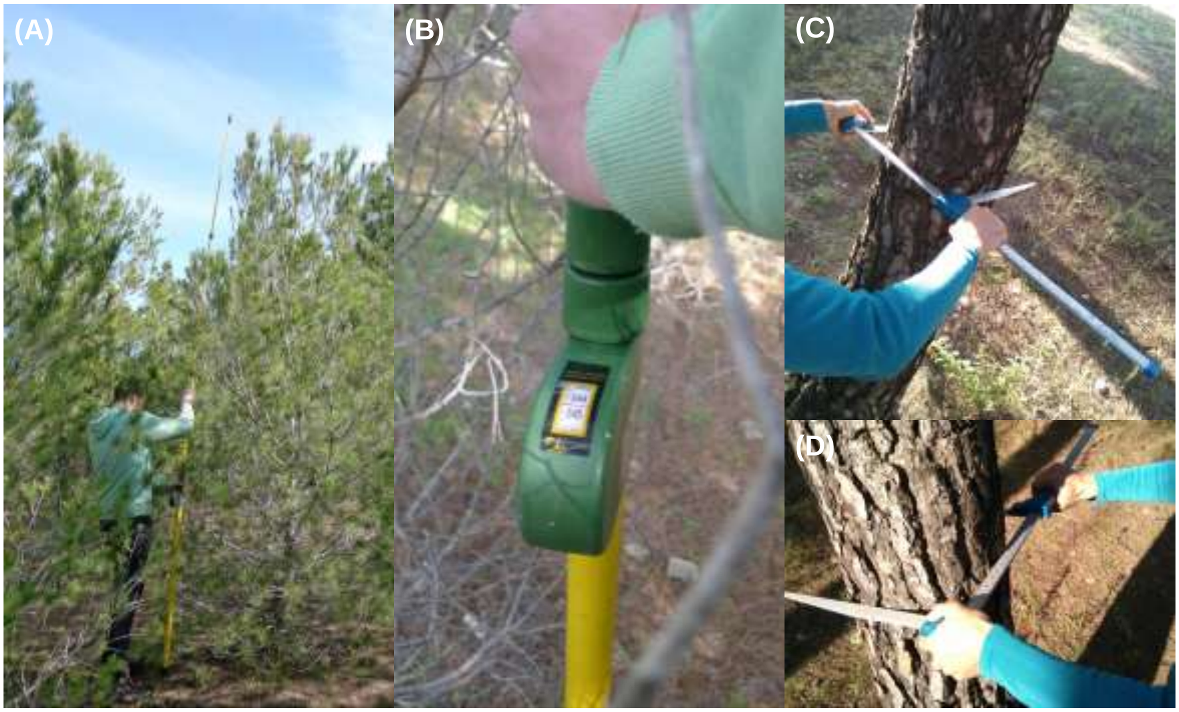

FIGURE 15 | Measuring total height and diameter of a *Pinus halepensis* tree. (A) Telescopic pole used for measuring total height and (B) detailed of the telescopic pole (© Eduardo Notivol). (C-D) Caliper used for measuring diameter (© R.C. Martín-Sanz).

3.2.2. Reproductive traits

Importantly, in *P. halepensis*, all female cones remain attached to the branches after ripening, either open or close. This characteristic makes *P. halepensis* a good model species for studying serotiny degree. Differences in size and colour allowed discrimination of several cohorts within tree crowns (Figure 16), thereby enabling a retrospective record of female reproduction (Ne'eman *et al.*, 2011). Female strobili are formed during spring and are red and small sized (~1 cm long). By the end of the first season their colour turns green and their size increases (2-3 cm long). During the second season they achieve their final size (~10 cm long) remaining green. Then gradually change their colour into bright brown and from the beginning of the third season they turn into pale grey (Gil *et al.*, 2009). We assessed the total number of female cones by retrospectively counting cone cohorts from age 16 (year 2013) back to the very first reproductive events. This was possible given the relatively small size of the trees in the common gardens.

FIGURE 16 **|** *Pinus halepensis* female cone developmental stages and male cone clusters. (A) Female strobili emerged in spring, (B) one-year old female conelets, (C) two-year old female cone, (D) serotinous cone and (E) immature male cone clusters. (Photographs A and E by ©Eduardo Notivol, photos B, C and D by © R.C. Martín-Sanz).

Male pollen cone clusters per tree (Figure 16E) when trees were 16 years old were approximated from counts during 15 seconds. This method has been previously used for assessing reproductive traits in different species (Knops and Koenig, 2012; Santos-del-Blanco *et al.,* 2014; Rodriguez-Quilón, 2017). It was chosen after tentative counts by different observers, aiming at combining a reasonable correlation with full male pollen cone clusters and a reasonable speed needed for measuring hundreds of trees with affordable field work schedules. Due to the great competition among trees at CUC site caused by the higher growth in this site, we considered current male reproduction biased due to shading of lower branches. Therefore, we used qualitative data taken in 2010, in which male reproduction was classified in a 1-4 scale.

3.2.3. Fire-adaptive traits

We assessed the proportion of closed cones (those grey coloured remaining closed after full maturation) with respect to the total fully ripe cones (open and closed) for each tree for an estimation of their degree of serotiny in 2013. We discarded the last two cone cohorts (easily distinguishable by their brown colour) since they did not have the chance to open naturally as cones over 2 years old do. The total number of closed cones per tree at age 16 was used as an estimation of the canopy cone bank (CCB), a variable related to the persistence ability of *P. halepensis*.

Bark thickness both at the tree base and at breast height (10 and 130 cm above the ground, respectively) were measured using a standard bark gauge (Figure 17). Since we were obliged to preserve the common garden we discarded more precise, but invasive or destructive bark measuring methods like extraction of bark portions (Jackson *et al.,* 1999; Paine *et al.,* 2010; Graves *et al.,* 2014) or cutting stem discs to remove the bark (Rosell *et al.,* 2014, 2015; Rosell, 2016). Therefore, by bark thickness (basal: BT10 or at breast height: BT130) we refer to total (inner and outer; more information can be found in the respective chapter) bark. We performed a preliminary sampling of a few trees at each test site measuring bark thickness at three points surrounding the bole. We found that measuring bark thickness at a single point was accurate enough given the uniformity of *P. halepensis* bark at this age. Thus, we measured bark at a single point of the bole, always in the south orientation.

FIGURE 17 **|** (A) Photograph of a standard bark gauge, (B) measuring bark thickness with the bark gauge and (C) standard bark gauge indicating the thickness of the measured bark. Photos by ©R.C. Martín-Sanz.

3.2.4. Derived variables

The threshold size for serotiny loss (TSSL) as the size at which the degree of serotiny becomes 50%, was estimated from logistic allometric models relating degree of serotiny to tree height (Wesselingh *et al.,* 1997; Méndez and Karlsson, 2004; Santos-del-Blanco *et al.,* 2010; *Study I*). As other derived variables, height-diameter allometry (H/DBH) and, female (fRA) and male reproductive allocation (mRA) were used in *Study IV*. Finally, individual age (Aff) and height (Hff) at first female flowering (recall that this species starts its reproductive phase as female, Santos-del-Blanco *et al.,* 2010) were obtained from annual cone counts and periodic height measurements following Climent *et al.* (2008) and were also used in *Study IV*.

3.3. Climatic and fire data

Environmental conditions from common garden sites were obtained from the climatic models by Gonzalo-Jiménez (2010), whereas environmental variables from the areas where sampled populations had evolved (populations' origin) were obtained from WorldClim v.1.4 (1 Km grid; Hijmans *et al*., 2005). We used both models because Gonzalo-Jiménez's model provides better estimates than others mostly due to the use of a denser data network, but it is only available for the Iberian Peninsula. From the nineteen original bioclimatic variables for populations' origin, we considered nine variables: annual precipitation, precipitation during the driest quarter (June, July and August), precipitation during the warmest quarter (March, April and May), precipitation during the wettest quarter (September, October and November), precipitation of the driest month, annual mean temperature, mean temperature of the warmest month, mean temperature of the coldest month, and a continental index (difference between mean temperature of the warmest month and mean temperature of the coldest month). Three spatial variables were also recorded for each population: longitude, latitude and altitude. We carried out a principal component analysis (PCA) with *varimax* rotation to reduce the number of climatic variables and allow a more synthetic interpretation. We decided the number of principal components to retain running a parallel analysis with 1000 iterations (Hayton *et al.,* 2004) and selecting those principal components with eigenvalues for observed data larger than those from simulations. The analysis was performed with psych package (Revelle, 2017) on the R software v3.3.2. (R Core Team, 2016). PCA loadings of retained components and variables with loadings above 0.80 were selected and used in subsequent analysis in different chapters of this thesis (see Supplementary Information 10.1. for the results of this analysis).

Natural fires frequency data (lightning fires, of relevance in the east part of Spain; Vázquez and Moreno, 1998) were defined as the number of fires in 90,000 ha of surface covered by *P. halepensis* forests each year during the period 1974-2010. A 10 x 10 km grid-unit was used to derive this fire frequency data for the 13 Iberian populations of *P. halepensis* used in *Studies III* and *IV*. Reliable fire information for the non-peninsular populations was lacking.

3.4. Data analysis and experiments

Diverse statistical methods using different packages implemented in R software version 3.2.3. (R Core Team, 2016) have been used in this thesis to perform data analysis.

3.4.1. Variation of serotiny degree and aerial seedbank among sites and populations (Study I)

3.4.1.1. Site effect on tree growth, survival and reproduction

The effect of site on tree height, survival, and reproduction (both as total number of female cones and as the ratio between cones and tree size as a proxy to allocation to reproduction; Barot *et al.,* 2005; Santos-del-Blanco *et al.,* 2013) were tested with linear (height) and generalized linear mixed models (survival, binomial, and reproduction, Poisson). Population was defined as random in each model.

3.4.1.2. Plastic, allometric and genetic effects on cone serotiny and canopy cone bank

When accounting for the allometry of serotiny in a partially serotinous species like *P. halepensis*, we must be aware of potentially confounding factors. First, the older the cone, the greater the probability to suffer weathering and be exposed to thermal extremes causing xeriscence. Second, the onset of female reproduction in *P. halepensis* varies among sites and populations from 3 to 10 yr of age or more (Santos-del-Blanco *et al.*, 2013). Precocious cone-bearing occurs in certain populations and in harsh sites; but, as already mentioned, an older serotinous cone is more likely to open than a younger one. To check the relevance of this effect in the variation of serotiny, we included the ratio between the number of cones older than 4 yr and the total number of cones at the last observation (16 yr).

Generalized linear mixed models (logit link function) were fitted to binomial data reflecting the number of open and closed cones at the individual tree level. Environmental effects were represented by trial site and allometric effects by tree size. To avoid confounding the allometric effects with other factors, we also included the total number of female cones and a proxy for cone age (see above). Moreover, we added a term for the interaction between tree size and trial site, reflecting potential allometric plasticity of serotiny. To avoid confounding genetic effects and make our results applicable to the species level, we included population × site interaction as a random term. We also fitted independent allometric models for serotiny at each individual site, i.e., serotiny as a function of tree size. In these models, population was also considered as random. We defined the threshold size for serotiny loss (TSSL) as the size at which the degree of serotiny becomes 50%, computed by dividing intercept by slope estimates (Wesselingh *et al.,* 1997; Méndez and Karlsson, 2004; Santos-del-Blanco *et al.,* 2010).

These models were run for 110,000 iterations, sampling from one out of 100 chains after discarding the first 10,000, thus generating 1000 posterior samples. We used weakly informative inverse Wishart priors for variance components and error terms. These settings were suitable to achieve good convergence and low autocorrelation (<0.1) for model parameters and derived estimates, i.e., TSSL. Posterior distributions are summarized by their mode followed by 95% credible intervals in brackets. Parameter estimates, or their differences, were considered statistically significant when their 95% credible intervals did not include zero. As estimates for variance components are bound to positive values, they cannot include the value zero. Instead, to estimate whether a variance component was significantly greater than zero, we assessed its probability distribution. We also estimated the percentage of explained variance, conditional on the fixed effects, by dividing variance components (population or population × site interaction) into total variance, which also included the error and logit link implicit variance ($\pi^2/3$). To check the effect of genotype × environment interaction on serotiny, once the allometric effects were accounted for, we compared the DIC (deviance information criterion) of a model containing population × site as a random term with another one containing population only. The significance of the fixed factors in these models was determined by an omnibus test (Mittell *et al.,* 2015; Foo *et al.,* 2016).

A brief interpretation of model parameters is as follows: a significant population effect indicates intraspecific genetic variability; significant differences between sites reflect phenotypic plasticity and significant site × population interaction indicates genetic variation for plasticity among populations. Besides, a significant effect of tree size reflects allometric effects and a significant site × tree size interaction reflects the existence of allometric plasticity for cone serotiny. Finally, a significant effect of the total number of female cones or the proxy for cone age allowed us to avoid confounding allometric effects with other factors. Thus, a higher total number of female cones and/or higher proxy for cone age corresponded to lower degree of serotiny.

Population (genotype), site (environment), and their interaction effects on the CCB (square-root transformed to improve residual distribution) were tested with linear mixed models using packages lme4 (Bates *et al.,* 2015) and lmerTest (Kuznetsova *et al.,* 2016) to get *P* values, defining block within site as a random effect.

Looking for ecotypic trends in CCB among-population variation, we checked the correlations between serotiny or CCB with summer precipitation and temperature oscillation (as a proxy of continentally). These two climatic variables have been identified as the most relevant climatic factors explaining the phenotypic variation of this species in previous works (Climent *et al.,* 2008; Voltas *et al.,* 2008).

All reported models and tests in this first study were performed using package MCMCglmm (Hadfield, 2010) for serotiny analysis, and lme4 (Bates *et al.,* 2015) for CCB analysis and preliminary analysis on height, survival and reproduction.

3.4.2. *Maintenance costs of serotiny (Study II)*

The second study of this thesis aimed to investigate the possible maintenance costs of serotiny in *P. halepensis*. For this, three experiments were carried out with different sampled material, protocols and objectives. Firstly, we needed to verify that peduncles of *P. halepensis* cones allowed physiological exchanges between the tree and the cones, that is, showing xylem with sapwood and living cortical tissues. Since smaller/younger trees are more serotinous (Tapias *et al.,* 2001; Martín-Sanz *et al.,* 2016), young individuals combine a high availability of serotinous cones and an easier access from the ground with just the help of standard telescopic pruning scissors.

3.4.2.1. Cone-opening laboratory screening experiment

– Plant material and protocol –

The aim of this experiment was to determine the effect of cone age on cone opening (Tapias *et al.,* 2001) and to investigate the existence of two different types of cones –serotinous and non-serotinous– which could be related to physical differences (Moya *et al.,* 2008; Salvatore *et al.,* 2010). We collected serotinous cones of *P. halepensis* from young trees (aged < 20 yr) in two planted stands in Central Spain (GUA and VAL; Figure 11 and Table 6) previously described (Table 3).

TABLE 6 **|** Summary of the samplings made at each site.

Plant stand	**Tree age (years)**	**Year^1**	**Year^2**	**Sampling type**	**Nº of samplings**	**Experiment**
GUA	15	-	-	Cone pairs	30	Manipulating water availability *ex situ*
VAL	17	-	-	Individual cones	161	Laboratory screening
Common garden						
CUC	17	14	15	Cone pairs	69	Manipulating tree to cone physical connection *in situ*
OLM	17	14	16	Cone pairs	87	

Notes: Year^1 is the year when the experiment was established. Year^2 is when the experiment finished.

First, we determined the age of each cone by wood-ring counting of the insertion branch disc (Tapias *et al.*, 2001; Figure 18). Then, we introduced individual cones in a chamber where both temperature and humidity were controlled. Temperature was increased from 36 to 60 ℃ while relative humidity was gradually decreased from 25 to 4% (see Supplementary Information 10.3., Table S10.3.3). Due to chamber space limitation, we performed this process seven times with 24 cones in each test cycle. Cone opening was recorded using a webcam in time-lapse format. The videos were visualized to obtain the opening time of each cone, thus enabling to retrieve values of temperature and humidity at the time of cone opening (i.e. combination of temperature and humidity that triggered cone opening). Determining cone weight at the time of their opening would have provided information on the possible loss of water prior to cone opening. However, to maintain the controlled conditions in the chamber, we discarded obtaining these data. After the experiment, we also calculated the accumulated temperature as the heat sum suffered by each cone inside the chamber until opening.

FIGURE 18 **|** Determination of cone age by counting wood rings at the branch section just below of the cone insertion (Tapias *et al.*, 2001). Photo by ©M. Callejas.

We further obtained the dry weight of five scales from the central part of each cone after oven-drying during 24 hours at 60 ℃ We additionally calculated scales volume by measuring the hydrostatic thrust of the submerged scales in a water vase (Figure 19) and finally estimated the scales density dividing scales volume by dry weight. Moisture content of each cone after the experiment was calculated by comparing its weight before and after the experiment by using Equation 1:

$$H = \frac{BW - AW}{AW} * 100 \tag{1}$$

where BW is the weight of the cones before the experiment and AW is their weight after the experiment. We measured cone weight after the experiment immediately after removing the cones from the chamber to prevent moisture changes.

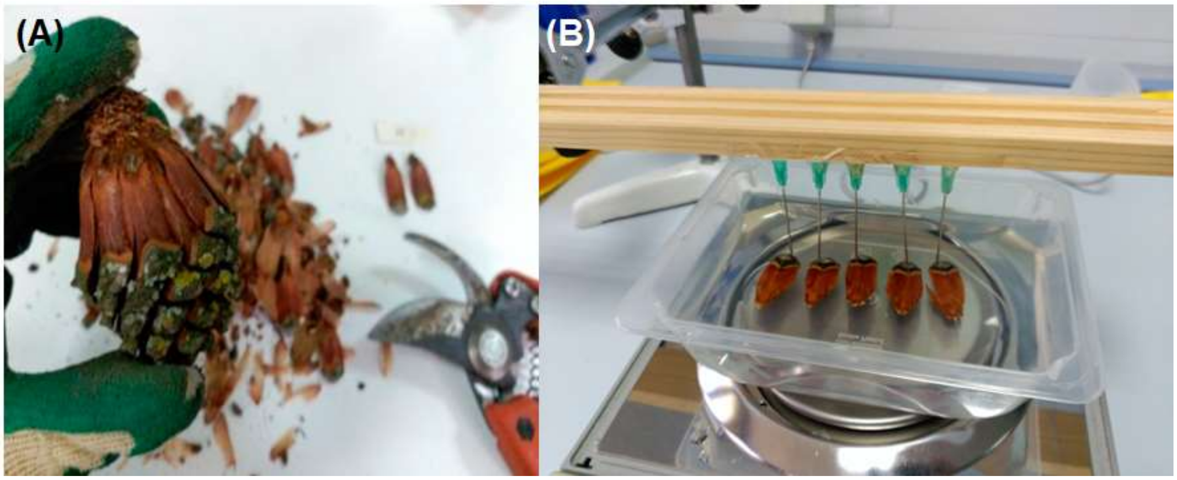

FIGURE 19 **|** (A) Obtaining central scales of a *Pinus halepensis* cone and (B) measuring the hydrostatic thrust of the submerged scales in water to calculate their volume (©M. Callejas).

– Data analysis –

Based on individual cones, we used Pearson's correlations to investigate the relationship among opening temperature of the cones and the other experimental variables (i.e. age of the cones, scales density and cone moisture after the experiment). Subsequently, we analyzed the effect of these variables on the opening accumulated temperature of the cones using linear mixed models (LMM) with test cycle as a random factor. Due to the results from this preliminary experiment, indicating the absence of two different types of cones in *P. halepensis* (serotinous and non-serotinous) and the significant effect of cone age, we decided to follow our investigation by comparing paired cones from the same whorl, i.e. two cones of the same age from the same branch (Figure 20A).

FIGURE 20 **|** Description of the *in situ* manipulative experiment. (A) Pair of closed serotinous cones of the same whorl of *P. halepensis*. The thick and long peduncles characteristic of this species are easily distinguishable. (B) ‘Closed’: both branched and detached cones of the pair remained closed; (C) ‘Positive’: detached cone opened earlier than its branched pair; (D) ‘Negative’: detached cone opened later than its branched pair; (E) ‘Opened’: both cones of the pair opened between observations. b, branched cone -control-; d, detached cone. Photos by ©R.C. Martín-Sanz.

3.4.2.2. Manipulating water availability of serotinous cones *ex situ*

– Plant material and protocol –

In this experiment, we sought to investigate the effect of water supply through cone peduncle on cone opening. We collected pairs of serotinous cones from the same whorl in the same two planted stands described in the first experiment (Tables 3 and 6). All cones were initially stored in a cold chamber with controlled temperature before the experiment. This second laboratory experiment was carried out in the controlled chamber previously described, accounting for temperature and relative humidity while providing or not water supply to the cones. The rationale behind is that the effect of water supply might be only detectable at intermediate temperatures, while at higher temperatures (ca. 60 °C) the effect of enhanced water supply would not be able to prevent cone dehiscence. Both cones of the pair were subjected to a controlled cycle of increasing temperature (from 24 to 60 °C) and decreasing relative humidity (from 40 to 4%) inside the chamber (Supplementary Information 10.3., Table S10.3.4). Cone opening was video-monitored with the same method used in the first experiment. We supplied external water to one cone of the pair (watered cones) while keeping the other cone dry (waterless cones). Water supply was provided through a thermoretractable sleeve with a rope inside, everything sealed within a Styrofoam box except the cones (Figures 21 and 22). This way we prevented both water evaporation and heating during the experiment. Cones were randomly distributed in the box. We also calculated the moisture content following Equation 1 and the accumulated temperature of each cone in this experiment as we did in the laboratory screening experiment.

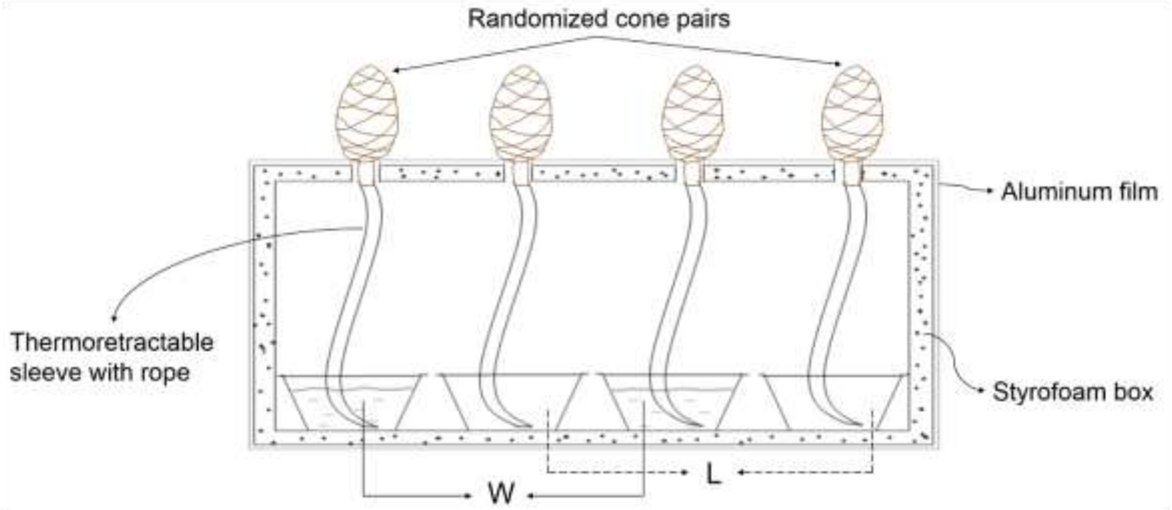

FIGURE 21 | Manipulating water availability in the ex situ experiment. Scheme of the experiment inside the Styrofoam box. W, watered cones; L, waterless cones.

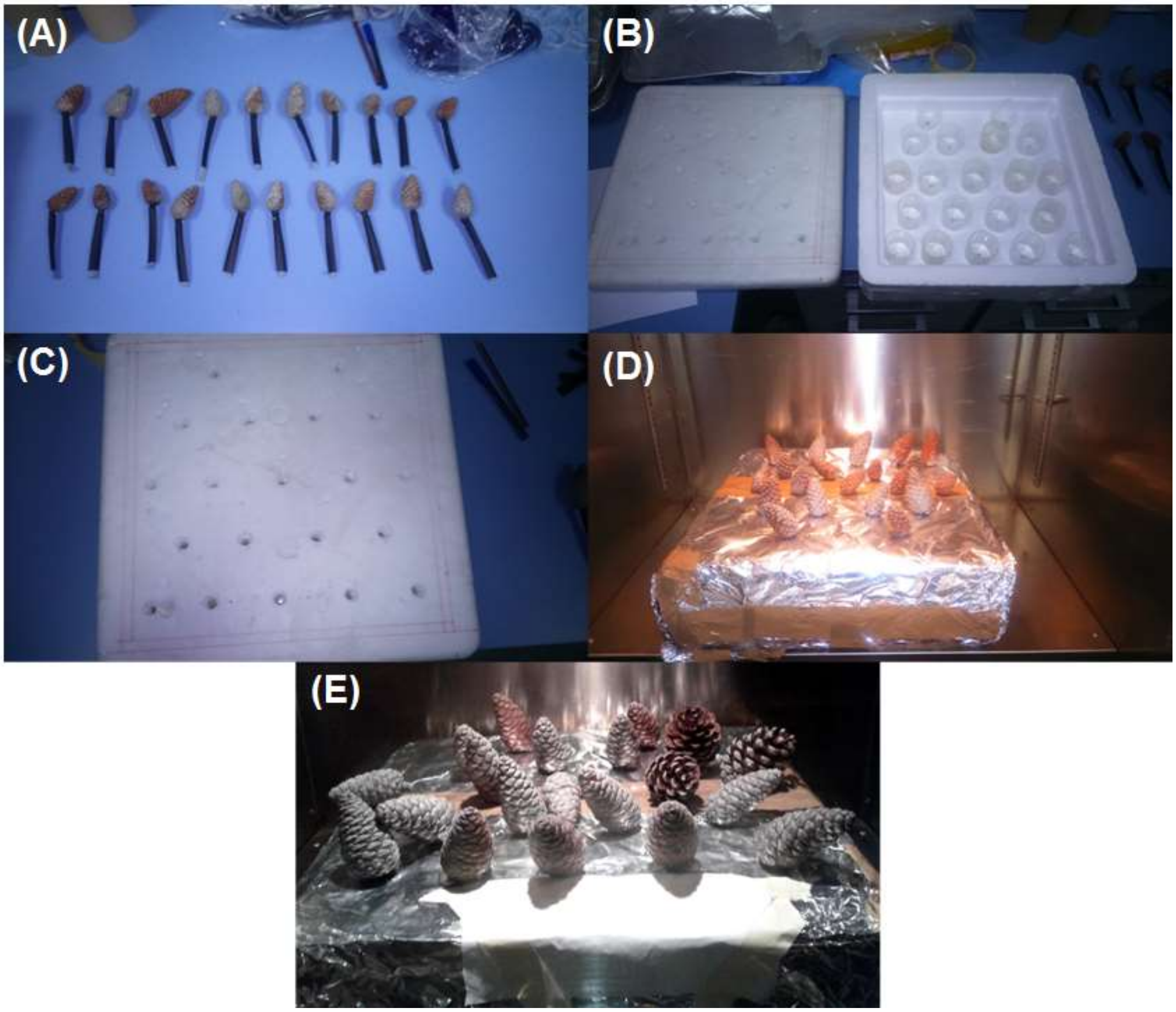

FIGURE 22 | Manipulating water availability ex situ experiment. (A) Preparation of cones with a thermoretractable sleeve and a rope inside. (B) Styrofoam box with vessels. (C) Closed Styrofoam box with holes for the thermoretractable sleeves. (D) Styrofoam box with closed cones recently placed inside the controlled conditions chamber. An aluminum film cover was used to further increase isolation and prevent water heating. (E) Photograph of the experiment in progress. There are few cones already open and the others are still closed. Photos by ©R.C. Martín-Sanz.

– Data analysis –

We used binomial generalized linear mixed models (GLMM) for analyzing the treatment effect between watered and waterless cones of each pair on their probability to open. We included treatment and accumulated temperature for cone opening as fixed factors, and cone within pair as random factor. In this experiment all the cones eventually opened, thus the binary cone opening variable was coded as follows: cones which opened before their pair and cones which opened later. Finally, to check whether the waterless cones lost or not more water than the watered cones at the end of the ex situ experiment, moisture content of the cones after the experiment was also evaluated using linear mixed models (LMM) with treatment as fixed factor, and pair and test cycle as random factors.

3.4.2.3. Manipulating tree to cone physical connection *in situ*

– Plant material and protocol –

This field manipulative experiment was carried out in February and April 2014, when trees were 17 years old, at two trial sites (CUC and OLM) of the common garden experiment previously described. As previously explained, all sites are composed of trees coming from the same natural populations of origin (provenances). Other sites characterized by harsher climatic conditions, and therefore potentially limiting growth and survival of this species were excluded due to a lower availability of serotinous cones. Cone serotiny is plastic in such a way that serotiny is much lower at harsher sites (Martín-Sanz *et al.,* 2016). We considered the two sites as replicates of the experiment rather than contrasting environments to reveal putative plasticity in the process of cone opening. They also were accessible and controlled, facilitating a long-lasting manipulative experiment. To account for variation among individuals, we selected highly reproductive and highly serotinous trees, marking three branches per tree with a pair of serotinous cones at each branch (Figure 20A). In total, we selected 84 pairs of serotinous cones from 29 trees at OLM site and 51 pairs of serotinous cones from 21 trees at CUC site. For each pair, one of the cones was excised by the peduncle as close to the branch as possible and placed in a wire basket hanged at the same place on the branch (detached cones, hereafter). Its 'twin' cone was kept untouched still physically connected to the branch (branched cones, hereafter control; Figure 20). Therefore, both cones experienced exactly the same external conditions, allowing us to discard this effect in our results. To avoid water loss, both cuts at the branch and the peduncle of the removed cone were immediately stoppered with pruning mastic (Lac Balsam, Compo S.A.). We monitored the experiment by recording each cone status (open or close) every few months during the first year, starting the tenth week after its establishment. The following years, we limited ourselves to one observation before summer and one after it, since we expected summer to be the period driving the greater variations in cone opening. We performed more frequent observations and for a longer period at OLM site, where the experiment was ended in September 2016 (lasting 125 weeks), whereas at CUC site we terminated the experiment in April 2015 (lasting 60 weeks).

The need for a minimum number of serotinous cones per tree obliged a non-random selection of the provenances, although individual tree selection was otherwise random. Due to this different representation of the provenances between sites (only eight provenances were common to both of them), accounting for this factor strongly restricted our analysis. Therefore, in order to include the genetic effect in our study, we did a global analysis with both site together and another with just OLM site, where we had more observations and a better representation of provenances. We created a new categorical variable that we called ‘group of provenances’ for using data from CUC and OLM sites together. This new variable grouped populations with similar behavior in reproduction, serotiny and growth in two highly contrasted groups (Northeast and Southwest). Southwest group comprises highly serotinous populations with a marked reproductive precocity and allocation, while populations of the Northeast group have lower serotiny and reproductive allocation (Climent et al., 2008; Santos-del-Blanco et al., 2010). This division is coherent with the weak geographical structure so far shown based in neutral markers (Ruiz-Daniels et al., in prep.). Thus, we firstly used the complete dataset for the two sites with the factor ‘group of provenances’ and secondly, we restricted ourselves to the trees that belonged to the common provenances at both sites using in this case the factor ‘provenances’. Our results were robust with these two options. However, due to a lower amount of data when using the eight common provenances, the models showed convergence problems so we finally decided to use the complete dataset. In addition, we also studied the genetic effects among populations using just the site with more prolonged observations −OLM site−. In this case, we also measured basal diameter and the number of serotinous cones counted in 15 seconds (Santos-del-Blanco et al., 2010) for each of the trees to account for allometric effects and putative competition for resources (Espelta et al., 2011).

– Data analysis –

In order to investigate the treatment effect (cone detached or not) on the probability of cone opening at both sites −CUC and OLM−, we used binomial generalized linear mixed models (GLMM) while accounting for data structure as paired serotinous cones. We used treatment, group of provenances, site and their interactions as fixed factors and individual tree as a random factor. To explore the treatment effect on the probability of cone opening at OLM site, we used binomial generalized linear models (GLM) with treatment, provenance and their interaction as fixed factors. We also included basal diameter and the number of serotinous cones as covariates. However, neither of these two variables displayed significant effects on cone opening; therefore both covariates were eliminated from the final models. Finally, we performed a non-parametric McNemar's test considering cone pairs as twin samples to evaluate the evolution of the treatment effect on cone opening at OLM site. This non-parametric statistical test is used to compare paired proportions or discordance of two dichotomous responses when pairs are independent (Altman, 1991). In our case, we compared the frequencies of four possible cases (Figure 20): “Close”, both cones of the pair remained closed; “Positive”, the detached cone opened before its branched pair; “Negative”, the branched cone opened earlier; “Open”, both cones opened between observations.

All the mixed models in this work were implemented in R using the lme4 package (Bates *et al*., 2015). We evaluated fixed terms significance of linear and generalized linear mixed models using means of likelihood ratio test (LRT) between the full model and reduced models without each variable, showing in the results section the chi-square, degrees of freedom and *P*-value for those likelihood ratio tests. To select the most parsimonious model for each experiment, we compared the Akaike's Information Criterion (AIC) of models with the most appropriate variables as random factors, as well as with a non-randomized model. We also ranked GLM models using AIC, selecting the model with the lowest value.

3.4.3. Bark absolute thickness and bark allocation variation among sites and populations (Study III)

3.4.3.1. Plastic and genetic effects on bark thickness at breast and basal height

We analyzed the effects of population (genetic effect), experimental site (environment) and their interaction on absolute bark thickness at breast height and at tree base with linear mixed models (LMM), including block within site as random factor. We compared the population per site variation of absolute bark thickness with critical thickness values –above which the cambium damage decreases considerably, therefore indicating a threshold for tree survival– both at breast height and at tree base. Due to the lack of specific data of critical bark thickness for *P. halepensis*, we used data published for other pine species: a high critical thickness of 20 mm, that would allow survival to moderate fires (Dehane *et al.,* 2015; Madrigal *et al.,* 2017) and a low critical thickness of 10 mm that would allow survival only to low-intensity fires (van Mantgem and Schwartz, 2003; VanderWeide and Hartnett, 2011).

With the mean bark thickness values per population and site extracted from the previous general linear models, we calculated the critical time for cambium kill (τ_c130 and τ_c10 –critical times at breast height and at tree base, respectively–) to compare the insulating ability of bark across populations and sites. We used the equation for one-dimensional heat flow through bark developed for conifers by Peterson and Ryan (1986), which assumed realistic values for lethal temperature of cambium (T_c = 60ºC), ambient temperature (T_0 = 20ºC), flame temperature (T_f = 500ºC) and bark thermal diffusivity (α = 0.060 cm^2/min):

$$\tau_c = 2.9x^2 \tag{2}$$

where τ_c is the time (in min) of fire exposure at 500ºC that would result in cambium death and x is bark thickness (cm).

3.4.3.2. Relationship of bark thickness with seed source environment

Looking for ecotypic trends in bark thickness, we tested for correlations among the mean values for bark thickness of our raw data (at breast and basal heights) and continuous environmental and geographical variables, as well as with fire frequency records from population origins for the Iberian populations. We used the previous selected environmental and geographic variables (see, Supplementary Information 10.1.). Then, Spearman correlations between PCA loadings of retain components and six selected variables were used in Spearman correlation analysis with bark thickness, as well as fire frequency data for the 13 Iberian populations of *P. halepensis*. These correlation were done with Hmisc package (Harrell, 2018), implemented in R software v3.3.2. (R Core Team, 2016).

3.4.3.3. Allometric, plastic and genetic effects on bark allocation

Focusing purely in the allometry of bark allocation, we based our analysis on the relationship of percentage of bark volume and total volume from tree base to breast height (Figure 23). Our sampled trees did not contain any heartwood (author's unpublished data), so total volume is bark and sapwood (functional xylem) volumes.

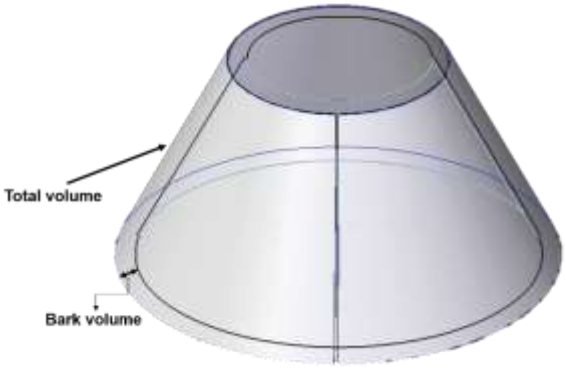

FIGURE 23 **|** Total and bark volumes of the cone trunk from the tree base to breast height. Scheme used for studying allometry of bark allocation.

Firstly, we calculated the total volume for the cone trunk from the tree base to breast height with the equation for a circular truncated cone (in dm^3):

$$V_T = \frac{\pi}{3} * h * \left(R_b^2 + R_b * R_{bh} + R_{bh}^2\right) \tag{3}$$

where h is the height difference between the base (10 cm above ground) and breast height (130 cm), R_b is the basal radius and R_{bh} is the radius at breast height.

Sapwood volume (dm^3) was computed with the following equation:

$$V_S = \frac{\pi}{3} * h * \left(SR_b^2 + SR_b * SR_{bh} + SR_{bh}^2\right) \tag{4}$$

where h is the height difference between the base (10 cm above ground) and breast height (130 cm), SR_b is the sapwood basal radius and SR_{bh} is the sapwood radius at breast height.

Finally, bark volume (dm^3) and percentage of bark volume were estimated by Equations 5 and 6, respectively:

$$V_B = V_T - V_S \tag{5}$$

$$\%V_B = \frac{V_B}{V_T} * 100 \tag{6}$$

We compared means of percentage of bark volume of the cone trunk from tree base to breast height (Equation 6) by site and populations using a LMM with environmental effect represented by trial sites and genetic effect by populations. In addition, we did the same model with total volume of the cone trunk as a covariate in order to look for tendencies in bark volume percentage.

All LMM for this study were fit with lmerTest package (Kuznetsova *et al.,* 2016) in R software. Models with different combination of variables were ranked using the Akaike's Information Criterion (AIC), selecting those models with lower AIC value. This criterion favors both model fit and simplicity, based on the principle of parsimony (fewer parameters in the model). Residuals of each model were examined for normality using diagnostic plots and statistical test.

3.4.4. Adaptive variation in P. halepensis: population differentiation and phenotypic integration (Study IV)

3.4.4.1. Trait trade-offs

To combine phenotypic data obtained at the two experimental sites, we had to correct field measurements by site effect. For this, independent Bayesian models were fitted for each trait using MCMCglmm package (Hadfield 2010) implemented in R software v3.3.2. (R Core Team, 2016). We used Gaussian models for growth traits data, bark thickness, male reproduction and female reproductive traits (log-transformed). Serotiny was analyzed with a multinomial family model adjusting an individual random factor to control for overdispersion. We applied inverse Wishart priors to all models. Markov Chain Monte Carlo (MCMC) chains were run a minimum of 5,500,000 times, sampling every 1,000 runs and with a burn-in period of 500,000 iterations. These settings were suitable to achieve good convergence and low autocorrelation (<0.1) for model parameters. Each MCMCglmm model was run three times to check if estimates were robust according to Kolmogorov-Smirnov tests. Analysis were performed on individual-tree data.

We fitted these models for both sites together, with site and block as fixed factors and provenance as random factor. Then, we tested the significance of site and the significance of block. If site and block were significant, we subtracted the fixed effect of the corresponding level to the individual field measurement of each individual, to remove the environmental effect as much as possible. Moreover, for some traits, we also included DBH and the interaction with site and block as fixed factors and tested their significance. If DBH or the interactions were significant, we also subtracted the effect of the corresponding level to the individual field measurement, to remove the allometric effect. This way we were able to get corrected values for all traits. Male reproduction was measured by qualitative data at the high-resource site and by quantitative data at the low-resource site, so we could not pool data of both sites together. In addition, because we had some phenotypic traits only measured at CUC site (see Table 5), we did also the analysis for each site separately. Thus, we include only block as fixed effect to eliminate the microenvironmental effect of the site as much as possible, or block and DBH when also accounting for the allometric effect. For serotiny degree and total male reproduction at the low-resource site (VED site), we used parameter expansion of the inverse Wishart prior to improve mixing, as variance estimates were low and tended to be trapped around zero. For male reproductive allocation at this former site, we used a prior informed by a previous reml model.

We used population corrected means (BLUPs) derived from previous models to test for possible correlations and trade-offs among phenotypic traits. First, we computed Pearson's correlation coefficient for each pair of traits at the population level. These correlations can be considered 'raw' genetic correlations. We also run a Principal Component Analysis (PCA) with varimax rotation for population means of all the studied traits to have a graphical and more comprehensive view of trait trade-offs. We used parallel analysis implemented in psych package (Revelle, 2017) implemented in R software (R Core Team, 2016) to decide the number of principal components to retain. We selected only those principal components with eigenvalues for observed data larger than those obtained in simulations (Hayton *et al*., 2004). Then, we ran de PCA based on the correlation matrix with the retained number of components. Correlations among plant traits can be interpreted as genetic correlations modified by common environmental effects. Correlations were conducted for both sites together (11 traits) and at each trial site separately (15 traits at the high-resource site and 13 traits at the low-resource site) to check whether trait-trait correlations and ecotypic trends of variation were site dependent, and due to we had traits not measured at both sites.

Furthermore, to get an estimate of genotype by environment interaction (GxE) in the studied traits we fitted a new LMM or GLMM for each trait (only for the 11 traits measured at both trial sites), with block as random factor and the interaction between site and provenance as fixed factors. The significance of the fixed factors in these models was determined by an omnibus test (Mittell *et al.,* 2015; Foo *et al.,* 2016). We considered the *P*-value of the interaction as an indicator of GxE.

3.4.4.2. Trait-environment associations

Firstly, the environmental conditions at the two sites of the common garden were used to compute ecological distances (Gower's distance; Gower 1971, Rutter and Fenster 2007) between the conditions of origin of the sampled populations and those at each trial site, using the six selected climatic variables. In this distance, values closer to 0 indicate greater similarity between the population of origin and the common garden site with respect to the environmental variables used for the calculation of the distance, while values closer to 1 indicate maximal dissimilarity. This was made using the gower package (van der Loo, 2017) implemented in R software (R Core Team, 2016). These two distances were also used in correlation analysis with phenotypic traits. Then, we computed graphical pairwise correlations among all the climatic variables (before selection) and the three geographical variables. Loadings of retained components of the PCA made for the environmental variables, together with the six selected climatic variables and the three geographical variables (see Supplementary Information 10.1.) were utilized in correlation analysis with plant traits, using provenance adjusted means (BLUPs) for each trait. We also used fire frequency data for the 13 Iberian populations (see page 78 for details on fire records) for correlation analysis with tree phenotypic traits. All correlations analysis were made by the Hmisc (Harrell 2018) and PerformanceAnalytics (Peterson and Carl 2018) packages in R software.

3.4.4.3. Quantitative genetic differentiation

Following the same Bayesian models explained in section *3.4.4.1.*, but fitted for data from each trial site separately and defining block as fixed factor, and population as random factor, we estimated the coefficient of variation for each phenotypic value, which was used to obtain the quantitative variability within a given population at each site. Phenotypic differentiation among populations (Q_{ST}) was calculated for all quantitative traits partitioning the total additive genetic variance into the between-population (σ^2_{Bpop}) and the within-population (σ^2_{Wpop}) components following Equation 7 for two sites separately:

$$Q_{ST} = \frac{\sigma^2_{Bpop}}{\sigma^2_{Bpop} + 2\sigma^2_{Wpop}} = \frac{V_{pop}}{V_{pop} + 2h^2 V_\varepsilon} \tag{7}$$

where the variance components were: variance of the population (V_{pop}), residual variance (V_{ε}) and h^2 is the narrow-sense heritability.

Due to the lack of progenies within provenances in our trial sites, we were not able to calculate the narrow-sense heritability of our studied traits for *P. halepensis*. Therefore, we carried out an intensive literature search of heritability data for this species. However, these data is scarce (Matziris 2000, Santos-del-Blanco et al 2010, Hernández-Serrano et al 2014), not covering the entire range of characters used in this chapter. Finally, we divided the heritability data into three groups based on their greater affinity to our data.

- Group 1: narrow-sense heritability was calculated in other experimental sites of *P. halepensis,* but with the same set of genetic materials (same provenances) and at similar tree age or size. The h^2 values of this level would be the most plausible for our study. In this case, we found mean values with their confidence intervals, so we used these three heritability values.
- Group 2: heritability was calculated for *P. halepensis*, but in other experiments (different genetic materials) and at different ages. Values of h^2 in this level are suitable for our study but less reliable than those of level 1. In this case, we just found two h^2 values for each traits, so we also used two other values, one 10 units below the minimum value found in the literature and another 10 units above the maximum.
- Group 3: narrow-sense heritability was calculated for other Mediterranean pine species (*P. pinaster*). This values of h^2 are less credible related to own data.

For height-diameter allometry (H/DBH) and aerial cone bank (CCB), we did not find any narrow-sense heritability value in the literature. Thus and due to these traits are completely related to height and female reproduction, respectively, we used for H/DBH the heritability values found for height, and those found for female reproduction were used for CCB (see Table 7). We also lacked heritability values for age and height of first female flowering (Aff and Hff, respectively). However, we used the values found for female reproductive precociousness relative to size, a trait very similar to the previous ones (Santos-del-Blanco et al 2010).

TABLE 7 | Narrow-sense heritability and confidence intervals for family estimates reported in the literature for several traits for *Pinus halepensis* and *Pinus pinaster*. Heritability values in parenthesis are assumed values not from previous works (see above text for explanation). 'Group' indicates the three groups in which we have divided the heritability values according to their greater plausibility with respect to our data.

Species	**Trait and (age)**	**Heritability (h^2)**	**95 % CI**	**Reference**	**Group**
Pinus halepensis	fRA (11-15)	0.40	0.27-0.63	Santos-del-Blanco et al. 2010	1
Pinus halepensis	SER (17)	0.20	0.09-0.40	Hernández-Serrano et al. 2014	1
Pinus halepensis	FTR (9-10)	(0.47) 0.57-0.68 (0.78)	-	Matziris, 2000	2
Pinus halepensis	H (9-10)	(0.32) 0.42 – 0.57 (0.67)	-	Matziris, 2000	2
Pinus halepensis	SBH (9-10)	(0.16) 0.26 – 0.48 (0.58)	-	Matziris 2000	2
Pinus halepensis	BT (9-10)	(0.05) 0.15 – 0.24 (0.34)	-	Matziris, 2000	2
Pinus pinaster	FPs (4.5)*	0.43	0.27-0.67	Santos-del-Blanco *et al.,* 2013	3
Pinus pinaster	mRA (4-5)	0.38	0.23-0.63	Santos-del-Blanco *et al.,* 2013	3
Pinus pinaster	MR (4-5)	0.48	0.30-0.70	Santos-del-Blanco *et al.,* 2013	3
No data	CCB	(0.47) 0.57 – 0.68 (0.78)	-	-	
No data	CCBa	(0.47) 0.57 – 0.68 (0.78)	-	-	
No data	H/DBH	(0.32) 0.42 – 0.57 (0.67)	-	-	

Notes: See explanation of trait's abbreviations in page 74. *FPs is female reproductive precociousness relative to size. Heritability values for this trait were used for age and height at first flowering reproduction.

3.4.4.4. Neutral vs. Adaptive differentiation

From a Single Nucleotide Polymorphism (SNP) database used for other more extensive population genetics studies (Serra-Varela *et al.,* 2017; Ruiz-Daniels *et al., in press*), the global neutral genetic differentiation (F_{ST}) was calculated for our studied populations using GDA software (Weir and Cockerham 1984; Lewis and Zaykin 2001). This way we obtained neutral molecular analogs of the previously calculated estimates of genetic differentiation among populations for phenotypic traits (Q_{ST}). To disentangle the effects of genetic drift from those of selection, confidence intervals for the two statistics estimations and $Q_{ST} – F_{ST}$ distributions were assessed with a parametric bootstrap procedure (1000 samples) following Whitlock and Guillaume (2009), implemented in R software v3.3.2. (R Core Team, 2016). Q_{ST} was considered to be statistically different from F_{ST} when the 95% confidence intervals of Q_{ST} did not overlap the 95% confidence intervals of F_{ST} (Sahli et al., 2008).

4. Results

4.1. Variation of serotiny degree and aerial seedbank among sites and populations *(Study I)*

4.1.1. Site effect on tree growth, survival and reproduction

We found highly significant differences in tree height and survival among the three sites ($\chi^2 = 11.08$, df = 38, $P < 0.001$; $\chi^2 = 21.04$, df = 2, $P < 0.001$, respectively). As expected, height and survival of the trees at the mild site were higher than at the cold and dry sites. However, while height was similarly low at the cold and dry sites, tree survival was significantly lower at the cold site (Figure 24A).

Female reproduction was also significantly different among sites, both in absolute terms ($\chi^2 = 23.98$, df = 2, $P < 0.001$) and relative to size ($\chi^2 = 35.03$, df = 2, $P < 0.001$). The highest values for both variables corresponded to the dry site. While the total number of female cones per tree was lower at the cold site, it was at the mild site where the relative investment in reproduction was lowest since here trees were much taller (Figure 24B).

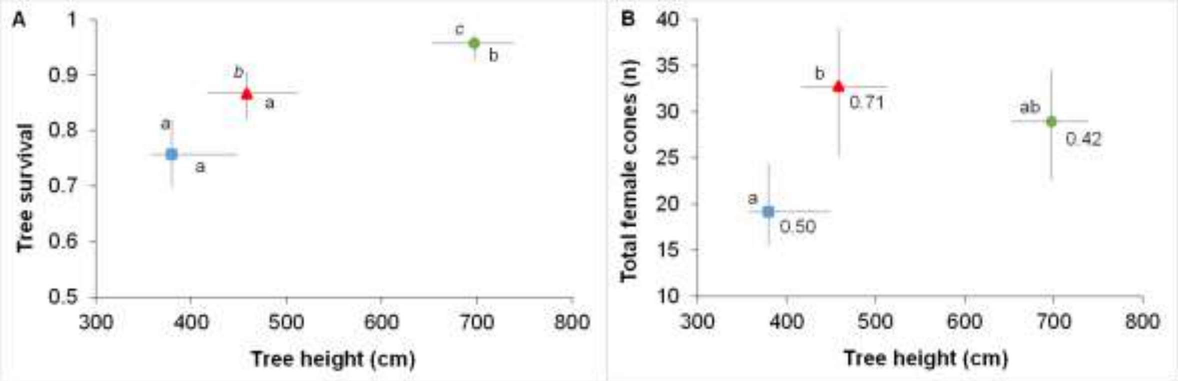

FIGURE 24 | Biplot of (A) average tree height and survival rate and (B) tree height and total female cones at the three *P. halepensis* trial sites (blue square: ADE –cold site–; red triangle: VED –dry site–; green circle: CUC –mild site–). In (A), letters indicate homogeneous groups for height and letters in italics for survival. In (B), letters indicate homogeneous groups for total female cones and insert values correspond to the ratio between cone number and tree size (cones per cm of height).

4.1.2. Plastic and allometric effects on cone serotiny

The overall expression of serotiny was very different among the three trial sites. Particularly, the number of highly serotinous trees (this is, those with more than 80% of cones closed) was significantly different among sites ($\chi^2 = 62.70$, df = 2, $P < 0.001$), being lower at the dry site compared to the mild and the cold sites, respectively-: 43% vs. 57% and 68%, respectively (Figure 25).

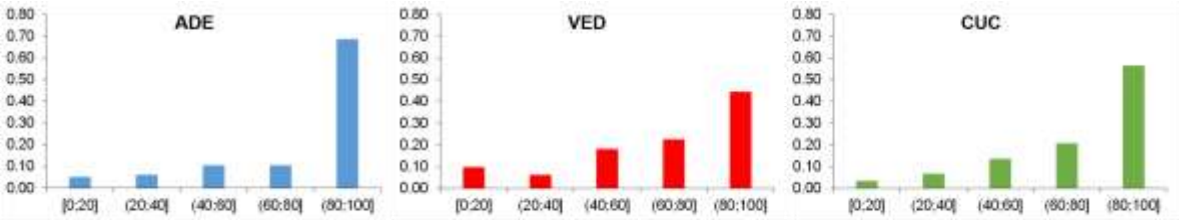

FIGURE 25 | Frequency distributions of degree of serotiny per trial site of *Pinus halepensis*. ADE is the cold site, CUC is the mild site, and VED is the dry site.

We found highly significant effects of the ratio of older to total cones ($\chi^2 = 10.60$, df = 1, $P = 0.002$), the total number of cones ($\chi^2 = 12.78$, df = 1, $P < 0.001$), tree size ($\chi^2 = 13.79$, df = 3, $P < 0.001$), trial site ($\chi^2 = 29.76$, df = 4, $P < 0.001$), and site × tree size interaction ($\chi^2 = 5.45$, df = 2, $P = 0.004$) on cone serotiny. As expected, both a higher ratio of older to total cones and/or a higher total number of female cones were related to lower degrees of serotiny across sites. Tree size was negatively related to the degree of serotiny across sites, but the interaction of tree size with trial site implied a different allometric effect on serotiny among sites. At the cold site, trees lost serotiny more rapidly in size terms compared to the mild and dry sites (Figure 26). However, at the dry site, the low frequency of highly serotinous trees blurred the significance of tree size on the variation of cone serotiny ($\chi^2 = 3.34$, df = 1, $P = 0.048$).

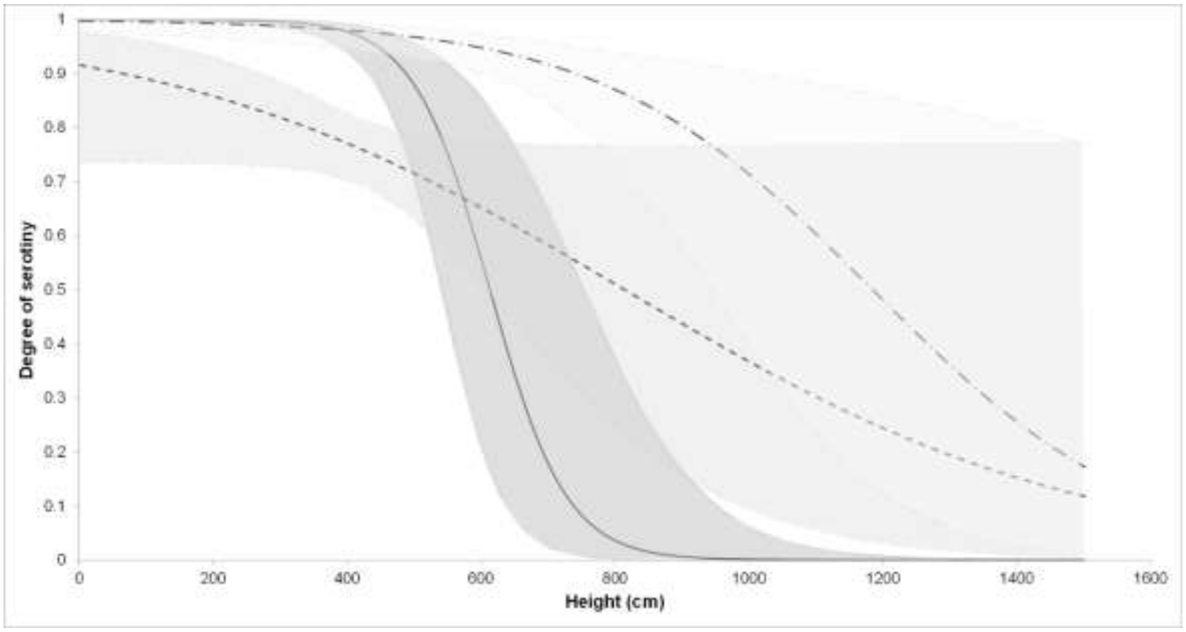

FIGURE 26 | Logistic models relating degree of serotiny to tree height in *Pinus halepensis* trees grown at three contrasting trial sites. Data include range-wide populations and are thus representative of the whole species. Solid line corresponds to ADE −cold site−, dashed line corresponds to VED −dry site− and dot-dash line corresponds to CUC −mild site−. Gray lines and shades represent the upper and lower 95% credible intervals of each model.

Varying allometric effects on serotiny among sites were reflected in the values of threshold size for serotiny loss (TSSL, i.e., the tree height corresponding to a degree of serotiny of 0.5), which confirmed a faster loss of serotiny at the cold site (TSSL = 596 cm; CI: 508–726) when compared to the dry (TSSL = 791 cm; CI: 497–3197) and mild sites (TSSL = 1132 cm; CI: 878–1911). Since the credible interval for TSSL was large at the dry site, only the difference between mild and cold sites was significant (Figure 26).

4.1.3. Population effects and interactions with site and tree size on cone serotiny

We found a significant population effect on the variation of cone serotiny. The variance explained by population at each site was similar at the mild and cold sites, 29.7% (12.9–50.4%) and 26.2% (15.0–56.9%), respectively, and slightly lower at the dry site (22.1%, 10.0–44.1%).

We considered the lower deviance information criterion (DIC, for MCMC simulations) of a model containing population × site as a random term -compared with a simpler one containing population only- as evidence for a significant genotype × environment interaction in the expression of serotiny while accounting for allometric effects. We tried to represent this interaction by fitting individual models for each population and site. However, due to both the low number of values and the distributions per population, these models were only significant for a few populations (Figures 27 and 28).

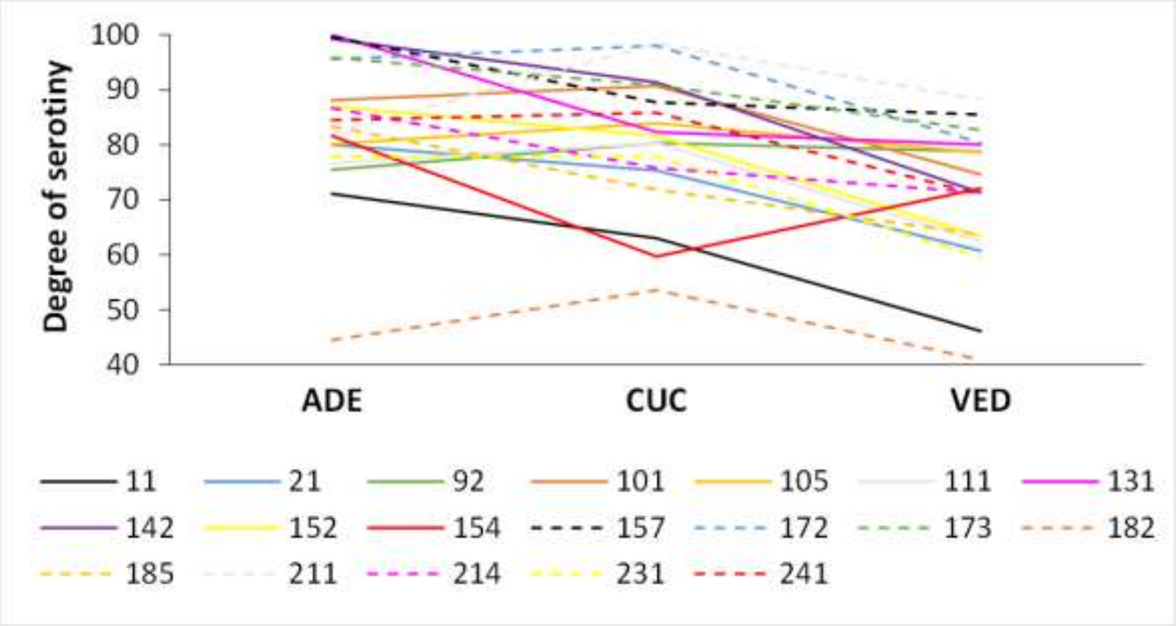

FIGURE 27 | Representation of the genotype x environment interaction in the degree of serotiny without taking into account the tree size effect. ADE: cold site, CUC: mild site, and VED: dry site. Numbers indicate provenances (see Supplementary Information 10.3., Table S10.3.1 for information about provenances).

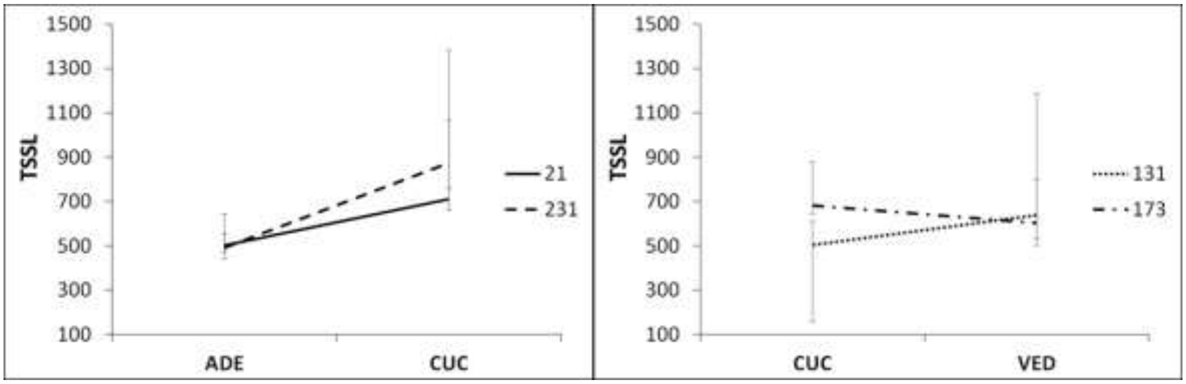

FIGURE 28 | Representation of the genotype x environment interaction in the TSSL. Populations shown are among the few in which the models adjusted for both sites and were significant. Comparison between ADE and VED sites was not possible because there were no fitted models for the same population on both sites. ADE is the cold site, CUC is the mild site and VED is the dry site.

4.1.4. Population x site effects on the canopy cone bank

Population ($F_{18,20} = 13.83$, $P < 0.001$) and site effects ($F_{2,20} = 4.88$, $P = 0.008$) on the canopy cone bank (CCB) were highly significant, but population × site interaction was not. Therefore, the site-related plasticity of the CCB did not differ among populations, and the population ranking for CCB did not differ among sites, i.e., those populations with the highest/lowest CCB at a given site, performed similarly at the other trial sites. Mean CCB varied as much as 5-fold among populations, with some from northeastern Mediterranean Spain (including Balearic Islands) having the fewest closed cones in the canopy, and those from Tunisia, Italy and southeastern Spain having the most (Figure 29A).

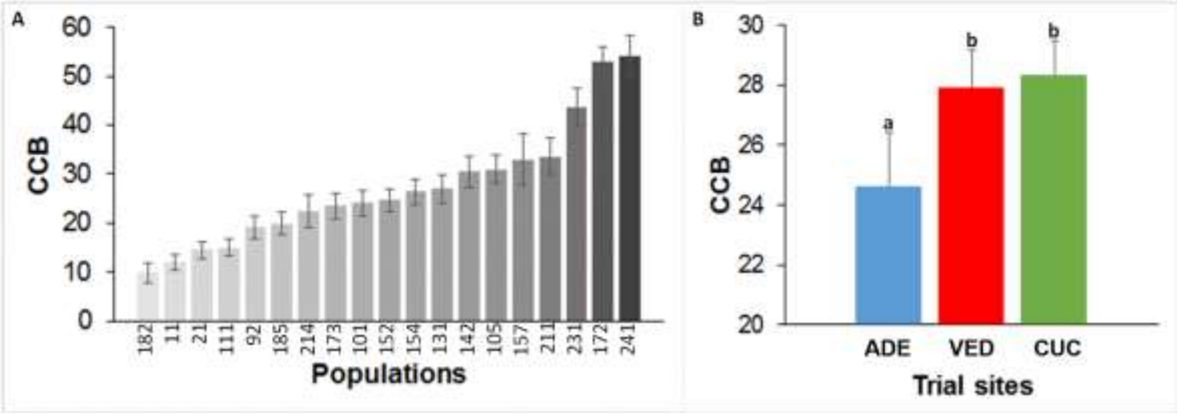

FIGURE 29 | (A) Mean canopy cone bank for 19 range-wide populations of *Pinus halepensis*. (B) Model estimates and confidence intervals for canopy cone bank at the three contrasting trial sites (ADE -cold site-, CUC -mild site- and VED -dry site-). Letters indicate homogeneous groups. Population codes can be checked in Supplementary Information 10.3., Table S10.3.1.

The CCB at the cold site was significantly lower than at the other two sites (Figure 29B). There were no further differences in CCB between the mild and dry sites, despite their significantly different reproductive output and serotiny degree. Serotiny degree and the CCB were negatively correlated with summer precipitation (Pearson r = −0.26, *P* = 0.080 and r = −0.38, *P* = 0.065, respectively). We also detected a significant positive relationship between serotiny and temperature oscillation (Pearson r = 0.38, *P* = 0.037) and between CCB and the same climatic variable (Pearson r = 0.55, *P* = 0.015). Moreover, serotiny and CCB were significantly correlated (Pearson r = 0.32, *P* < 0.001).

4.2. Maintenance costs of serotiny *(Study II)*

4.2.1. Physiological condition of serotinous cone peduncles in Pinus halepensis

All *P. halepensis* cones used in this study that were detached from the tree (ca. 540) had peduncles showing sapwood xylem, clear-coloured and wet when cut, and living cortical tissues (Figure 30A). Only two of the examined cones showed peduncles with partial heartwood formation –easily distinguishable by its reddish brown colour (Figure 30B-D)– and were eliminated from the following experiments. We also cut longitudinally several cones of *P. halepensis* to verify the connection through the peduncle between the interior of the cone and the tree branch (Figure 30E).

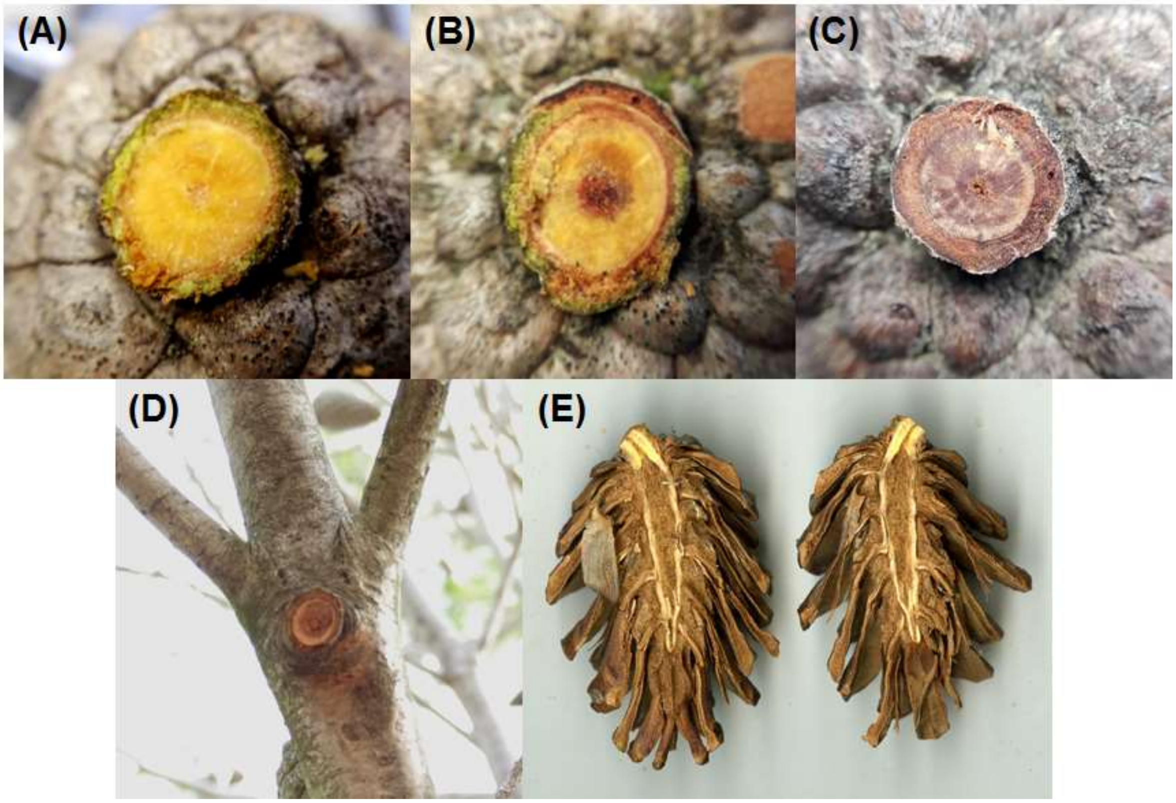

FIGURE 30 | Peduncle sections pertaining to serotinous cones of > 5 years from *P. halepensis* (A, B) and *P. brutia* (C). Xylem of (A) peduncle is entirely sapwood; (B) xylem has a typical incipient heartwood starting from the pith and (C) is entirely heartwood. (A) and (B) peduncles show living cortical tissues (phloem and cortical parenchyma), but not (C). 99% of *P. halepensis* cones sampled in this study were in the '(A)' status. (D) Section of the peduncle close to the tree branch of one of the few *P. halepensis* cones with heartwood. (E) Longitudinal section of a *P. halepensis* cone. The peduncle shows a light colored sapwood with connection to the cone scales. ©R.C. Martín-Sanz.

4.2.2. Cone-opening laboratory screening experiment

Confirming our hypotheses, older cones opened at lower accumulated temperature ($r = -0.33$; $P < 0.0001$) and displayed lower moisture content after the experiment than younger cones ($r = -0.24$; $P = 0.005$). However, older and younger cones did not differ in scale density. Scale density was uncorrelated with their respective opening temperature, but it was related to cone moisture, with the densest cones showing a lower moisture content after the experiment ($r = -0.19$; $P = 0.023$). Moreover, we found a rather continuous variation for both cone opening temperature and scales density (Figure 31).

We found a significant negative effect of cone age on cone opening accumulated temperature (χ^2 = 7.85, DF = 1, P = 0.005), but scale density and moisture content did not affect significantly the cone opening temperature (χ^2 = 1.00, DF = 1, P = 0.317 and χ^2 = 1.07, DF = 1, P = 0.301, respectively).

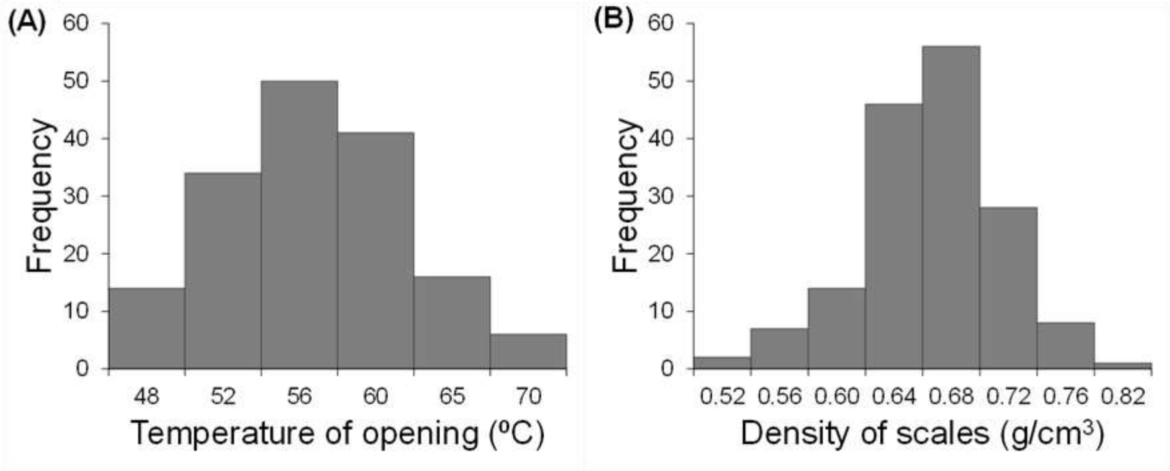

FIGURE 31 | Frequency distribution (A) for temperature of cone opening and (B) for density of the scales of the cones in the laboratory screening experiment.

4.2.3. Manipulating water availability of serotinous cones ex situ

The cones that received water supply through their peduncles opened after their waterless twin cone (χ^2 = 17.74, DF = 1, P < 0.0001; Figure 32). Meaningfully, the waterless cones lost significantly more water throughout the experiment than their watered pairs (χ^2 = 33.13, DF = 1, P < 0.0001). The accumulated temperature had a negative effect in cone opening (χ^2 = 4.71, DF = 1, P = 0.023) showing a decreasing effect of the treatment along the experiment. Unlike in the field experiment, all cones opened in response to the controlled extreme conditions. Although 14.8% of cone pairs opened almost at the same time, and 18.5% of the 'wet' cones opened before its 'dry' pair, the majority of watered cones (66.7%) opened -as expected- after their dry pair.

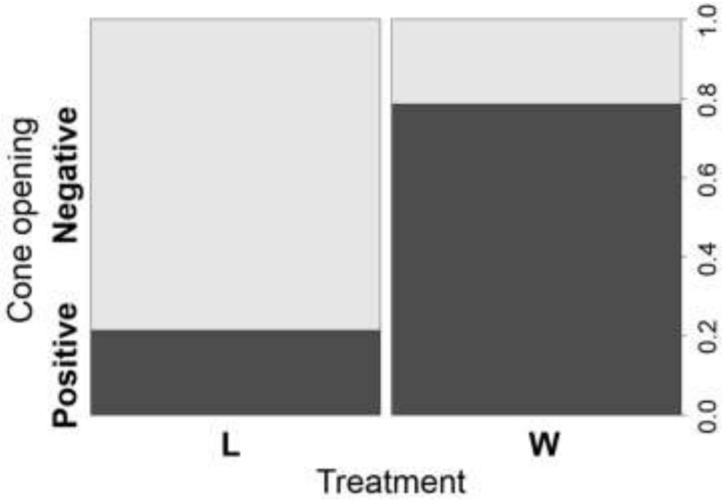

FIGURE 32 | Cone opening by treatment for the manipulating water availability ex situ experiment. Categories meaning is the following: ‘Positive’, cones opened after its pair, i.e. cones remained closed longer; ‘Negative’, cones opened before its pair; L, waterless cones; W, watered cones.

4.2.4. Manipulating tree to cone physical connection in situ

The results for both test sites showed that the detached cones exhibited a strongly significant higher probability of opening than their respective paired cones that remained attached to the tree ($\chi^2 = 49.09$, DF = 1, $P < 0.0001$). Site was slightly significant on cone opening ($\chi^2 = 4.34$, DF = 1, $P = 0.037$), as well as group of provenances ($\chi^2 = 6.37$, DF = 1, $P = 0.012$), with trees from the Northeast group showing faster opening compared to the Southwest group (Figure 33A). The estimated opening percentage for the Northeast group varied between 38 to 60% at CUC and OLM sites respectively, while for the Southwest group, the percentage of cone opening was lower at both sites (17 to 34%, respectively). However, none of the interactions were significant, not even the interaction between treatment and provenance group or between provenance group and site ($\chi^2 = 0.22$, DF = 1, $P = 0.637$, $\chi^2 = 3.24$, DF = 1, $P = 0.072$, respectively).

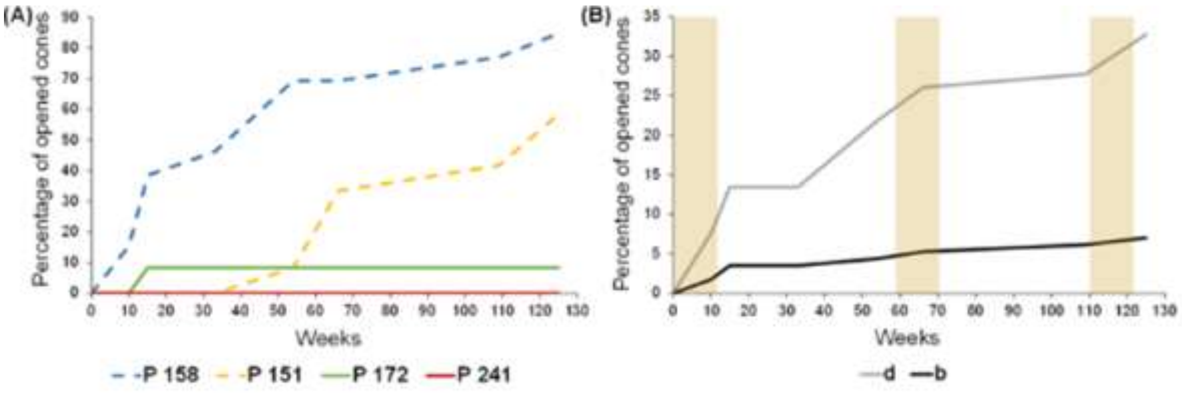

FIGURE 33 | Cone opening at OLM site by provenance and treatment. (A) Percentage of cone opening for some provenances with contrasting behavior pertaining to the two different provenance groups. Provenances 152 and 158 are part of the Northeast group; provenances 172 and 241 are part of the Southwest group. This plot illustrates the significant treatment by provenance interaction found at this site. (B) Percentage of cone opening during the experiment at OLM site, by treatment. Brown shadows indicate summer seasons. b, branched cones -control-; d, detached cones.

The GLM restricted to OLM site showed also a highly significant effect of the treatment ($F_{1,170} = 32.11$, $P < 0.0001$), as well as a significant effect of the provenance ($F_{12,158} = 30.62$, $P = 0.002$). The interaction between treatment and provenance was also significant ($F_{12,146} = 23.12$, $P = 0.023$).

At OLM site –where longer observations were performed–, the focal cones opened progressively since the observation onset, but still 72% of them remained closed after 31 months. Additionally, only 36% of cones that remained closed corresponded to detached cones. As expected, the greater changes in the percentage of open cones occurred during the summers (Figure 33B). The non-parametric test (McNemar's) for OLM site showed a significant treatment effect (i.e. attached vs. detached cones) since the onset of the experiment ($\chi^2 = 3.27$, DF = 1, two-tailed $P = 0.070$), continuously increasing the strength of the treatment effect until the last observation in September 2016 ($\chi^2 = 20.93$, DF = 1, two-tailed $P < 0.0001$; Figure 34).

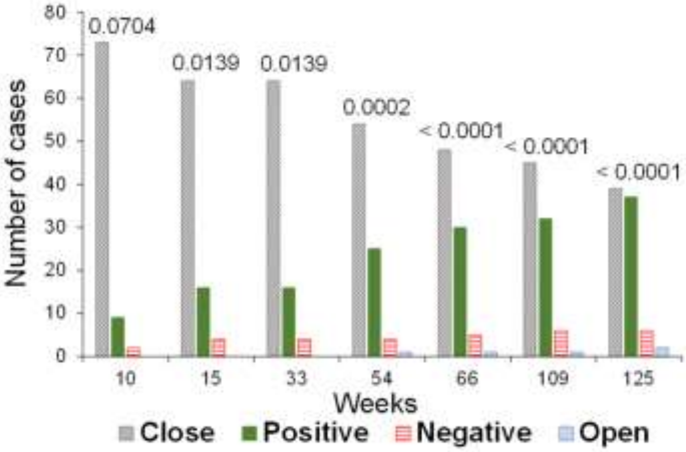

FIGURE 34 | Progress of the field manipulative experiment at OLM site from 10 to 125 weeks after its setting up. Categories meaning is the following: 'Close', both cones of the pair remained closed; 'Positive', the detached cone opened earlier; 'Negative', the attached cone opened earlier; 'Open', both cones opened between observations. Numbers in the bars correspond to the significance of McNemar's test.

4.3. Bark absolute thickness and bark allocation variation among sites and populations *(Study III)*

4.3.1. Plastic and genetic effects on bark thickness at breast and basal height

Absolute bark thickness, both at breast height and at the tree base, was significantly different between sites ($F_{1,6}$ = 171.02, $P < 0.0001$ and $F_{1,6}$ = 57.58, $P = 0.0003$, respectively) and among populations ($F_{18,468}$ = 10.55, $P < 0.0001$ and $F_{18,468}$ = 2.07, $P = 0.006$, respectively). However, site by population interaction was not significant in any height ($F_{18,468}$ = 0.99, $P = 0.473$ at breast height and $F_{18,468}$ = 1.37, $P = 0.143$ at the tree base). Mean bark thickness was significantly higher at CUC (mild conditions and high-resource availability) for the two measured heights (BT130 = 12.5 mm [CI 11.8–13.1] and BT10 = 23.5 mm [CI 22.8–24.2] at CUC site; BT130 = 8.1 mm [CI 7.6–8.5] and BT10 = 20.6 mm [CI 20.1–21.2] at VED site). We performed this analysis for all 19 populations and specifically for the 13 Iberian populations (for which we have fire record data). Results for the Iberian populations followed the same pattern as those for all populations, so we only show the results for all populations.

At the tree base, all populations showed bark thicker than the low critical value (10 mm) at both experimental sites (Figure 35A). By contrast, not all populations reached the high threshold thickness of 20 mm at VED site, where resources were lower, but all of them exceed that critical value at CUC site. As for the bark thickness at breast height, only three populations exceed the 10 mm thickness threshold at VED site. At CUC site, most of the populations showed a bark thickness higher than 10 mm, but below 20 mm –only two populations did not reach the low critical value of 10 mm–. At breast height, the maximum range of differences among populations within sites was more than two-fold. Moreover, the ranking of populations for bark thickness at breast height remained rather stable: populations with higher growth and lower reproduction (from Greece and Italy) had thicker bark, while the populations of lower growth and greater reproduction and serotiny (Tunisia and southern Spain) showed lower thicknesses at both test sites. However, at the tree base the populations ranking varied considerably between VED and CUC sites (low-resources and high-resources sites, respectively), without clear patterns. Only one of the faster-growing populations (211) had thick bark at both test sites and one population from southern Spain remained below the average at both sites (population 172, Supplementary Information 10.3., Table S10.3.5).

Attaining the critical minimum bark thickness at breast height seemed closely linked to mean population sapwood diameter, whereas bark thickness at the tree base was little correlated to sapwood diameter, especially at VED site (Figure 35A). To help visualizing the plasticity on bark thickness between sites, we used three extreme provenances: one that did not reach the minimum critical bark thickness even at CUC site –high-resource site– (152), another one in which the plasticity conducted to a different critical bark thickness between sites (185), and finally a third provenance that achieved the critical bark thickness even at the low-resource site –VED– (211, Figure 35B).

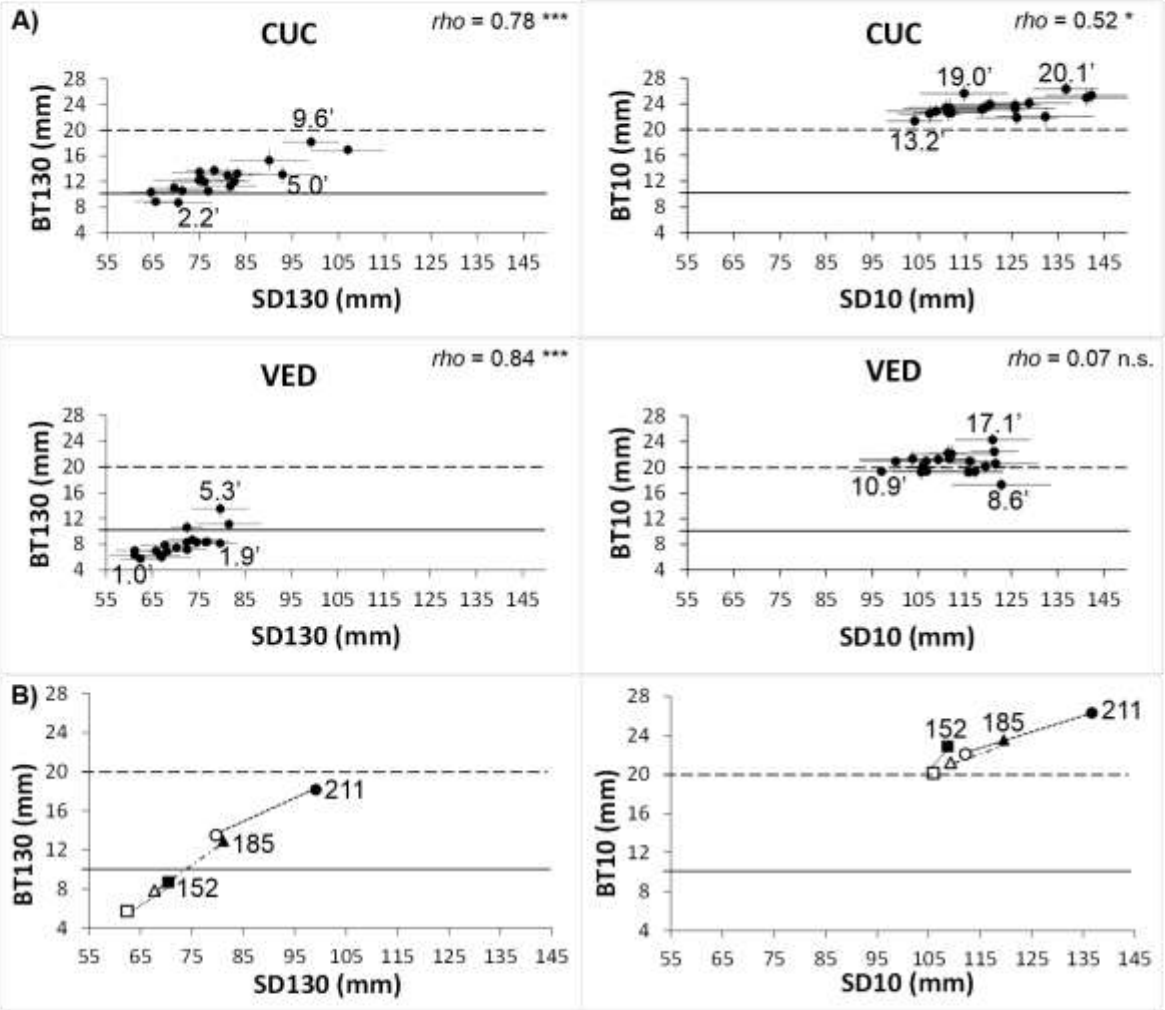

FIGURE 35 | A) Bark thickness vs sapwood diameter of *P. halepensis* populations at each experimental site. BT130 and BT10 are bark thickness at breast height and at the tree base, respectively; SD130 and SD10 are sapwood diameter at breast height and at the tree base, respectively. Horizontal black lines represent the assumed values of critical bark thickness for cambium survival (solid line: 10 mm; dashed-line: 20 mm). Numbers indicate the critical time for cambium kill (τ_c) for some divergent populations (Table S10.3.5). Significance for Spearman correlations between bark thickness and sapwood diameter at: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, n.s. = no significant. B) Variation between sites of the relationship between critical bark thickness and sapwood diameter of three representative *P. halepensis* populations. Numbers are the population code as in Supplementary Information 10.3., Table S10.3.1. Close symbols indicate CUC site (high-resource site) and open symbols indicate VED site (low-resource site).

The insulating ability of the bark was greater at CUC site and at the base (τ_{c130} = 4.7 min [CI 3.3-6.3] and τ_{c10} = 16.1 min [CI 13.0-19.5] at CUC; τ_{c130} = 2.0 min [CI 1.4-2.7] and τ_{c10} = 12.4 min [CI 10.3-14.8] at VED).

4.3.2. Relationship of bark thickness with seed source environment

Bark thickness at the tree base, pooling together data of both sites, was correlated (at 90% confidence, $P = 0.10$) with summer rainfall and precipitation of the driest month (Spearman *rho* = 0.46, $P = 0.049$, *rho* = 0.42, $P = 0.072$, respectively). Basal bark thickness just at the low-resource site (VED site) was correlated with summer and spring rainfall, and driest month rainfall (*rho* = 0.50, $P = 0.031$, *rho* = 0.42, $P = 0.074$ and *rho* = 0.53, $P = 0.020$, respectively). At the high-resource site (CUC site) we did not find any association between bark thickness and environmental variables. Correlations with loadings of principal components were neither found. Natural fires frequency was positively correlated with bark thickness at the tree base at VED site (Spearman *rho* = 0.62, $P = 0.024$; Supplementary Information 10.3., Table S10.3.6).

4.3.3. Allometric, plastic and genetic effects on bark allocation

We found significant site and population effects on the percentage of bark volume ($F_{1,6} = 23.87$, $P = 0.003$; $F_{18,468} = 1.87$, $P = 0.016$, respectively), but site by population interaction was not significant ($F_{18,468} = 0.88$, $P = 0.608$). The high-resource site (CUC) showed a higher percentage of bark volume (mean $\%V_B$ at CUC site: 46.59 % [CI 45.23-47.89], mean $\%V_B$ at VED site: 42.92 % [CI 41.62-44.22]). Regarding populations, there was no clear trend concerning behavior groups, although the population with the highest growth (population 211) showed also the highest percentage of bark volume (Supplementary Information 10.3., Table S10.3.7).

When including the total volume as a covariate, we found a high allometric effect in bark percentage (total volume: $F_{1,430} = 112.98$, $P < 0.0001$), as well as volume x site interaction ($F_{1,430} = 7.61$, $P = 0.006$). The rest of factors and interactions were not significant (site: $F_{1,16} = 2.19$, $P = 0.190$; population: $F_{18,430} = 1.35$, $P = 0.152$; site x population: $F_{18,430} = 1.42$, $P = 0.115$; total volume x population: $F_{18,430} = 1.11$, $P = 0.343$; total volume x site x population: $F_{18,430} = 1.50$, $P = 0.087$). The allometric effect on bark percentage was negative, i.e. the higher the total volume, the lower the bark percentage (Figure 36).

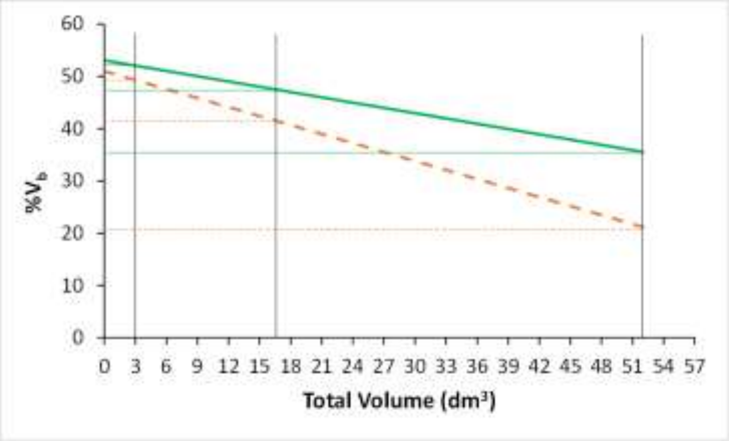

FIGURE 36 | Percentage of bark volume vs. total volume per site for the mean total volume of the cone trunk from the tree base to breast height -between 10 and 130 cm height- (16.8 dm^3), and the minimum and maximum total volume (∼3 dm^3 and ∼52 dm^3, respectively). At higher total volume, the differences between sites are magnified. Green line represents CUC site –mild conditions and high resource availability– and orange dashed-line is VED site –dry conditions and low resource availability–.

4.4. Adaptive variation in *P. halepensis*: population differentiation and phenotypic integration *(Study IV)*

4.4.1. Trait trade-offs

Models with site and block as fixed factors and provenance as random factor, showed that site and block were significant for CCB and SER so the fixed effect for each site and block were subtracted from the original field measurement, to get corrected estimates and be able to mix data from the two experimental sites. Site effect was subtracted from the original measurements for height, DBH, total female reproduction and bark thickness at both tree base and breast height, while for height-diameter allometry and female reproductive allocation we also subtracted the tree size effect. Regarding models for each site separately, tree size was significant for height-diameter allometry, female reproductive allocation, canopy cone bank and male reproductive allocation at both sites. For serotiny we subtracted the block effect from the original field measurements, as well as for canopy cone bank, age and height of first female flowering and qualitative male reproduction at CUC site.

Pooling data of both sites together, tree provenance explained a substantial amount of variation in serotiny degree (variance explained of 33.5%), bark thickness at breast height

(23.3%), some reproductive traits (25.9% for the canopy cone bank taking into account tree size and 22.9% for female reproductive allocation) and growth (22.7% for height and 15.9% for DBH). By contrast, tree provenance explained only little of bark thickness at tree base variation and of height-diameter allometry variation (1.9% and 8.0%, respectively; see Tables S10.3.8, S10.3.9 and S10.3.11 for more information).

Trait mean values at the species level are provided in Table 8, and means and boxplots at the population level in Figure 37. We found significant differences among populations for all studied traits. Populations from Greece and Italy showed higher growth and bark thickness at breast height, while basal bark was thicker in northern Spain populations. Populations from Tunisia and southern Spain were significantly different from others regarding female reproduction, exhibiting a higher number of female cones as well as higher reproductive allocation. Serotiny degree was especially higher in the most southern Spanish populations (172).

TABLE 8 **|** Mean values and SE for the 11 traits studied in *Pinus halepensis* at the two experimental sites, and factor significance based on LMMs and GLMMs for each trait, including site, provenance and site x provenance (GxE) as fixed effects, as well as tree size to account for the allometric effect. Significance: ****P* < 0.001, ***P* < 0.01, **P* < 0.05.

Trait	**Units**	**Mean**	**SE**	**Site effect**	**Provenance effect**	**GxE**	**Tree size effect**
H	cm	527.29	7.340	2.2.E-16***	2.2E-16***	9.0E-07***	-
DBH	cm	85.02	1.715	2.2E-16***	2.2E-16***	0.375	-
H/DBH	-	527.74	3.635	2.2E-16***	3.7E-07***	5.6E-10***	2.2E-16***
BT130	mm	10.18	0.271	2.2E-16***	2.2E16***	0.633	-
BT10	mm	21.58	0.013	0.0006***	0.047**	0.346	-
SER	Nº open and close cones	0.57	0.009	2.2E-16***	2.2E-16***	0.018*	-
SERa	Nº open and close cones	0.59	0.009	2.2E-16***	2.2E-16***	0.035*	2.2E-16***
TFR	Nº cones	14.52	0.046	2.2E-16***	3.2E-11***	0.002**	-
fRA	Nº cones	15.42	0.043	2.2E-16***	2.2E-16***	0.001**	2.2E-16***
CCB	Nº close cones	10.24	0.055	2.2E-16***	2.2E-16***	0.016**	-
CCBa	Nº close cones	10.64	0.054	2.2E-16***	2.2E-16***	0.004**	0.0002**

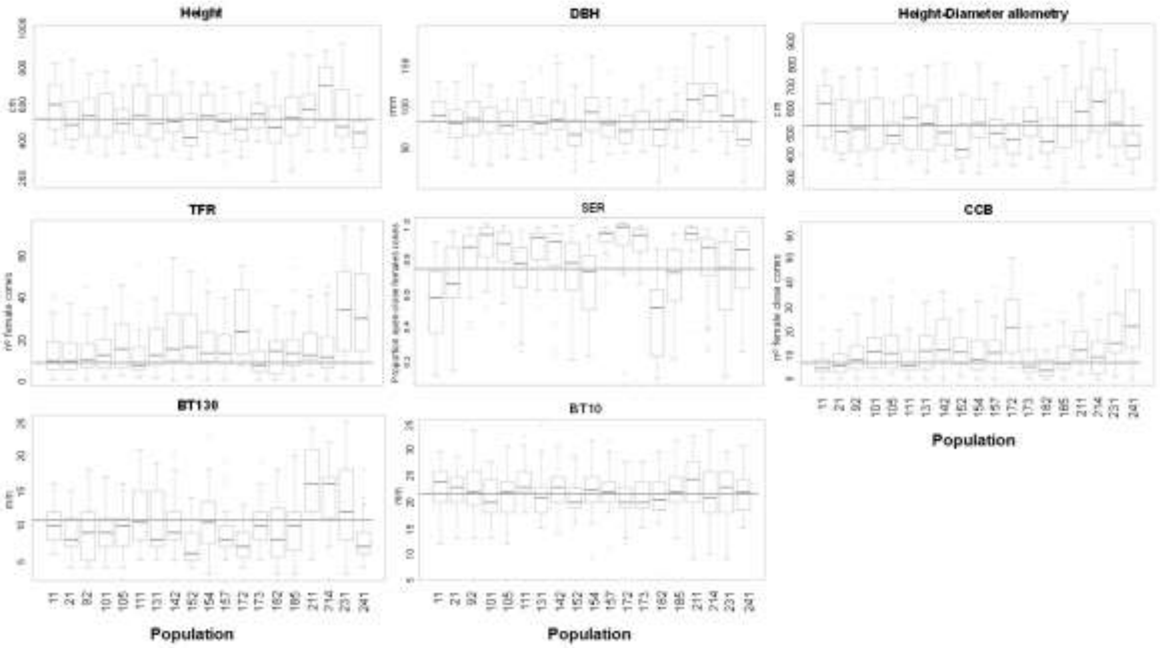

FIGURE 37 | Boxplots showing variation in measured traits within each *Pinus halepensis* population. The grey line corresponds to the trait mean estimate across populations.

Models including site by provenance interaction as fixed effect, revealed high plastic effects (site effect) and genetic effects (provenance effect) for all traits. The genotype by environment interaction was highly significant for all reproductive traits, as well as for serotiny degree and for growth traits except diameter. Bark thickness at both the breast height and the tree base did not show significant genotype by environment interaction either (Table 8).

We found significant correlations among traits of the same functional group, except for the fire-adaptive traits: serotiny and bark thickness (Figure 38). Growth traits were positively correlated with bark thickness at breast height, and negatively with reproductive traits. Bark thickness at breast height also showed a significant negative correlation with reproductive traits, but basal bark thickness did not showed significant correlations with any other trait. As expected, serotiny degree and reproductive traits were significantly and positively related to the canopy cone bank (Figure 38 and Supplementary Information 10.3., Figure S10.3.2). Regarding correlations at CUC site, where we included four traits not measured at VED site: qualitative male reproduction (QMR), male reproductive allocation (mRA), and age (Aff) and height (Hff) of first female flowering, we also found significant correlations among traits (Figure 38). Growth traits showed significant negative relation with male and female reproduction. Growth traits were positively correlated to bark thickness at breast height and the tree base, and with height and age at first female flowering; the last two showed significant negative correlations with female and male reproduction. In addition, thicker bark at both measured heights correlated positively with height at first female flowering (this, implying a negative correlation between bark thickness and reproductive precocity), and negatively with female and male reproductive allocation. Serotiny degree was again positively related with female reproductive traits, but female and male reproductive traits did not show any significant correlation (Figure 38 and Supplementary Information 10.3., Figure S10.3.3). Finally, correlations made just for trees at VED site revealed similar patterns among traits. In this case, we had 13 phenotypic traits, the 11 measured at both test sites plus total male reproduction (TMR) and male reproductive allocation (tmRA). As previously found, growth traits showed negative correlations with female and male reproduction, and were positively related to bark thickness at breast height, which was negatively correlated with female and male reproductive allocation. At this site, basal bark thickness and serotiny degree did not show any correlation. Finally, female reproductive traits were positive related to male reproductive traits (Figure 38 and Supplementary Information 10.3., Figure S10.3.4).

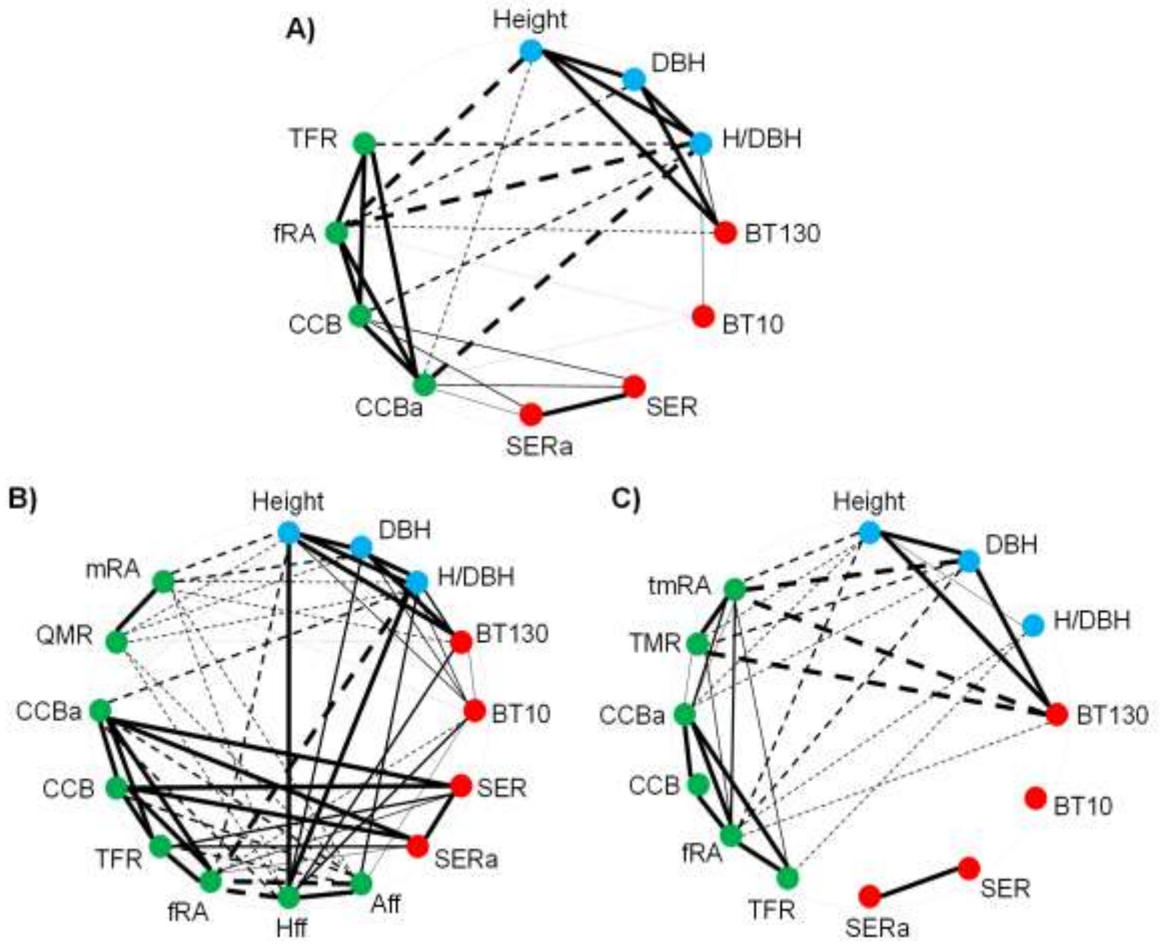

FIGURE 38 | Plots indicating phenotypic integration in *Pinus halepensis*. A) Data for both sites together. B) Correlations at the high-resource site (CUC site). C) Correlations at the low-resource site (VED site). Solid lines indicate positive correlation, dashed lines indicate negative correlation. Line thickness is proportional to the significance level (***$P < 0.001$, **$P < 0.01$, *$P < 0.5$, ·$P < 0.1$). Colours group traits in functional groups: growth traits in blue color, fire-adaptive traits in red colour and reproductive traits in green colour.

The multi-trait PCA based on population data for both sites confirmed a positive relationship among traits of the same group of vital functions and trade-offs between growth and reproduction. The first PC (44% of variance explained) was mainly influenced by growth traits and PC2 (34% variance explained) by female reproductive traits (Figure 39A and Supplementary Information S10.3., Figure S10.3.5 and Table S10.3.11). The PCAs carried out for each experimental site also verified the same patterns of trade-offs and correlations (Figures 39B-C and Figure S10.3.5 and Table S10.3.12 for the high-resource site, and Figure S10.3.6 and Table S10.3.13 for the low-resource site).

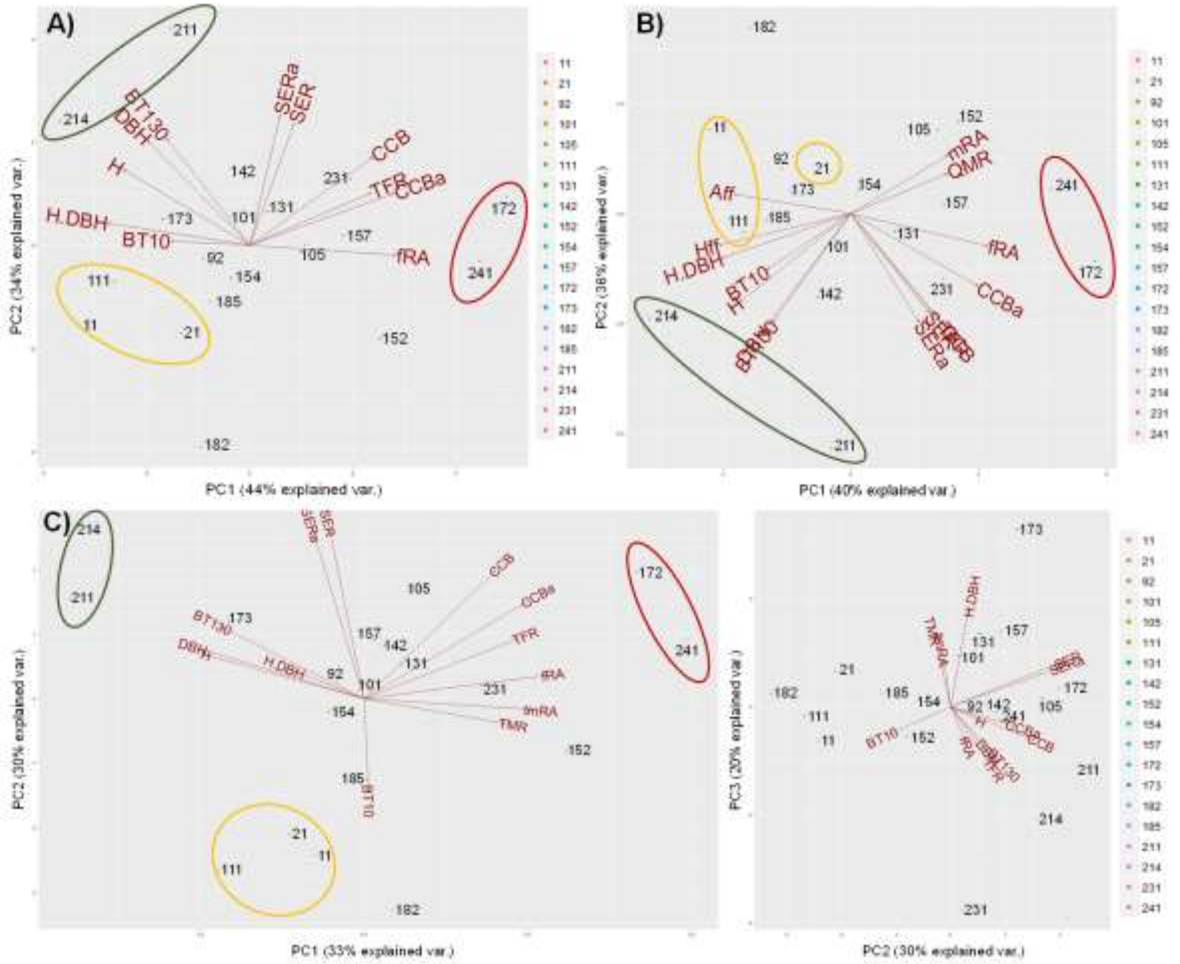

FIGURE 39 | PCAs based on population trait means in *Pinus halepensis* for (A) the 11 phenotypic traits studied at both sites, (B) the 15 traits studied at the high-resource site (CUC site) and (C) the 13 traits studied at the low-resource site (VED site). Red circle group the Tunisian and southern Spain populations; green circle cluster the Greek populations, and yellow circle bunch some of the northern Spanish populations. Details on PCAs can be found in Supplementary Information 10.3.

4.4.2. Trait-environment associations

The Gower's environmental distance between each population to each experimental site varied between 0.14 for population 111 to 0.51 for population 172 at CUC site, and between 0.21 for population 92 to 0.63 for population 172 at VED site. As might be expected, the environmental distance of Spanish peninsular populations to both test sites was smaller in general, and the same pattern was found for the Greek and Italian populations which environments were quite similar to that of the two test sites (Figure 40). However, populations of southern Spain presented higher distances to both test sites, particularly in the continental, drier and more stressful site (VED). Importantly, we did not find any significant correlation between Gower's distances to each test site and the studied raw phenotypic traits (see Supplementary Information 10.3., Table S10.3.14).

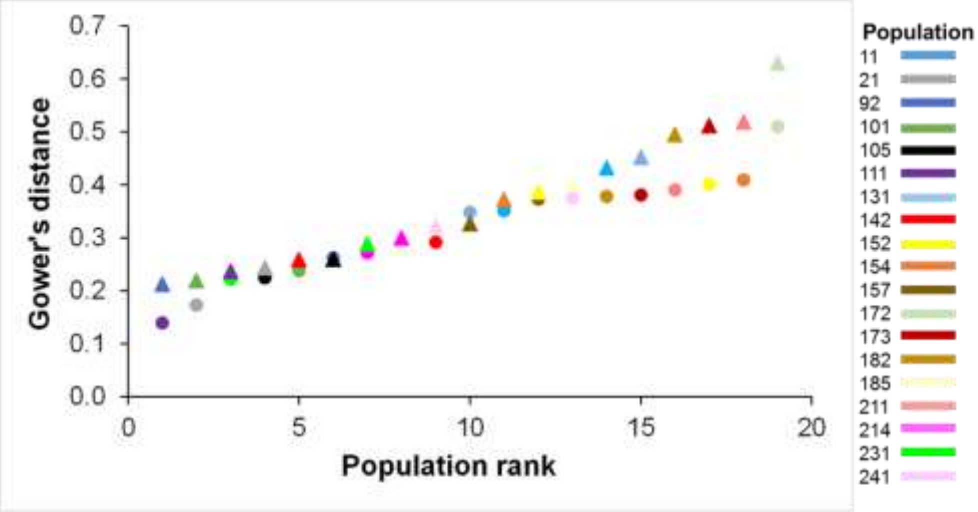

FIGURE 40 | Gower's (absolute) environmental distance between each population to the location of the two sites of the common garden. Circles indicate distance to CUC site (favorable environment) and triangles indicate distance to VED site (harsh environment).

Pairwise correlations among the raw environmental variables and the three geographical variables are shown in Table S10.3.15 in Supplementary Information 10.3. As expected, altitude showed a negative correlation with mean annual temperature, mean temperature of the coldest month (the higher the altitude, the lower the temperature of coldest month) and positive with continentally index (the higher the altitude, the higher the oscillation in annual temperature), altitude was also negatively correlated with precipitation of the wettest season. Latitude exhibited a highly significant positive correlation with summer and spring rainfall, as well as with precipitation of the driest month, and was negatively related with continentally index and mean temperature of the warmest month.

The first principal components retained in the PCA parallel analysis was related to precipitation of the driest and warmest seasons (summer and spring rainfall; explained variance PC1: 45%), while the second principal component was mainly influenced by temperature of the coldest month and continentally index, as well as precipitation of the wettest season (autumn rainfall; explained variance PC2: 36%; see Supplementary Information 10.1., Table S10.1.1 for more information about this PCA analysis).

Correlations between environmental variables and plant traits measured at both sites together, were higher for height-diameter allometry, canopy cone bank and serotiny degree (Table 9). Height and diameter were significantly correlated with latitude and longitude, while H/DBH showed significant positive correlation with summer and spring rainfall, precipitation of the driest month and PC1 (related to these precipitations), as well as with latitude, and was negatively correlated with continentally index and altitude. Bark thickness at breast height was related positively to longitude and negatively to altitude, whereas basal bark thickness was significantly and positively related to natural fire frequency (for the 13 Iberian populations with fire record data). Serotiny degree was negatively correlated to spring rainfall and latitude, both accounting or not for tree size. In this last case, serotiny degree was also negatively related to PC1. Female reproductive allocation was negatively correlated to latitude, as well as CCB, which was also negatively related to spring precipitation and positively to continentally index, either considering allometric effects or not.

TABLE 9 **|** Correlations of trait population means with geographic and environmental variables at the populations' origin, the two most important principal components from the environmental variables PCA and the natural fire frequency at populations' origin. Data from both experimental sites pooled together. Cont.Index: continentally index; TCM: mean temperature of the coldest month; Psp: precipitation of the warmest quarter (spring); Ps: precipitation of the driest quarter (summer); Pa: precipitation of the wettest quarter (autumn); PDM: precipitation of the driest month; Lat: latitude; Long: longitude; Alt: altitude; FF: natural fires frequency (see information of this variables in Materials and Methods).

	Cont.Index	**TCM**	**Psp**	**Ps**	**Pa**	**PDM**	**Lat**	**Long**	**Alt**	**PC1**	**PC2**	**FF[‡]**
H	-0.24	0.00	0.28	0.34	0.06	0.45·	**0.60****	**0.57***	-0.38	0.37	0.08	0.08
DBH	-0.09	-0.04	0.09	0.20	0.08	0.35	0.45·	**0.67****	-0.32	0.21	0.03	0.01
H/DBH	-0.43·	0.11	**0.51***	**0.48***	0.13	**0.47***	**0.63****	0.27	-0.40·	**0.53***	0.21	0.24
BT130	-0.12	0.22	-0.09	-0.04	0.09	0.13	0.29	**0.79*****	**-0.50***	-0.04	0.16	0.16
BT10	-0.14	-0.03	0.44·	0.44·	-0.23	0.39	0.40·	0.05	-0.12	0.36	-0.07	**0.57***
SER	0.37	-0.18	**-0.54***	-0.42·	0.02	-0.33	**-0.49***	0.12	0.23	-0.44	-0.16	-0.18
SERa	0.39	-0.19	**-0.56***	-0.43·	-0.02	-0.33	**-0.45***	0.20	0.19	**-0.47***	-0.19	-0.22
TFR	0.44·	-0.14	-0.32	-0.17	0.03	-0.04	-0.38	0.22	0.24	-0.27	-0.17	-0.26
fRA	0.35	-0.07	-0.27	-0.23	0.05	-0.20	**-0.50***	-0.14	0.33	-0.29	-0.10	-0.19
CCB	**0.52***	-0.21	**-0.51***	-0.36	-0.02	-0.22	**-0.53***	0.15	0.33	-0.44·	-0.24	-0.27
CCBa	**0.53***	-0.20	**-0.50***	-0.38	-0.01	-0.30	**-0.64****	-0.05	0.42·	**-0.46***	-0.23	-0.24

Notes: [‡]Correlations for 13 Iberian populations with fire record data. Abbreviations of trait names as in page 74.

TABLE 10 | Correlations of trait population means with geographic and environmental variables at the populations' origin, the two most important principal components from the environmental variables PCA and the natural fire frequency at populations' origin. Data from the high-resource site (CUC).

	Cont.Index	**TCM**	**Psp**	**Ps**	**Pa**	**PDM**	**Lat**	**Long**	**Alt**	**PC1**	**PC2**	**FF‡**
H	-0.30	0.09	0.29	0.32	0.12	0.41·	**0.61****	**0.60****	-0.45·	0.36	0.16	0.06
DBH	-0.14	0.03	0.06	0.13	0.10	0.27	0.45·	**0.67****	-0.36	0.16	0.08	-0.07
H/DBH	**-0.47***	0.17	**0.50***	0.44·	0.11	0.45·	**0.67****	0.33	**-0.46***	**0.49***	0.24	0.17
BT130	-0.14	0.19	-0.04	0.00	0.04	0.16	0.35	**0.76*****	**-0.49***	-0.01	0.13	0.14
BT10	-0.04	-0.11	0.10	0.12	-0.04	0.07	0.23	0.19	-0.14	0.11	-0.05	0.23
SER	0.39·	-0.17	**-0.54***	-0.40·	0.16	-0.31	**-0.55***	0.09	0.28	-0.42·	-0.11	-0.18
SERa	0.33	-0.12	**-0.52***	-0.38	0.23	-0.26	**-0.47***	0.18	0.20	-0.38	-0.04	-0.26
Aff	**-0.53***	0.20	0.28	0.14	0.06	0.06	0.36	-0.16	-0.20	0.29	0.25	0.25
Hff	**-0.53***	0.34	0.23	0.15	0.16	0.17	**0.49***	0.30	**-0.51***	0.26	0.36	0.25
TFR	0.35	-0.03	-0.43·	-0.30	0.04	-0.18	-0.32	0.31	0.08	-0.38	-0.08	-0.03
fRA	**0.46***	-0.06	**-0.48***	-0.42·	0.03	-0.37	**-0.62****	-0.04	0.32	**-0.50***	-0.13	-0.09
CCB	0.38	-0.05	**-0.48***	-0.37	0.06	-0.24	-0.43·	0.26	0.15	-0.43·	-0.09	-0.15
CCBa	0.44·	-0.06	**-0.53***	**-0.45***	0.04	-0.38	**-0.62****	0.02	0.28	**-0.52***	-0.11	-0.14
QMR	0.17	0.04	-0.27	-0.19	0.08	-0.14	-0.24	-0.31	0.03	-0.23	0.02	-0.30
mRA	0.20	0.03	-0.24	-0.16	0.00	-0.15	-0.28	-0.36	0.07	-0.24	-0.03	-0.23

Notes: ‡Correlations for 13 Iberian populations with fire record data. Abbreviations of trait names as in page74. Environmental and geographical variables as in Table 9.

TABLE 11 | Correlations of trait population means with geographic and environmental variables at the populations' origin, the two most important principal components from the environmental variables PCA and the natural fire frequency at populations' origin. Data from the low-resource site (VED).

	Cont.Index	**TCM**	**Psp**	**Ps**	**Pa**	**PDM**	**Lat**	**Long**	**Alt**	**PC1**	**PC2**	**FF‡**
H	-0.13	-0.04	0.08	0.19	0.06	0.29	0.38	0.40·	-0.29	0.21	0.02	0.10
DBH	-0.13	-0.01	0.08	0.21	0.02	0.37	**0.48***	**0.56***	-0.34	0.21	0.02	0.25
H/DBH	0.02	0.02	-0.20	-0.22	0.09	-0.30	-0.31	-0.31	0.08	-0.20	0.04	-0.14
BT130	-0.07	0.12	-0.09	0.01	0.20	0.18	0.27	**0.79*****	-0.44·	0.02	0.15	0.13
BT10	-0.12	-0.06	**0.51***	**0.52***	-0.28	**0.49***	0.35	-0.12	0.04	0.43·	-0.12	**0.70****
SER	**0.47***	-0.25	**-0.61****	**-0.48***	-0.2	-0.42·	**-0.52***	0.05	0.29	**-0.55***	-0.31	-0.24
SERa	0.43·	-0.24	**-0.56***	-0.44·	-0.22	-0.36	-0.44·	0.07	0.25	**-0.51***	-0.3	-0.16
TFR	0.36	-0.15	-0.21	-0.08	0.11	0.04	-0.34	0.10	0.27	-0.13	-0.13	-0.27
fRA	0.26	-0.05	-0.17	-0.13	0.06	-0.09	-0.40·	-0.12	0.28	-0.17	-0.07	-0.31
CCB	**0.61****	-0.35	-0.45·	-0.26	-0.10	-0.13	**-0.54***	0.06	**0.46***	-0.37	-0.37	-0.30
CCBa	**0.52***	-0.24	-0.42·	-0.3	-0.08	-0.23	**-0.60****	-0.11	**0.45***	-0.39·	-0.28	-0.35
TMR	0.12	-0.01	-0.07	-0.09	-0.02	-0.18	-0.44·	**-0.78*****	0.35	-0.12	-0.04	**-0.58***
tmRA	0.20	-0.02	-0.15	-0.16	0.03	-0.23	**-0.53***	**-0.66****	0.38	-0.19	-0.04	**-0.57***

Notes: ‡Correlations for 13 Iberian populations with fire record data. Abbreviations of trait names as in page 74. Environmental and geographical variables as in Table 9.

We found similar correlations when analyzing each trial site separately for bark thickness at breast height, serotiny degree and female reproduction traits (Table 10). Growth traits showed also similar correlations with environmental and geographic variables when pooling together data of both sites and just for trees at CUC site, while at VED site growth traits were fewer related to environmental variables. Basal bark thickness also showed different correlation patterns, with no correlations at CUC site and a significant positive relation with fire frequency, precipitation of the driest month, and summer and spring rainfall at VED site. Qualitative male reproduction at CUC site was not related to any environmental neither geographic variable, while quantitative male reproduction data at VED site was significantly negatively correlated to longitude, latitude and natural fires frequency. Finally, age (Aff) and height (Hff) at first female flowering at CUC site were negatively related to continentally index, and Hff also to altitude, as well as positively to latitude (Table 11).

4.4.3. Neutral vs. Adaptive differentiation

The overall estimate of neutral genetic differentiation was moderate (F_{ST} = 0.136, CI: 0.126-0.147), in accordance with previous studies for a broader number of population (Rodríguez-Quilón, 2017; Ruiz-Daniels et al., *in press*).

Taking into account the heritability values reported by previous studies, traits related to growth had in general less genetic differentiation among populations (Q_{ST}) than reproductive traits. In addition, the latter showed higher genetic differentiation at the most stressful environment site (VED site), while growth traits showed higher Q_{ST} at the most favorable site (CUC site; Figure 41, Table 12 and Supplementary Information 10.3., Tables S10.3.16 and S10.3.17). Results obtained for male reproduction and male reproductive allocation were not completely comparable between sites since at VED we had quantitative data for the year 2015 and at CUC, qualitative data for the year 2010. However, at both sites Q_{ST} values for male reproduction as well as for male reproductive allocation were not different from F_{ST}. Considering the most plausible values of narrow sense heritability, population quantitative differentiation was higher than neutral differentiation at both sites for serotiny degree taking into account tree size, for bark thickness at breast height, and just at the harsher environment site (VED) for female reproductive allocation and serotiny degree uncorrected by size (except with the highest h^2 value, for which Q_{ST} was equal to F_{ST}. Q_{ST} for female reproduction and canopy cone bank (considering or not tree size) was not significantly different from F_{ST}. The same happened for growth traits, except for height at CUC site, and for age and height of first female flowering (Figure 41 and Table 12).

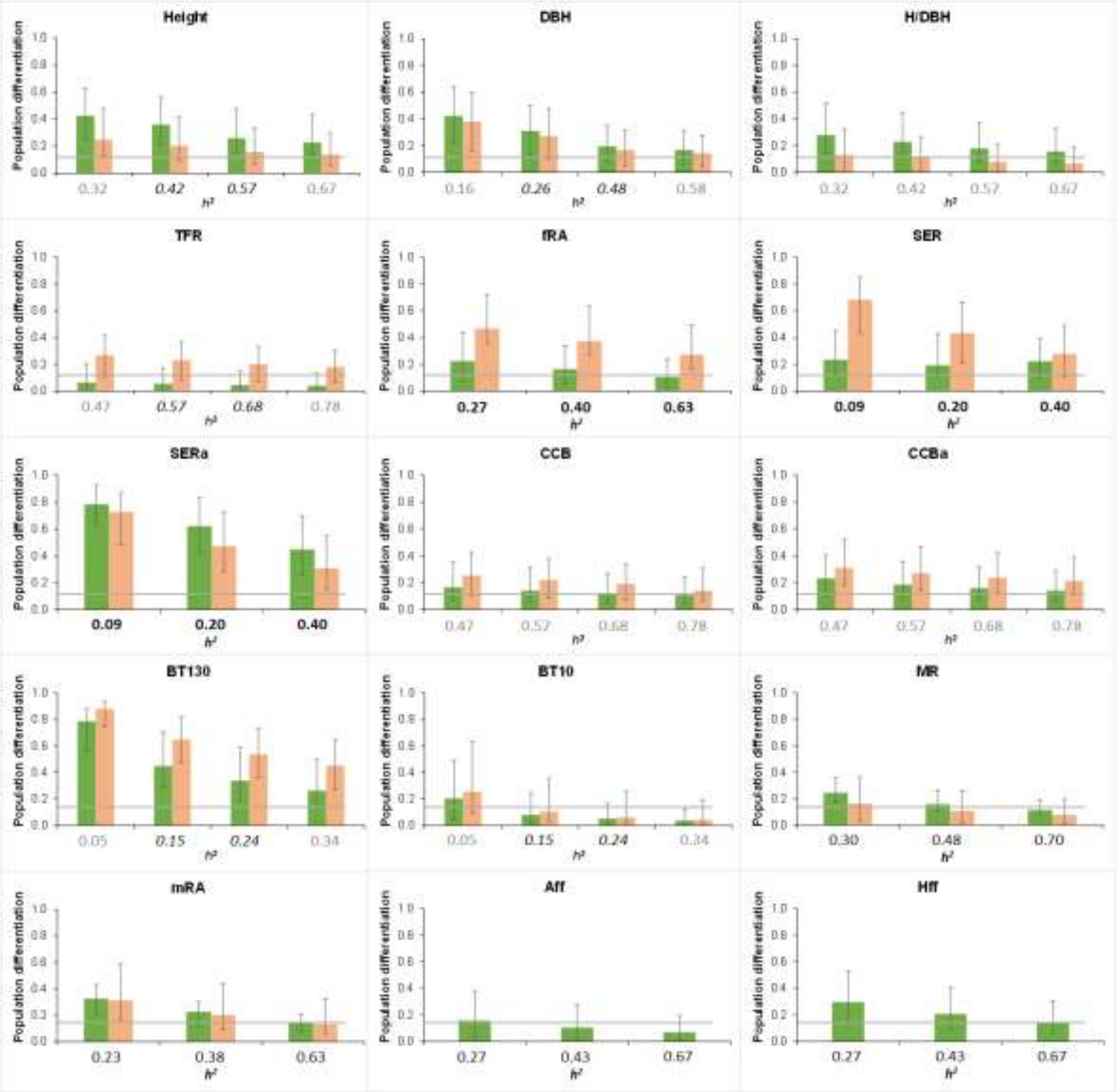

FIGURE 41 | Among-population differentiation (Q_{ST}) calculated for different heritability values (h^2) for the traits studied in 19 *Pinus halepensis* populations growing in two trial sites with contrasting environmental conditions (CUC in green colour, VED in orange colour). Grey line indicates mean F_{ST}. Meaning of trait abbreviations as in Table 5. Graphs for MR and mRA shows qualitative data for 2010 at CUC site and quantitative data for 2015 at VED site. Aff and Hff were only measured at CUC site. Heritability values in bold case are from group 1 according to their greater plausibility with respect to our data, h^2 values in italics belong to group 2, h^2 in black belong to group 3 and h^2 in grey colour are assumed values (see Table 7).

TABLE 12 | Summary of the probability of $Q_{ST} > F_{ST}$ for each trait studied.

Trait	$Q_{ST} > F_{ST}$
H	Yes, under high-resource conditions and $h^2 < 0.57$
DBH	Yes, when $h^2 < 0.16$
H/DBH	No
TFR	No
fRA	Yes, under low-resource conditions
SER	Yes, when $h^2 < 0.20$
SERa	Yes
CCB	No
CCBa	Yes, under low-resource conditions and $h^2 < 0.47$
BT130	Yes
BT10	No
MR*	Yes, under high-resource conditions and $h^2 < 0.30$
mRA*	Yes, when $h^2 < 0.23$
Aff**	No
Hff**	Yes, when $h^2 < 0.27$

Notes: *MR and mRA include qualitative data for 2010 at CUC site (high-resources) and quantitative data for 2015 at VED site (low-resources). **Aff and Hff were just measured at CUC site.

5. Discussion

In this thesis, we provide the first experimental quantitative evidence of plasticity for serotiny degree, canopy cone bank and bark thickness in a conifer, traits closely related to fitness in fire-prone ecosystems. We render also the first direct experimental evidence that endogenous conditions play a critical role in maintaining closed the serotinous cones in a conifer with variable serotiny. Moreover, we provide signals of local adaptation in some key life-history traits, underlying the importance of taking into account correlations and trade-offs among traits, and considering integrated phenotypes.

A replicated common garden avoided the problem of confounding environmental, allometric, and genetic effects. The highly contrasted sites induced both different survival rates, tree form, slenderness, as well as different relative allocation to growth and reproduction.

5.1. Variation of serotiny degree and aerial seedbank among sites and populations *(Study I)*

Results indicated that environmental stress can exacerbate the release of the aerial seedbank without fire. While the ecotypic variation on female reproductive investment and serotiny led to a high population differentiation across sites, in line with previous work (Hernández-Serrano *et al.*, 2013, 2014; Santos-del-Blanco *et al.*, 2013; Castellanos *et al.,* 2015), the phenotypic plasticity between sites played also a major role. By adjusting logistic allometric models relating degree of serotiny to tree height, we proposed a new parameter: the threshold size for serotiny loss (TSSL, tree height corresponding to 50% of serotinous cones), which allows a direct quantification of allometric plasticity for serotiny. Based on the TSSL values, the fastest loss of serotiny corresponded to the cold site, not to the dry-warm one. The lower degree of serotiny at dry conditions would be expected if the only effects involved in the opening of serotinous cones of this species were exogenous, i.e. direct environmental effects, but the fastest loss of serotiny relative to tree size for the same replicated populations under cold conditions reveals allometric plasticity due to a complex direct and indirect environmental effects involving costs of maintenance of closed cones as previously postulated for different species and confirmed for *P. halepensis* in *Study II* of this thesis (Lev-Yadun, 1995; Cramer and Midgley, 2009; Harris and Pannell, 2010; Espelta *et al.*, 2011; Tonnabel *et al.*, 2012; Martín-Sanz *et al.,* 2017). In fact, the total cone number also affected negatively the proportion of serotinous cones beyond tree size and site conditions, suggesting that the resources available to the tree likely impose trade-offs of resource allocation limiting the amount of cones that a tree can maintain closed. An alternative adaptive role of the precocious seed release in *P. halepensis* has been proposed, considering that severe drought could enhance interfire recruitment (Nathan *et al.*, 1999; Espelta *et al.*, 2011). However, this idea is based on the likely increase of favorable niches due to competition depletion related to tree mortality, but we lack experimental data supporting this, and the same environmental stress could in turn hinder pine recruitment (Alfaro-Sánchez *et al.*, 2015).

Surprisingly, literature on the variation of canopy seedbanks is far scarcer than that for serotiny. However, the amount of closed fruits or cones containing viable seeds determines the persistence ability of plant communities in fire-prone ecosystems when adult survival is low (Lamont and Enright, 2000; Tapias *et al.,* 2004). Among *P. halepensis* populations, drier and more continental environments of origin correlated with both higher degree of serotiny (Hernández-Serrano *et al.*, 2014) and higher reproductive allocation (Climent *et al.*, 2008). Consequently, in this thesis we have confirmed a higher canopy cone bank in dry continental populations. Hence, the plasticity trend for serotiny shown by our data could be acting in an opposite way to the differential selection among populations implied in local adaptation, i.e., counter-gradient plasticity (Kremer *et al.*, 2014). Although counter-gradient plasticity has been repeatedly mentioned in reviews (see, for example, Alpert and Simms, 2002), experimental evidence in plants is scarce (Valladares *et al.*, 2007; Merilä and Hendry, 2014). We should bear in mind that the canopy cone bank is a complex trait affected by reproductive precocity and reproductive allocation as well as by serotiny itself (Zammit and Westoby, 1988; Tapias *et al.,* 2001; De las Heras *et al.,* 2007). Furthermore, both fecundity and serotiny variation could be also strongly influence by growth rates and allocation to other functions. In fact, some *P. halepensis* populations achieved high aerial seedbank through early or/and intense reproduction, even with a low serotiny degree, and vice versa (Figure 42). Therefore, considering that close-to-fitness traits are the major target of adaptive evolution via natural selection (Stearns, 1977), looking solely to serotiny is not enough to predict the environmental effects on the amount of seed available for postfire recruitment.

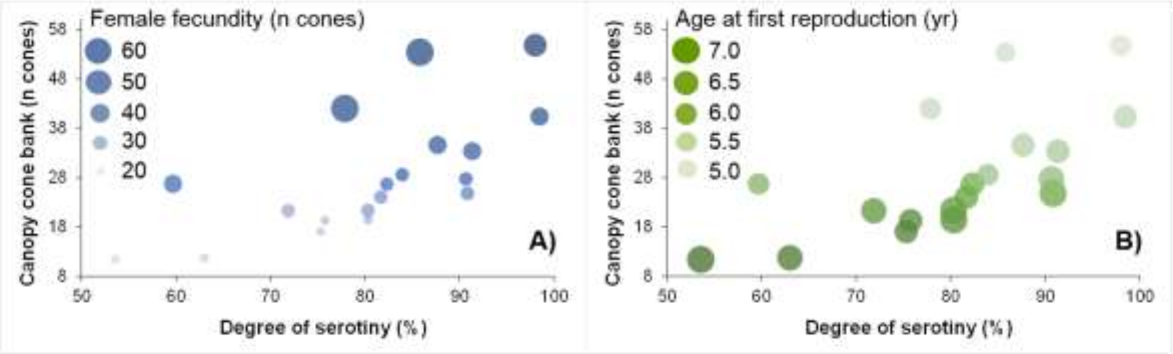

FIGURE 42 | Biplots of the canopy cone bank (CCB) against degree of serotiny among range-wide *Pinus halepensis* provenances (r = 0.67**). A) Bubble size represents female fecundity, through classes of total number of female cones produced during 16 years. Female fecundity is highly correlated with CCB (r = 0.93***). B) Bubble size represents classes of ages at first reproduction which is negatively correlated with CCB (r = -0.74***).

5.2. Maintenance costs of serotiny *(Study II)*

Our experiments showed that the duration of serotiny in *P. halepensis* involves the allocation of water to the cones, supporting that maintenance cost of serotiny can be more important than previously appreciated. We found continuous variation both in the opening temperature and in the scales density of the cones, which does not support the existence of two different types of cones as was previously revealed (Moya *et al.,* 2008; Salvatore *et al.,* 2010). Considering the effect of age on cone opening (older cones are more prompt to open; as in Tapias *et al.,* 2001) and that they also lost more water during our experiment, besides that our trees were fairly young, the higher serotiny of younger trees is not due to a higher inherent serotiny of the first cone cohorts but, very likely, due to a physical degradation (weathering) of cuticles and other compounds as the cone remains in the crown, like reported in other pine species (Elliot, 1988; Benkman *et al.,* 2003).

Contrary to what was previously admitted as a general rule for pines, our study reveals that the peduncle xylem of cones in *P. halepensis* remains as sapwood for a long time, therefore enabling the interaction between the tree and the cones (Figure 30A). Thick peduncles occur in some other serotinous pines, like *Pinus coulteri* D. Don (Borchet *et al.,* 2003), whereas in many others –including all other serotinous pines of the subsection *Pinaster*, the closest relatives to *P. halepensis*- the peduncles does not enlarge and transforms into heartwood. Other conifers like *Cupressus* or *Sequoia* are also well known to display living cones with sapwood peduncles (Lev-Yadun, 1995).

Once that age and other sources of variation were kept invariable thanks to the use of paired cones from the same whorl, both field manipulative experiment and chamber experiments confirmed that water intake through the peduncles affected significantly the pace of cone opening, such that lack of water supply or lack of connection with the bearing branch speeded up cone dehiscence. In addition, in the chamber experiment, waterless cones lost more water than their watered pairs further confirming that our water deprivation treatment was actually affecting the internal condition of the cones. However, most cones opened in a quite narrow temperature interval, i.e. the treatment effect between the two-paired cones was shown as different timing at the same temperature, not as different opening temperature. Confirming our expectations, the effect of watering the cone through the peduncle was detectable only at the lower range of accumulated temperatures, while higher temperatures (higher than those occurring naturally in the field in the absence of fire) provoked an abrupt opening that blurred the effect of water intake. On the other hand, our simple and straightforward field manipulative experiment seems to be the first of its kind and permitted eliminating all microenvironmental variation between the branched and the detached cones. Treatment effect increased over time and cone opening shot up during or after each summer season, especially for the detached cones. This demonstrated that the xeriscence condition of *P. halepensis* cones (i.e. only considering external environment; Nathan *et al.,* 1999) is clearly modified by the internal environment. Consistently with the ecotypic variation in the degree of serotiny confirmed in the first study of the thesis, treatment effect was significantly different among populations. While the effect was marked for populations ranging from weakly to moderately serotinous (Northeast group: less serotinous and reproductive), cones of trees from the few highly serotinous populations (Southwest group) virtually did not respond at all to the treatment, likely because of a stronger sealing of the cone scales.

These results open the field for more precise ecophysiological studies, since we did not measure actual water transfer via xylem or other possible interchange via phloem in the connected cones. Moreover, experiments investigating re-allocation of resources could help highlight the cost at the scale of the tree entailed by water allocation to the serotinous cones: for example, by assuming that tree physiology is partitioned between different branches, one could examine allocation to vegetative tissues and serotiny duration in branches where most cones would have been harvested compared to branches that remain intact. The existence of maintenance costs of serotinous cones has strong implications on the effects of climate change (combining more frequent severe droughts and wildfires) in the resilience of natural populations, via modifications of the canopy seedbanks and recruitment after stand-replacing fires. Moreover, evolutionary models for serotiny in *P. halepensis* must take into account the significant contribution of maintenance costs to the complex interaction between genotype and the environment.

5.3. Bark absolute thickness and bark allocation variation among sites and populations *(Study III)*

We found a marked phenotypic plasticity linked to site effects on bark traits. Our results confirmed the expectation that the growth-limiting dry and continental environment hampered bark thickness, such that at 18 years of age (close to an average-low fire return interval for this species) trees did not achieve the critical thickness necessary to survive fires. This could be detrimental for the resilience of this species' populations under increased drought and more frequent and intense forest fires driven by ongoing climate change. Moreover, our results confirmed a strong population genetic effect on absolute bark thickness, with Eastern Europe populations, which have higher growth and lower reproduction showing thicker bark, and populations from North Africa and Southern Spain –with lower growth, and more precocious and intense reproduction– displaying thinner absolute bark.

The vital strategy of *P. halepensis*, as an obligate seeder, is the maintenance of an aerial seedbank sufficient for the persistence of the population in case of fire (Tapias *et al.,* 2001; Ne’eman *et al.,* 2004). Therefore, it is essential that trees can survive low or moderately intense fires until reaching a sufficient aerial seedbank, which is usually achieved when *P. halepensis* trees are between 15 and 20 years old (Moreira *et al.,* 2011). We found that our trees generally reached the critical bark thickness at the base, but not at breast height. This is consistent with a greater thermal insulation at the stem base and a steep bark tapering along the bole found in several Mediterranean pine species (De Ronde, 1982; Pageaud, 1991; Jackson *et al.*, 1999). This can be regarded as an adaptive solution to reduce immaturity risk (Lamont *et al*., 1991; Keeley *et al.,* 1999). As predicted, the plasticity associated to different resource availability (mostly water availability during the vegetative period, since light, nutrients and CO_2 are not limiting in our study sites) clearly affects the probability of *P. halepensis* to survive fires; the lesser resources, the lower expected survival facing fire.

The maximum life expectancy of *P. halepensis* due to fire return interval is usually estimated between 30 and 50 years, while the average is about 25 years (Agee, 1998; Vázquez and Moreno, 1998). However, fire interval could be as short as 6 years in some areas of its natural distribution (Tessler, 2012; Tessler *et al*., 2014), due to Mediterranean forest fires are mainly caused by human ignitions. When fire interval is shorter than 15 years, *P. halepensis* recruitment would be limited, and its populations may totally disappear (Roitemberg and Ne'eman, 2000; Eugenio *et al*., 2006; Herman, 2009). Therefore, the age of our sampled trees –18 years– is highly meaningful considering the species' life-history. Bearing this in mind, the lower probability of survival associated with a lower canopy seedbank at low-resource environments due to much lower serotiny degree derived from cone xeriscence and costs of maintenance (Martín-Sanz *et al.,* 2016, 2017) would lead to a lower recruitment after fire under environmental limitations.

When studying allocation to bark, we followed a new methodological approach intending to be closer to the real allometry between the bark and the rest of the tree, instead of using the usual ratio with stem diameter (see for example, Paine *et al.,* 2010; Pausas, 2015b; Midgley and Lawes, 2016). We considered this better defined by comparing the percentage of bark volume versus the total volume of the trunk, taking also into account that *P. halepensis* trees do not show heartwood at the age of our trees. As expected, bark volume was closely associated to total volume. This allometric effect implies that the evolutionary forces acting on plant size will produce changes in bark thickness (Rosell, 2016). Due to this relationship, when the goal is to compare differential allocation to the bark among species or populations, plant size must be taken into account (Hempson *et al*., 2014; Poorter *et al.,* 2014; Rosell and Olson, 2014). The size effect is implicit in the percentage of bark, so the site effect on this percentage indicated a significant plasticity, although moderate in value, in the allocation of resources to the bark. Besides, total volume by site interaction showed the change in plasticity due to size. These results indicated that at the same tree size and under the scarcity of resources, trees have both thinner absolute bark and less bark percentage. While differential relative allocation to bark is essential under a functional-evolutionary perspective, survival to fire clearly depends on absolute bark thickness (Midgley *et al.,* 2010; Lawes *et al.,* 2011a). Considering the strong selective pressure of differential survival to fire, the allometry of bark allocation could be different depending on whether trees have reached the critical bark thickness or not. Thus, our study indicated that *P. halepensis* allocates resources to bark until attaining a minimum thickness that would allow individual survival under surface and moderately-intense fires when resource availability allows it. This suggests a more variable adaptive strategy to cope with fire than has been considered so far (see Ducrey *et al.,* 1996; Rigolot, 2004; Fernandes *et al.,* 2008).

To our knowledge, this is the first study providing experimental evidence of plasticity for this key adaptive trait, in interaction with population differentiation, and combining fire resistance and allometric allocation approaches. We still lack direct experimental data on the critical bark thickness for *P. halepensis* survival, and on the possible differences in bark morphology, its internal structure (separating between inner and outer bark) and the rate of bark thickness tapering along the entire bole; aspects that can be critical for tree survival (Harmon 1984). Moreover, the possible trade-offs with other key processes (namely reproduction or defense) deserve further investigation under a climate change scenario.

5.4. Adaptive variation in *P. halepensis*: population differentiation and phenotypic integration *(Study IV)*

We tried to find signs of local adaptation in the population differentiation of life-history traits in *P. halepensis*. Phenotypic variability in our studied life-history traits of this species was largely the result of phenotypic plasticity, but with apparent ecotypic trends for most of them. Moreover, we found relevant correlations between traits related to growth, reproduction and defense against fire at the population level in this species, consistently with previous studies and counterpartying theoretical allocation predictions and life-history theories of the species. Therefore, the highest growing populations (those in the east of the distribution) also had thicker bark and less investment in reproduction. Besides, populations that delay female flowering (in age and size), also showed lower male and female reproduction, which ultimately means lower aerial cone bank. Following results on other species (*Banksia sp*., Clarke *et al.,* 2016), we did not find trade-offs between the two different fire strategies (serotiny degree and bark thickness), nor between serotiny and growth. Importantly, the combined analysis between vegetative growth and reproductive traits with fire-adaptive traits has never been performed in pine species. Summarizing, we observed a strong east-west and north-south geographical pattern for the studied traits, conditioned by the incidence of climatic variables associated with water stress (see below), and pointing towards the combined effect of the pattern of post-glacial recolonization and the environmental heterogeneity of the Mediterranean basin as modulators of the current adaptive structure of this species.

We also tested the relationship between the ecogeographic variables and the different adaptive phenotypes, with results in line with previous studies (see Climent *et al.,* 2008; Voltas *et al.,* 2008). The multivariate analysis of population climatic variables for *P. halepensis* showed that the first principal component was mainly influenced by rainfall (especially summer precipitation) and the second principal component was correlated with temperature (largely temperature oscillation), contrary to commonly found results, in which genetic clines (and the first principal component) are better explained by geographic variation in temperature, while the second principal component is correlated with moisture variables (Rehfeldt, 1995; Rehfeldt *et al.,* 2002; Wang *et al.,* 2006; Bower and Aitken, 2007, but see Rehfeldt *et al.,* 1999; St Clair *et al.,* 2005 for the contrary pattern). Drought is an important limiting factor for Mediterranean species (Baquedano *et al.,* 2008; Benito-Garzón *et al.,* 2011), and thus a correlation between rainfall and life-history traits was expected, although growth cessation and cold hardiness is also key for the survival of forest trees (Tanino *et al.,* 2010; Cooke *et al.,* 2012). Accordingly, continentally index (annual temperature oscillation) also correlated with reproductive traits and serotiny degree, but in an opposite way. In fact, given that both cold temperatures and drought trigger similar physiological responses in plants (Bigras and Colombo, 2001; Seki *et al.,* 2002; Blödner *et al.,* 2005; Pot *et al.,* 2005), the same genes have been found to control responses to both types of stress (Urano *et al.,* 2010; Perdiguero *et al.,* 2013; Jaramillo-Correa *et al.,* 2015). Actually, the importance of precipitation is often stronger in its interaction with temperature (Andalo *et al.,* 2005), so the variation among populations in precipitation explains higher phenotypic variation in populations of continental than of maritime climates and at lower than at higher altitudes (Aitken *et al.,* 2008). Adaptation to these climatic variables is critical for ensuring survival in plants (Condit *et al.,* 1995), so that maladaptation to increasing drought and temperature can magnify vulnerability for forests under climate change (Carnicer *et al.,* 2011; Choat *et al.,* 2012). Moreover, summer rainfall is correlated with the area burnt in forest fires (Pausas, 2004; Pausas and Paula, 2012; Hernández-Serrano *et al.,* 2013), so it was also linked to fire-related traits such as bark thickness. Higher fire frequency selected for thicker basal bark, but not bark at breast height. In addition, the higher the frequency of fire, the lower the male reproductive allocation. It must be borne in mind that we only used data for the origin areas of the Iberian populations, due to the lack of fire records for non-Iberian populations. In summary, precocious and highly reproductive populations with thin bark mainly originated in areas with intense and long droughts (lower spring and summer rainfall), and high annual temperature oscillation that define short growing seasons. Less precocious populations with less intense reproduction, faster vegetative growth and thicker bark generally corresponded to natural areas with fewer and less strong droughts and milder temperatures that define long growing seasons. This pattern could be due to the necessity of outcoming neighboring trees, thus involving more investment in vegetative growth to favor a competitive strategy (Grime, 1977; Falster and Westoby, 2003). Serotiny degree follows the same pattern in relation to precipitation, but it was hampered by a greater thermal oscillation as we previously detected. These results completely agree with assumptions derived from life-history theory, predicting a delayed reproduction in environments that favor vegetative growth (Roff, 1992).

Finally, our results proved evidence of clear genetic differences in key life-history traits among populations of *P. halepensis* sampled across a wide range of environments as well as substantial phenotypic variation among populations, in line with previous studies (Climent *et al.,* 2008; Santos-del-Blanco *et al.,* 2010, 2013). The global F_{ST} (0.136) was moderate and similar to previous values reported for this species (Rodriguez-Quilón, 2017). Phenotypic differentiation among populations was higher than or equal to that estimated with molecular markers, varying considerably between sites and traits. The quantitative genetic differentiation calculated with the most plausible h^2 values was consistently higher than that estimated with molecular markers across environments for key traits such as serotiny degree and bark thickness at breast height. This suggests that populations show higher differentiation in those traits than that expected only by drift or gene flow, so adaptive phenotypic variation is related to environmental conditions where populations have evolved (reviewed in Alberto *et al.*, 2013), implying that very likely local adaptation processes have occurred. These measurements of among-population divergence, i.e. Q_{ST} values, are inherently dependent on the heritability of the character. Therefore, the calculated values of Q_{ST} are more or less probable depending on the plausibility of the heritability values used in this thesis (more credible h^2 values from groups 1 and 2; Tables 7 and 12). For instance, the fact that $Q_{ST} > F_{ST}$ is highly probable in female reproductive allocation (h^2 from Santos-del-Blanco *et al.*, 2010) under harsher environments, in height (h^2 from Matziris, 2000) as long as h^2 < 0.57 or in DBH (h^2 from Matziris, 2000) when h^2 < 0.16. For serotiny degree, the narrow sense heritability of 0.20 (Hernández-Serrano *et al.*, 2014) is the limit for which phenotypic differentiation is higher than neutral genetic differentiation. Obviously, performing these analyses in provenance-progeny or progeny trials that allow the estimation of heritability values for the studied traits, would provide much more accurate results. However, experiments with sufficient number of provenances and progenies within them are extremely rare, due to their size and cost[1]. In addition, to calculate and compare Q_{ST} and F_{ST} parameters it would be ideal to carry out the sampling of quantitative traits and molecular markers, both on the same populations and on the same individuals. Moreover, although it is widely used, this approach has limitations (Leinonen *et al.*, 2013; Tiffin and Ross-Ibarra, 2014) and the results of $Q_{ST} > F_{ST}$ comparison should not be considered as a definite signal of local adaptation and directional natural selection. Firstly, maternal effects, while little studied in conifers (Zas *et al.*, 2013), can affect early life traits such as germination rates (Castro, 1999; Castro *et al.*, 2008; De Kort *et al.*, 2013). Secondly, molecular markers might overestimate F_{ST} if they are non-neutral, or underestimate it if they are highly polymorphic. And finally, non-additive genetic interactions may affect Q_{ST} -F_{ST} estimates when the trait studied is controlled by several genes (i.e. polygenic). In general, population genetic approaches are widely recognized that can lead to a large number of false positives, since population structures and evolutionary histories are generally not considered to be arise from the expected neutral F_{ST} values, and Q_{ST} may be hindered by environmental effects (Le Corre and Kremer, 2012; Leinonen *et al.*, 2013). Therefore, in order to understand the evolutionary forces acting on *P. halepensis* populations and ensure that the ecotypic patterns found are due to selection, should be bear in mind that correlations with climatic variables might be biased if those variables are spatially structured or partially match the genetic structure of the species (Jaramillo-Correa *et al.,* 2015). Consequently, to really know if the ecotypic patterns indicate local adaptation it is essential to correct them by eliminating the species' neutral genetic structure (which for *P. halepensis* will be soon available). In addition, it is still essential to unravel the genetic architecture of the adaptive

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[1] To our knowledge, there is a single provenance-progeny trial of *P. halepensis* (Hernández-Serrano *et al.*, 2014), with two fundamental problems: 1) it includes a small number of progenies within the provenances (only 3 to 5 progenies), and 2) the absence of extreme populations in this species (those from the east of the distribution), including only Spanish Iberian populations in which the differences in life-history traits are much less pronounced.

traits in this species, for which the integration of quantitative genetic knowledge and new phenotype-genotype association methods is required.

As might be expected, the greatest environmental dissimilarity occurred between the environments of origin of the populations and the harsh, low-resource site. However, correlations between phenotypic traits and Gower’s distance to each of the test sites were not significant, pointing out that our results are not biased by the particular environments of the test sites, that is, the environments of the trial sites are not favoring/hampering some provenances and not others. Therefore, our results could be extrapolated to any other trial site. In addition, Gower's ecological distance can be considered as another way to interpret the genotype x environment interaction in phenotypic traits, from a qualitative perspective. The genotype-by-environment interaction is paramount since it allows to understand how organisms can respond to the ongoing climate change, and can also indicate local adaptation if populations have a better performance in the site most similar to the conditions of their site of origin (Vergeer and Kunin, 2013). Life-history traits, in comparison with morphological characters, tend to show higher additive genetic and nonadditive and nongenetic variability (Houle, 1992; Hansen *et al*., 2011). We found that variation in plasticity among populations was greater for vegetative growth traits than for reproductive traits and serotiny degree, consistent with findings in different plant genera (reviewed in Weiner et al., 2009). Although our results showed the existence of G x E for almost all the studied traits, correlations with Gower's distance were not significant, thus indicating that G x E interactions were non-cross-over (i.e., there were just variance differences; El-Soda *et al.,* 2014; Heslot *et al.,* 2014; Roles *et al.,* 2016; Saltz *et al*., 2018), being of lesser relevance due to the lack of changes in population rank (El-Soda *et al.*, 2014).

Importantly, although bark thickness would be expected as a marginal trait in *P. halepensis*, due to its obligate-seeder nature, our results suggested higher relevance of this trait. Bark thickness, both at breast height as at tree base, presented a strong phenotypic plasticity and differentiation among provenances, but insignificant genotype-by-environment interaction. This lack of population differentiation for plasticity in bark thickness is an extreme case in which the ranking of populations and the differences of variance among them were extremely similar between both test sites, despite the great ecological and climatic differences. This could be due to non-exclusive causes such as a close dependence of bark thickness on vegetative traits, the perception of environmental heterogeneity, selecting for plasticity, which differs between traits related with different functions, or a strong stabilizing selection for plasticity of this trait among populations. The latter seems to occur in basal bark thickness where the lack of ecotypic patterns might indicate that all individuals, regardless of the population, must achieve a minimum basal bark thickness in order to survive surface or moderate intense fires until they reach a sufficient aerial seedbank for the population persistence under wildfires. In addition, the quantitative differentiation among populations for bark thickness at breast height is consistently higher than that of neutral genetic differentiation, thus showing a clear ecotypic pattern with strong evidence of local adaptation.

5.5. Implications for adaptive forest management and conservation

Under a scenario of increased disturbances such as forest fires and extreme droughts (Lindner *et al.,* 2010), life expectancy of trees might be lower than in the present, particularly in the western Mediterranean basin. Moreover, fire regimes change over time and space both because of the randomness of ignitions at short scales (Moritz *et al.,* 2012), and due to the interaction between fire drivers ─fuels, ignitions, and weather conditions─ which are largely affected by the ongoing global change (IPCC 2007, 2013). These changes in fire regime are the most dramatic threat to serotinous species by reducing growth rates and thereby increasing immaturity risk by decreasing the frequency of favorable recruitment years and by reducing fire intervals (Enright *et al.,* 2015).

By contrast, human-driven changes of fire regimes in some areas may imply fire suppression helped by fuel control and rapid extinction, which could particularly affect to obligate-seeder species due to the lack of suitable niches and competitive advantage of alternative life-history strategies. Although designing silviculture to promote aerial seedbanks is not frequent, except when seeds have high commercial value, like in the non-serotinous stone pines (e.g. *Pinus pinea*, Pasalodos-Tato *et al*., 2016), forest management practices can directly increase or deplete aerial seedbanks. Most traditional forest management methods include the modification of competition and micro-environment by selectively eliminating undesired species or the same species (clearings), and in some cases can even affect the individual aerial biomass allocation by pruning. In addition, different types of prescribed burning normally used to decrease the risk of crown fires by depleting fuels accumulated in forest understory can lead to very different and even totally conflicting results (Elliot and Vose, 2005; Sharpe *et al.,* 2017). Finally, serotiny genetic variability both among populations and within them has strong implications for management. As evolvable traits that can respond both to natural or artificial selection, serotiny and aerial seedbanks could be managed for (or against), either by in-situ selection or breeding. Moreover, genetic correlations among traits will determine how selection for one trait will affect another. So far, an example of accidentally wrong provenance choice regarding serotiny has been reported in *Pinus pinaster* (Gil *et al.,* 2009), emphasizing the importance of taking into account the origin of the seed when carrying out reforestations. Recently emerged adaptive forest management, aiming to maintain evolutionary processes and adaptive potential of managed populations (Koskela *et al.,* 2013; Lefèvre *et al.,* 2013) should take into account reproductive traits and serotiny degree to finally adequately manage aerial seedbanks. Managers should bear in mind that reducing competition and pruning as well as different prescribed burning types can affect the aerial seedbanks in a rather complex way depending on the particular species, developmental stage and site conditions. Moreover, serotiny and aerial seedbanks should be considered in genetic conservation and assisted migration programs that target adaptation to future climate scenarios (Oney *et al.,* 2012; Lefèvre *et al.,* 2014).

6. Conclusions

1. *Pinus halepensis* populations vary significantly in terms of life-history traits related to adaptation to fire like serotiny degree, aerial cone bank and bark thickness.

2. Smaller *P. halepensis* trees were more serotinous than larger ones due to the strong allometric effect found in serotiny degree, a pattern considered adaptive for plants confronting short fire-return intervals. However, the threshold size for serotiny loss (TSSL), a new parameter which allows a direct quantification of allometric plasticity for serotiny, indicated a faster loss of serotiny in areas with higher temperature oscillation (higher continentally index).

3. Growth-limiting environments exacerbated the precocious release of seeds from serotinous cones, contrary to the ecotypic trend found for the aerial cone bank. This counter-gradient plastic response is potentially maladaptive under a scenario of frequent wildfires.

4. Cone serotiny in *P. halepensis* involves the allocation of water to the cones, which implies that maintenance costs of serotiny in this species can be more important than previously thought. This fact supports that the observed plasticity on *P. halepensis* serotiny degree depends both on exogenous and endogenous factors.

5. It would be necessary to clarify the xeriscent nature that is currently attributed to *P. halepensis*, being more accurate to talk about variable serotiny among populations, modulated both by the direct environmental effect and by the hydric status of the tree.

6. Harsh environments with low resource availability hampered bark thickness on *P. halepensis* such that trees did not achieve the critical bark thickness necessary to survive fires at 18 years of age, a time close to an average-low fire return interval for this species.

7. The combined findings in plasticity of aerial cone bank –including maintenance costs of serotiny– and bark thickness, could have strong implications in the resilience of natural *P. halepensis* populations. The risk of immaturity, i.e. death by fire before achieving sufficient aerial cone bank ensuring recruitment, could increase dramatically under more frequent and intense droughts and forest fires driven by ongoing climate change*.*

8. We found high genetic differentiation in all life-history traits studied in *P. halepensis,* while genotype-by-environment interaction was greater in vegetative growth traits than in reproductive or fire-adaptive characters. In addition, this interaction indicated variance differences among populations, but not changes in population ranking.

9. In *P. halepensis*, the existence of local adaptation is supported by the correlation between eco-geographical variables and phenotypes integrated by multiple adaptive traits. Allocation to adaptive traits related to growth and bark thickness, and reproduction traded-off against each other, agreeing theoretical predictions from allocation and life-history theories. Serotiny degree positively correlated with reproduction but did not show trade-offs with other functions.

10. Neutral genetic differentiation (F_{ST}) found among populations was lower than the quantitative differentiation (Q_{ST}) on serotiny degree and bark thickness at breast height across contrasting environments. This suggests spatially divergent selection, which points out evidence of local adaptation.

11. Under the current global change, reproductive traits and serotiny degree must be taken into account to adequately manage the aerial seedbanks in forest management and conservation, trying also to apply silvicultural treatments that reduce the immaturity risk of the trees.

7. Conclusiones

1. Las poblaciones de *Pinus halepensis* varían significativamente en los caracteres de historia vital relacionados con la defensa contra incendios como el grado de serotinia, el banco aéreo de conos y el espesor de la corteza.

2. Los árboles más pequeños de *P. halepensis* fueron más serótinos que los más grandes debido al fuerte efecto alométrico encontrado en el grado de serotinia, un patrón considerado adaptativo para las plantas que se enfrentan a cortos intervalos de retorno del fuego. Sin embargo, el tamaño umbral para la pérdida de la serotinia (TSSL), un nuevo parámetro que permite una cuantificación directa de la plasticidad alométrica para este carácter, indicó una pérdida más rápida de la serotinia en áreas con mayor oscilación térmica (índice de continentalidad más alto).

3. Los ambientes limitantes para el crecimiento de *P. halepensis* agravaron la liberación precoz de semillas de conos serótinos, de forma contraria a la tendencia ecotípica encontrada para el banco aéreo de conos. Esta respuesta plástica contra-gradiente es potencialmente desadaptativa bajo un escenario de frecuentes incendios forestales.

4. La serotinia de *P. halepensis* implica la asignación de agua a los conos, lo que sugiere que los costes de mantenimiento de la serotinia en esta especie son más importantes de lo que se pensaba. Este hecho confirma que la plasticidad observada en el grado de serotinia en *P. halepensis* parece depender tanto de factores exógenos como endógenos.

5. Sería necesario aclarar la naturaleza xeriscente que actualmente se atribuye a *P. halepensis*, siendo más preciso hablar de serotinia variable entre poblaciones, modulada tanto por el efecto ambiental directo como por el estado hídrico del árbol.

6. Los entornos desfavorables con baja disponibilidad de recursos limitaron el crecimiento en espesor de la corteza en *P. halepensis*, de modo que los árboles no alcanzaron el espesor crítico de corteza necesario para sobrevivir a los incendios a los 18 años, una edad cercana a un intervalo de retorno de incendios medio-bajo para esta especie.

7. Los resultados combinados de plasticidad del banco aéreo de conos –incluidos los costes de mantenimiento de la serotinia– y el espesor de corteza, podrían tener fuertes implicaciones en la capacidad de recuperación de las poblaciones naturales de *P. halepensis*. El riesgo de inmadurez, es decir, la muerte por fuego antes de lograr un banco aéreo de conos suficiente que asegure el reclutamiento, podría aumentar drásticamente bajo las fuertes y más frecuentes sequías e incendios forestales provocados por el cambio climático actual.

8. Encontramos elevada diferenciación genética en todos los caracteres de historia vital estudiados en *P. halepensis*, mientras que la interacción genotipo por ambiente fue mayor en caracteres de crecimiento vegetativo que en los reproductivos o de adaptación al fuego. Además, esta interacción indicó diferencias de varianza entre poblaciones, pero no cambios de ranking entre ellas.

9. En *P. halepensis*, la existencia de adaptación local está respaldada por la correlación entre las variables ecogeográficas y los fenotipos integrados por múltiples rasgos adaptativos. La asignación de recursos a los caracteres adaptativos relacionados con el crecimiento y el espesor de la corteza, y la reproducción se compensan entre sí, coincidiendo con las predicciones de las teorías de la asignación y de la historia de vida. El grado de serotinia se correlacionó positivamente con la reproducción, pero no mostró compensaciones con otras funciones.

10. La diferenciación genética neutra (F_{ST}) encontrada entre las poblaciones fue menor que la diferenciación cuantitativa (Q_{ST}) en el grado de serotinia y en el espesor de corteza a la altura del pecho en ambientes contrastantes en

cuanto a disponibilidad de recursos. Esto sugiere una selección espacialmente divergente, señalando evidencias de adaptación local.

11. Bajo el cambio global actual, los rasgos reproductivos y el grado de serotinia deben ser tomados en cuenta para administrar adecuadamente los bancos de semillas aéreos en el manejo y conservación forestal, tratando también de aplicar tratamientos silvícolas que reduzcan el riesgo de inmadurez de los árboles.

8. Perspectives

8.1. Construction and maintenance costs of serotiny

We have confirmed that the supply of water through the thick living peduncles of the cones strongly influences the opening speed of serotinous cones in *P. halepensis*, suggesting maintenance costs of serotiny in this species. Despite its relevance, maintenance costs of serotiny have been poorly examined, besides even considering that conifers do not face these costs. However, recent studies have indicated evidence of putative maintenance costs of serotiny not only in Proteaceae species (the most studied species in this sense) but also in various conifer species. Moreover, it is broadly assumed that serotiny implies increased costs per seed than soil-stored seedbanks (carbon, water, and canopy growth constraints; Lamont and Enright, 2000), but empirical evidence of these higher costs is also meagre. Other potential or theoretical costs of serotiny, like architectural and biomechanical costs (closely related to the total costs of reproduction) are still mostly uninvestigated. Therefore, what the construction costs of the cones in terms of the amount of C, N or other allocated compounds are, or what the real maintenance costs of serotinous cones are, that is, how much water or other compounds these cones consume in relation to leave transpiration or other functions, have not been studied directly. Consequently, performing experiments that would allow the estimation of these costs, both construction and maintenance, is fundamental to understand the variability and evolution of serotiny. Furthermore, the fact that peduncles of cones in *P. halepensis*, as well as other conifers, present xylem with sapwood and living cortical tissues, which allow a vascular interaction between the cones and the tree is fundamental, but it has never been studied. A broad comparative study on this feature among different serotinous species and in particular across genus *Pinus* is essential to understand the maintenance costs of serotinous cones.

8.2. Physiological production costs of bark

In this thesis we proved the existence of plasticity for bark thickness in *Pinus halepensis* with dry continental environments hampering this trait, such that trees need much more time to achieve a critical bark thickness essential to survive fires. In addition, our results indicated that this species allocates resources to bark until attaining that minimum thickness that could allow individual survival under surface and moderately-intense fires when resource availability allows it, suggesting that bark thickness in *P. halepensis* is a trait not as marginal as one would expect from the life history of this species. However, we still lack direct experimental data on the critical bark thickness for *P. halepensis* survival, as well as detailed studies on the bark internal structure, differentiating between inner and outer bark. Furthermore, construction costs of bark are highly important concerning resource allocation but have never been studied, as neither the comparison between the production costs of wood and bark. To better understand the ecotypic patterns and phenotypic plasticity found in bark thickness, as well as the patterns of resource allocation among populations of the same species or even among different species, determining the construction costs of the bark and wood becomes indispensable. Defining these construction costs could be as simple as performing a C/N analysis and an ashing/nitrate determination afterwards, since carbon concentration is usually a good indicator of construction costs.

8.3. Further insights in reproductive strategies

Although results of this thesis have considerably increased our understanding about the adaptive genetic variation in a Mediterranean conifer, and fundamentally in the causes of variation of the aerial seedbank, we still lack studies on the possible epigenetic, maternal or masting effects on the aerial seedbanks.

Compared with pines typically seen as masting species, *P. halepensis* interannual variability in reproduction is scarce (the interval between large crops has been shown to be just one year; Tapias *et al.*, 2004), but actually, whether it is a masting species or not has not been studied in depth. Possible mast seeding of this species is highly relevant considering the previously detected costs of reproduction (Santos-del-Blanco *et al.*, 2014). To our knowledge, just one work has considered masting with other key adaptive traits in Mediterranean pine species (Tapias *et al.*, 2004). Here, authors found that masting correlated positively with age of first flowering in *P. halepensis* and traded-off with the percentage of serotinous cones and cone persistence in the canopy.

Genetic effects of serotiny and total female reproduction might influence the genetic composition of the aerial seedbank (Barrett *et al.,* 2005) and/or the post-fire recruitment (Gershberg *et al.,* 2016). Moreover, maternal and epigenetic effects would be added to those genetic effects within the aerial seedbanks. Allocation of resources to the female function in serotinous species is so large that has been compared to cases of maternal care in animals (Harris and Pannell, 2010; Tonnabel *et al.,* 2012), but maternal effects in conifers are still under-investigated (Zas et al., 2013). These maternal effects are based on seed size and above all, affect the germination rate and the early seedling development (Castro, 1999; Castro *et al.*, 2008; Donohue, 2009; De Kort *et al.*, 2013). Although empirical results so far indicate negligible genetic differences attributable to different degrees of serotiny, theoretical approaches on the evolutionary implications of seedbanks endorse an exciting, yet highly challenging field of research (Vitalis *et al.,* 2004). Possibly a combination of high resolution genetic and epigenetic molecular markers could give some relevant insights in a near future.

8.4. Fire regimes

To enhance our understanding of the role of fire in shaping plant life-history traits and how climate change and new disturbance events will affect forests, we need more detailed fire regime information. By fire regime, it is understood the complex combination of fire characteristics that predominate in a given ecosystem (Keeley *et al.*, 2012; Pausas and Keeley, 2014). It includes frequency -fire return interval in relation to plant longevity-, intensity -flame height in relation to the height of canopy fuels-, seasonality and type of fuels consumed which includes two contrasted fire types (understory or surface fires: flame height is lower than the overstory, and crown fires; Pausas, 2015a, 2017).

All these characteristics (temporal variation in fire regimes, spatial pattern –size and spatial complexity– and magnitude –intensity, severity and type of fire–) are important factors that have shaped the evolution of life-histories in plants. For example, the patchiness derived from higher heterogeneity in fire intensity should select against serotiny (Buma *et al.,* 2013), or the standardization of fire regimes can likely unify the diversity of life history strategies (Tonnabel *et al.,* 2012; Olivieri *et al.,* 2016). Such possible effects deserve more research in the future in order to help our understanding of human impacts in serotinous species and in aerial seedbanks. For this, obtaining more and more accurate amount of data of wildfires, trying to cover as much as possible all aspects of fire regime would be essential.

8.5. Genetic population structure and local adaptation

To deepen in the understanding of local adaptation in plant species, beyond just Q_{ST}-F_{ST} comparison, which has several disadvantages, an appropriate option is to use the neutral genetic structure of the species. However, this structure for *P. halepensis* is not yet available, although researchers of our group, within the framework of other projects, are working on it and will be published shortly (by D. Grivet and R. Ruiz-Daniels). For this, they have genotyped a wide number of *P. halepensis* populations. Using their SNPs dataset and the Bayesian clustering method STRUCTURE (Pritchard *et al.,* 2000), I have re-analyzed the data to obtain the neutral genetic structure of the studied populations on this thesis. In this way, we can compare if our result, with fewer populations, follows the neutral genetic structure that will be obtained in the near-future for the whole species. Once information about the neutral genetic structure of *P. halepensis* will be published for the entire population sample, it will be used to correct correlations between environmental and geographical variables, and phenotypic traits, in order to check whether the ecotypic patterns found for different traits in this species along this thesis are actually due to natural selection rather than to drift or historical effects, which would show the existence of local adaptation.

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10. Supplementary Information

10.1. PCA analysis for environmental variables

In this first section of Supplementary Information, we show the results of the PCA analysis performed to reduce the number of environmental variables. The parallel analysis associated with the PCA for environmental variables at the populations origins revealed that two principal components should be retained (Figure S10.1.1; variance explained: PC1 = 45%, PC2 = 36%). Overall and taking into account loadings above 0.80, the first principal component was positively related to precipitation of the warmest (March, April, May) and driest seasons (June, July, August), and precipitation of the driest month. PC2 was negatively related to the continentally index and positively to wettest season rainfall (September, October and November; Table S10.1.1).

FIGURE S10.1.1 **|** Scree plot for the PCA of the nine environmental variables.

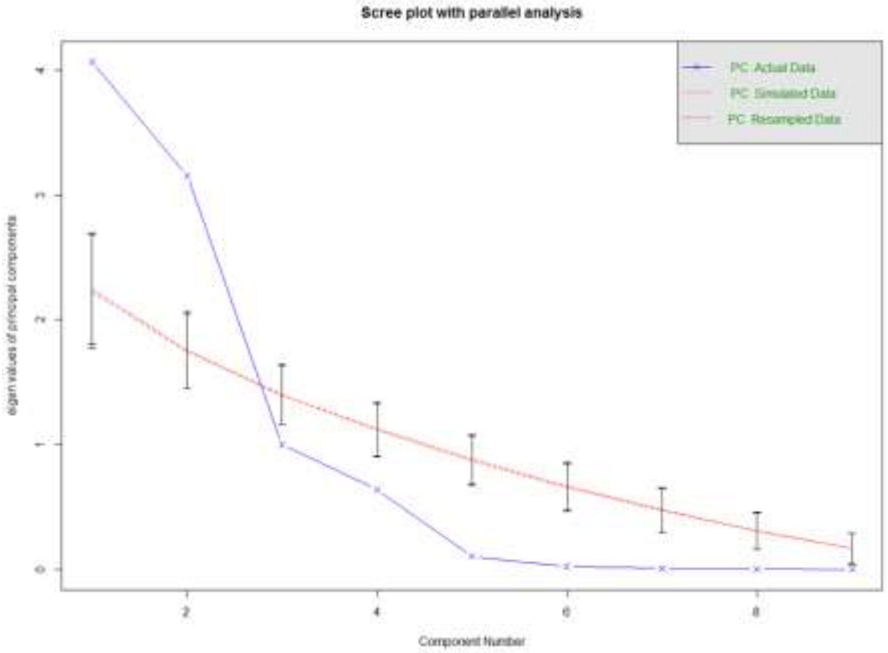

TABLE S10.1.1 | Results from Principal Component Analysis applied to environmental data from 19 *Pinus halepensis* source populations. Variables with loadings > |0.80| in bold case.

Variable	PC1	PC2
Rainfall of driest[†] season	**0.95**	-0.16
Rainfall of warmest[⁕] season	**0.94**	0.05
Rainfall of wettest[✹] season	0.09	**0.82**
Rainfall of driest month	**0.90**	-0.21
Annual rainfall	0.61	0.51
Mean temperature of warmest month	-0.79	-0.30
Mean temperature of coldest month	-0.15	**0.94**
Continentally index[*]	-0.43	**-0.82**
Annual mean temperature	-0.47	0.75
Importance of components		
Proportion of variance explained	0.45	0.36
Cumulative proportion of variance	0.45	0.80

Notes: [†] Driest season: June, July and August. [⁕] Warmest season: March, April and May. [✹] Wettest season: September, October and November. [*] Difference between mean temperature of the warmest month and mean temperature of the coldest month.

10.2. Allometric analysis for bark thickness

Here, we detail the methodology followed to perform the allometric analysis of bark thickness and the obtained results.

10.2.1. Methods

Allometric analyses are strongly accepted among forestry researchers and their parameters allow general studies of comparison among species. Several researchers (Henry and Aarssen, 1999; Niklas, 2006; Warton *et al.,* 2006) have discussed that model II regression, e.g. standardized major axis (SMA), is more suitable than traditional least squares for adjusting allometric data. Therefore, we used SMA to examine the bark volume regarding the total volume of the cone trunk from the tree base to breast height. The classical allometric model is $VB = aVT^b$, and is usually fit as $\log VB = \log10\ a + b\ \log10\ VT$ (Huxley, 1932; Niklas, 1994; Ter-Mikaelian and Korzukhin, 1997). Parameter *a* is the elevation or allometric coefficient and parameter *b* is the regression slope or allometric exponent. An exponent significantly different from 1 indicates an allometric relationship between the variables studied (increasing or decreasing with size, non-constant).

The allometric exponents of each population at each site were compared with the isometric coefficient ($b = 1$) and to one another by multiple post hoc comparisons. We also checked for differences in the allometric coefficient or elevation. These analyses were carried out with the smatr package (Warton *et al.,* 2012) implemented in R software v3.3.2. (R Core Team, 2016). As the SMA analysis indicated that slopes differed among populations and sites, a general SMA test of elevation (allometric parameter) differences was not necessary.

10.2.2. Results

Most of the populations showed constant investment in bark with tree size ($b = 1$) at the high-resource site, i.e. isometric exponent, except three populations showing decreasing investment in bark (allometric exponent: $b < 1$; Table S7). At the low-resource site, half of the populations had isometric exponents ($b = 1$) and the other half decreasing investment with tree size ($b < 1$). In general, populations at the high-resource site showed slopes closer to 1 (mean $b = 0.92$) than the same populations at the low-resource site (mean $b = 0.86$;

differences between slopes were significant: LRT = 5.56, df = 1, P-value = 0.018). However, population ranking changed between sites (population by site interaction was significant: LRT = 55.06, df = 37, *P*-value = 0.028) , so that high reproductive populations had higher slopes at the high-resource site and lower slopes at the low-resource site, in which high growth populations had bigger slopes.

TABLE S10.2.1 | Bark allometric exponents (b) for bark volume of each population at the two experimental sites (confidence intervals in brackets). Significant *P* value indicates that b is different from 1 (isometric coefficient). r^2 and significance for the standardized major axis regression (SMA).

	Allometric exponent (α)		***P* value**		**r^2**	
Code	**High-resources**	**Low-resources**	**High-resources**	**Low-resources**	**High-resources**	**Low-resources**
11	0.87 [0.71-1.08]	0.89 [0.70-1.12]	0.184	0.280	0.82***	0.87***
21	0.80 [0.68-0.94]	0.99 [0.78-1.25]	**0.011**	0.896	0.97***	0.86***
92	0.93 [0.85-1.02]	0.77 [0.40-1.49]	0.098	0.410	0.99***	0.82***
101	0.85 [0.66-1.10]	0.97 [0.77-1.22]	0.201	0.795	0.79***	0.91***
105	0.98 [0.83-1.16]	0.97 [0.61-1.56]	0.808	0.900	0.92***	0.45**
111	0.87 [0.74-1.01]	0.86 [0.75-0.98]	0.068	**0.027**	0.93***	0.94***
131	0.90 [0.78-1.04]	0.95 [0.76-1.17]	0.131	0.577	0.94***	0.94***
142	0.94 [0.84-1.04]	0.87 [0.72-1.06]	0.190	0.144	0.98***	0.84***
152	0.82 [0.76-0.89]	0.75 [0.61-0.92]	**<0.001**	**0.009**	0.98***	0.88***
154	0.98 [0.86-1.11]	0.79 [0.63-0.98]	0.725	**0.038**	0.97***	0.93***
157	0.92 [0.82-1.03]	0.73 [0.56-0.97]	0.147	**0.034**	0.98***	0.84***
172	0.98 [0.88-1.10]	0.86 [0.58-1.27]	0.761	0.422	0.97***	0.81***
173	1.02 [0.92-1.13]	0.72 [0.55-0.95]	0.711	**0.025**	0.97***	0.91***
182	0.84 [0.75-0.95]	0.78 [0.69-0.87]	**0.008**	**0.001**	0.98***	0.95***
185	0.96 [0.83-1.12]	0.82 [0.72-0.94]	0.613	**0.009**	0.97***	0.98***
211	0.83 [0.61-1.13]	1.01 [0.85-1.20]	0.212	0.881	0.82***	0.93***
214	0.74 [0.53-1.03]	0.77 [0.59-1.00]	0.073	**0.050**	0.88***	0.88***
231	0.98 [0.83-1.16]	1.42 [1.00-2.04]	0.802	0.052	0.86***	0.70**
241	0.92 [0.76-1.12]	0.79 [0.67-0.94]	0.396	**0.012**	0.90***	0.95***

Notes: Code is population code (see Table S10.1.1). The 13 Iberian populations are in black font and the non-Iberian populations are in grey font. Significant *P* values in bold case ($P < 0.001$).

*** < 0.001, ** < 0.01, * < 0.05.

10.2.3. References

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10.3. Supplementary figures and tables

TABLE S10.3.1 | Geographic and climatic information about the 19 native populations of *Pinus halepensis* used throughout this thesis.

Country	Code	Region	Location	Longitude	Latitude	Altitude (m)	T (ºC)	TAR (ºC)	P (mm)	SP (mm)
Spain	11	Cataluña	Cabanellas	2º47'00''E	42º14'51''N	221	14.2	27	858	208
Spain	21	Cataluña	Tivissa	0º50'06''E	42º19'46''N	368	15.3	27.4	566	75
Spain	92	Maestrazgo - Los Serranos	Tuéjar	1º09'33''W	39º49'06''N	665	15	32.2	385	91
Spain	111	Maestrazgo - Los Serranos	Benicasim	0º01'33''E	40º04'37''N	449	14.2	21.9	699	90
Spain	101	Levante Interior	Tibi	0º38'55''W	38º31'08''N	993	13	27.2	503	58
Spain	105	Levante Interior	Bicorp	0º51'30''W	39º06'12''N	619	14.75	27	598.5	80.5
Spain	131	Levante Interior	Villajoyosa	0º18'14''W	38º29'44''N	98	16.8	23.8	431	51
Spain	142	Bética Septentrional	Monovar	0º57'27''W	38º23'05''N	760	14.7	28	387	51
Spain	152	Bética Meridional	Benamaurel	2º44'19''W	37º42'05''N	914	14.3	31.8	394	44
Spain	154	Bética Meridional	Santiago de la Espada	2º28'03''W	38º13'35''N	761	13.6	32.6	608	66
Spain	157	Bética Meridional	Alhama de Murcia	3º01'19''W	37º45'10''N	818	15.6	32.6	433	29
Spain	172	South	Carratraca	4º50'01''W	36º50'32''N	643	14.85	28.6	699.5	33
Spain	173	South	Frigiliana	3º55'13''W	36º49'03''N	583	15.2	24.6	696	26
Spain	182	Mallorca	Palma de Mallorca	2º56'26''E	39º08'56''N	46	16.7	21.9	563	40
Spain	185*	Mallorca	Son Marti/Calviá	2º29'13''E	39º35'14''N	272	15.8	22.3	526	55
Greece	211*	Greece	Istaia-eyboia	23º30'46''E	38º44'29''N	27	17.47	27.2	506	35
Greece	214	Greece	Kassandra	23º54'27''E	40º05'13''N	173	14.37	27.7	510	61
Italy	231	Italy	Litorale Tarantino	17º07'04''E	40º37'08''N	106	15.17	25.5	551	76
Tunisia	241	Tunisia	Thala	8º39'00''E	35º33'60''N	527	14.89	33.6	467	63

Notes: T: Annual mean temperature, TAR: Temperature annual range, P: Annual precipitation, SP: Summer precipitation. The 13 Iberian populations are in black font and the non-Iberian populations are in grey font. *Indicates two populations not genotyped and thus, not used in Chapter 4.

TABLE S10.3.2 **|** Number of trees from each population that were alive in 2013 at each of the four common garden sites used in this thesis.

	SITE			
Population	**ADE**	**CUC**	**OLM**	**VED**
11	14	15	14	13
21	13	16	13	14
92	12	15	12	12
101	13	15	14	12
105	14	16	14	14
111	10	16	9	14
131	7	13	16	13
142	13	15	16	16
152	10	16	15	15
154	12	16	13	13
157	13	16	14	10
172	9	15	15	14
173	12	13	14	13
182	11	16	14	11
185	9	11	14	15
211	5	9	15	14
214	3	13	14	12
231	16	14	12	11
241	10	16	12	16

Notes: The 13 Iberian populations are in black font and the non-Iberian populations are in grey font.

FIGURE S10.3.1 | Representative map of the Spanish genetic trial network for *Pinus halepensis* and aerial photographs of the four common garden sites used throughout this thesis. From www.genfored.es.

TABLE S10.3.3 | Cycle of controlled temperature and relative humidity used in the screening laboratory experiment with individual cones.

Time (min)	Temperature (℃)	Relative humidity (%)
5	36	25
60	36	25
5	36 – 40	25 – 20
120	40	20
5	40 – 44	20 – 15
120	44	15
5	48 – 52	10 – 8
300	48	10
5	48 – 52	10 – 8
240	52	8
5	52 – 56	6 – 4
300	56	6
5	56 – 60	6 – 4
240	60	4

TABLE S10.3.4 | Cycle of controlled temperature and relative humidity used in the manipulating water availability ex situ experiment with pairs of cones from the same whorl.

Time (min)	Temperature (℃)	Relative humidity (%)
15	0 – 24	40
120	24	40
5	24 – 28	40 – 35
120	28	35
5	28 – 32	35 – 30
240	32	30
5	32 – 36	30 – 25
240	36	25
5	36 – 40	25 – 20
360	40	20
5	40 – 44	20 – 15
360	44	15
5	44 – 48	15 – 10
360	48	10
5	48 – 52	10 – 8
360	52	8
5	52 – 56	8 – 6
240	56	6
5	56 – 60	6 – 4
240	60	4

TABLE S10.3.5 **|** Mean bark thickness and confidence intervals at breast height (BT130) and at the tree base (BT10) for each population and site (CUC: high-resources and VED: low-resources) obtained through general linear mixed models, and the calculated critical times for cambium kill, both at breast height (τ_{c130}) and at the tree base (τ_{c10}).

	BT130 [CI] (mm)		**τ_{c130} (min)**		**BT10 [CI] (mm)**		**τ_{c10} (min)**	
Code	**CUC**	**VED**	**CUC**	**VED**	**CUC**	**VED**	**CUC**	**VED**
11	12.2 [11.1-13.2]	8.2 [7.3-9.0]	4.3	1.9	24.6 [23.1-26.2]	22.5 [21.0-23.9]	17.6	14.6
21	10.5 [9.0-12.0]	6.9 [6.1-7.6]	3.2	1.4	25.6 [23.0-28.2]	20.9 [18.9-22.9]	19.0	12.7
92	11.9 [9.2-14.7]	6.0 [4.9-7.1]	4.1	1.0	23.3 [20.5-26.2]	21.3 [18.8-23.8]	15.8	13.2
101	11.3 [9.5-13.0]	7.5 [6.3-8.6]	3.7	1.6	21.9 [19.4-24.3]	19.3 [17.3-21.4]	13.9	10.8
105	11.0 [9.2-12.9]	8.4 [7.2-9.6]	3.5	2.0	22.5 [19.8-25.2]	20.1 [17.9-22.3]	14.6	11.8
111	13.8 [11.9-15.6]	8.3 [6.9-9.7]	5.5	2.0	24.1 [22.1-26.2]	24.3 [22.4-26.2]	16.9	17.1
131	13.5 [11.3-15.6]	7.0 [5.9-8.1]	5.3	1.4	22.6 [20.8-24.4]	19.3 [16.9-21.6]	14.8	10.8
142	13.2 [10.2-16.1]	8.3 [7.4-9.2]	5.0	2.0	25.5 [23.0-28.1]	20.9 [19.4-22.4]	18.9	12.7
152	8.7 [7.1-10.4]	5.7 [5.0-6.4]	2.2	1.0	22.9 [21.0-24.7]	20.1 [19.0-21.2]	15.2	11.8
154	13.2 [11.0-15.4]	8.3 [7.0-9.7]	5.1	2.0	23.3 [20.8-25.7]	20.6 [18.2-23.0]	15.7	12.3
157	10.5 [8.4-12.7]	7.2 [6.4-7.9]	3.2	1.5	23.4 [21.9-25.9]	21.3 [19.7-22.9]	15.9	13.2
172	8.9 [7.5-10.2]	6.4 [5.2-7.6]	2.3	1.2	21.4 [19.1-23.6]	20.0 [18.8-21.2]	13.2	11.6
173	12.3 [9.7-15.0]	8.7 [7.3-10.1]	4.4	2.2	23.2 [20.9-25.4]	19.2 [17.9-20.6]	15.6	10.7
182	12.2 [10.0-14.5]	7.0 [5.9-8.1]	4.3	1.4	23.9 [21.6-26.2]	19.4 [18.1-20.6]	16.6	10.9
185	12.9 [10.6-15.3]	7.9 [6.5-9.2]	4.9	1.8	23.6 [21.6-25.6]	21.2 [19.6-22.8]	16.1	13.1
211	18.2 [16.1-20.3]	13.5 [10.8-16.2]	9.6	5.3	26.3 [24.2-28.4]	22.1 [19.2-25.0]	20.1	14.2
214	16.9 [15.1-18.8]	11.1 [9.5-12.7]	8.3	3.6	25.3 [22.8-27.8]	17.3 [15.2-19.3]	18.6	8.6
231	15.3 [12.2-18.4]	10.6 [8.6-12.6]	6.8	3.3	22.0 [19.0-25.0]	22.2 [19.7-24.6]	14.0	14.3
241	10.3 [8.1-12.5]	6.3 [5.5-7.1]	3.1	1.2	22.6 [20.0-25.3]	20.9 [19.5-22.4]	14.9	12.7

Notes: Code is population code (see Table S10.3.1). The 13 Iberian populations are in black font and the non-Iberian populations are in grey font.

TABLE S10.3.6 **|** Correlations between mean bark thickness values (at breast height: BT130 and at tree base: BT10) from *Pinus halepensis* trees grown in a common garden experiment replicated in two contrasting sites (CUC: high-resources and VED: low-resources) and the first two Principal Components derived from a PCA analysis for nine environmental variables, as well as six environmental variables with PCA loadings > |0.80| representing average conditions in source populations. Significant correlations (< 0.10) are indicated in bold.

PC / Variable	**CUC**		**VED**		**2 SITES**	
	BT130	**BT10**	**BT130**	**BT10**	**BT130**	**BT10**
PC1	-0.01	0.13	0.06	0.38	0.03	0.32
PC2	0.32	0.14	0.12	0.02	0.26	0.04
Rainfall of driest† season	0.03	0.11	0.08	**0.50**	0.07	**0.42**
Rainfall of warmest✳ season	0.02	0.16	-0.01	**0.42**	0.05	0.37
Rainfall of wettest✹ season	0.23	0.26	0.26	-0.12	0.24	0.01
Rainfall of driest month	0.12	0.16	0.24	**0.53**	0.17	**0.46**
Mean temperature of coldest month	0.20	0.13	0.07	0.15	0.23	0.13
Continentally index*	-0.20	-0.14	0.00	-0.15	-0.15	-0.21
Fire frequency	0.15	0.14	0.19	**0.62**	0.16	0.37

Notes: See Table S10.1.1 for symbols clarification.

TABLE S10.3.7 | Mean percentage of bark volume (%VB) and confidence intervals for each site and population (significant factors in the model) obtained through general linear mixed models.

Site	**%V_B [CI]**	**Code**	**%V_B [CI]**
CUC	46.6 [45.3-47.9]	11	43.5 [41.8-45.2]
VED	42.3 [41.6-44.2]	21	45.9 [43.9-47.9]
		92	46.0 [43.2-48.8]
		101	41.9 [39.2-44.6]
		105	44.2 [41.7-46.7]
		111	46.3 [44.2-48.3]
		131	45.2 [43.3-47.1]
		142	43.0 [40.8-45.2]
		152	45.0 [42.7-47.4]
		154	43.2 [41.2-45.2]
		157	45.2 [43.5-46.8]
		172	43.8 [42.0-45.7]
		173	43.9 [42.0-45.8]
		182	46.4 [44.2-48.6]
		185	45.6 [43.8-47.5]
		211	47.4 [44.9-49.9]
		214	42.2 [39.4-44.9]
		231	44.8 [42.0-47.6]
		241	46.7 [44.4-49.1]

Notes: Code is population code (see Table S10.3.1). The 13 Iberian populations are in black font and the non-Iberian populations are in grey font.

TABLE S10.3.8 **|** Variance explained by provenance for each phenotypic studied trait, pooling together data of both sites (CUC: high-resource and VED: low-resource). , for the high-resource site or for the low-resource site. Details about phenotypic traits in Table 5.

Trait	**Exp. Var. (%)**	**CI**
H	22.67	11.68-38.91
DBH	15.88	8.31-32.50
H/DBH	8.00	3.26-20.60
TFR	10.99	5.59-25.07
fRA	22.90	11.89-40.02
SER	33.54	18.00-51.78
SERa	32.48	19.14-54.17
CCB	19.25	8.66-32.92
CCBa	25.95	13.27-42.42
BT130	23.31	13.92-42.77
BT10	1.87	0.48-7.00

TABLE S10.3.9 **|** Variance explained by provenance for each phenotypic studied trait, for data from the high-resource site (CUC). Details about phenotypic traits in Table 5.

Trait	**Exp. Var. (%)**	**CI**
H	30.44	17.81-51.45
DBH	18.12	6.85-35.13
H/DBH	20.37	9.73-40.28
TFR	7.67	0.05-18.97
fRA	10.65	3.88-28.97
SER	40.63	20.47-63.34
SERa	41.67	20.57-63.87
CCB	18.40	6.42-34.46
CCBa	19.46	10.38-39.82
BT130	20.01	9.18-38.72
BT10	1.73	0.33-8.67
Aff	11.22	2.74-24.29
Hff	19.63	8.56-37.70
QMR	16.45	10.67-25.30
mRA	14.98	9.56-24.24

TABLE S10.3.10 | Variance explained by provenance for each phenotypic studied trait, for data from the low-resource site (VED). Details about phenotypic traits in Table 5.

Trait	**Exp. Var. (%)**	**CI**
H	15.92	6.61-34.49
DBH	14.38	5.69-32.40
H/DBH	0.07	0.00-23.27
TFR	20.35	10.63-41.60
fRA	36.55	20.47-56.16
SER	23.01	11.43-47.25
SERa	24.98	12.64-49.84
CCB	21.16	10.72-40.97
CCBa	29.99	15.26-49.16
BT130	35.20	20.00-55.08
BT10	3.62	0.79-13.89
TMR	12.18	1.54-26.18
mRA	17.14	7.56-37.66

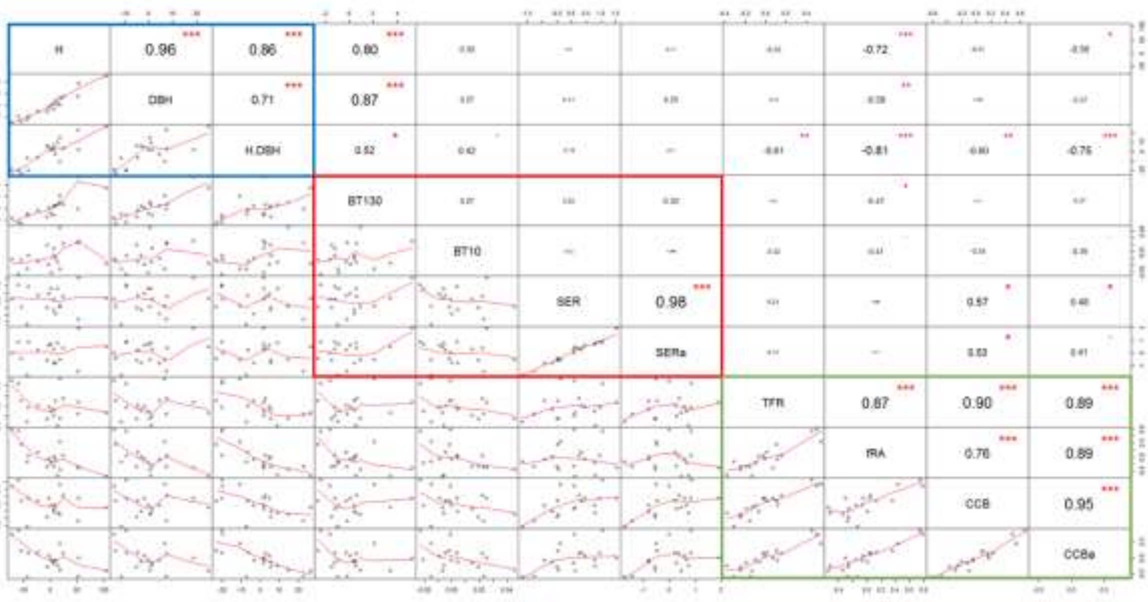

FIGURE S10.3.2 | Graphical pairwise correlations, correlation coefficients and significance of the correlation at the population level for the 11 studied phenotypic traits at both test sites in *Pinus halepensis.* Blue box marks growth traits, red box indicates fire-adaptive traits and green box grouped reproductive traits. Below diagonal, graphical pairwise relationships are shown and above diagonal, pairwise Pearson's correlation coefficients and level of significance of the correlation (***$P < 0.001$, **$P < 0.01$, *$P < 0.5$, ·$P < 0.1$).

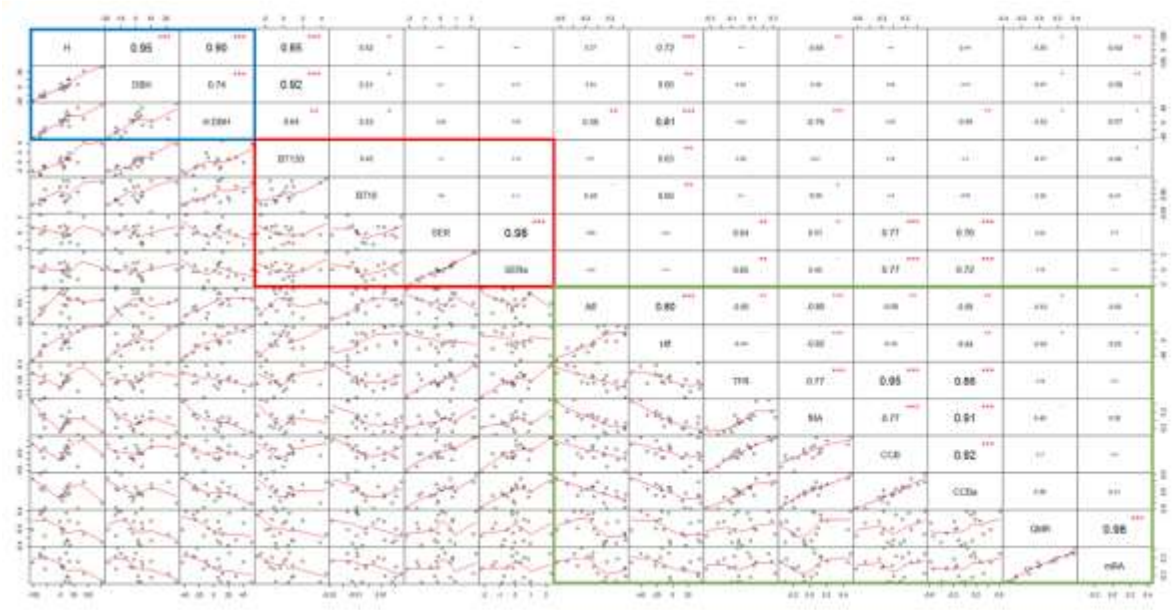

FIGURE S10.3.3 | Graphical pairwise correlations, correlation coefficients and significance of the correlation at the population level for the 15 studied phenotypic traits at the high-resource site (CUC site) in *Pinus halepensis.* Blue box marks growth traits, red box indicates fire-adaptive traits and green box grouped reproductive traits. Below diagonal, graphical pairwise relationships are shown and above diagonal, pairwise Pearson's correlation coefficients and level of significance of the correlation (****P* < 0.001, ***P* < 0.01, **P* < 0.5, ·*P* < 0.1).

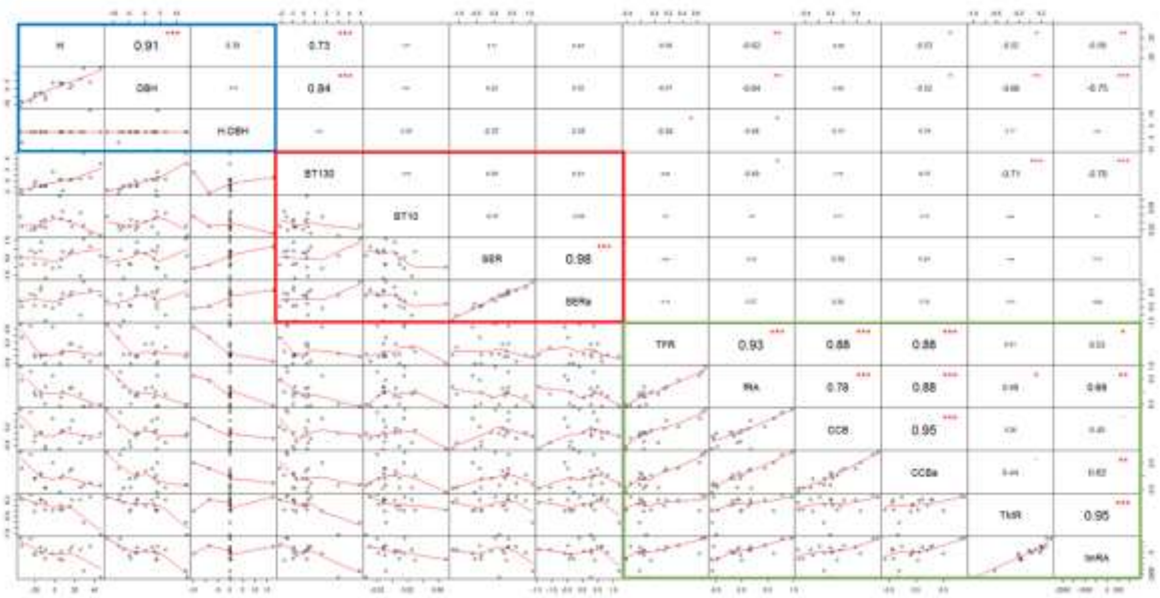

FIGURE S10.3.4 | Graphical pairwise correlations, correlation coefficients and significance of the correlation at the population level for the 13 studied phenotypic traits at the low-resource site (VED site) in *Pinus halepensis.* Blue box marks growth traits, red box indicates fire-adaptive traits and green box grouped reproductive traits. Below diagonal, graphical pairwise relationships are shown and above diagonal, pairwise Pearson's correlation coefficients and level of significance of the correlation (***$P < 0.001$, **$P < 0.01$, *$P < 0.5$, ·$P < 0.1$).

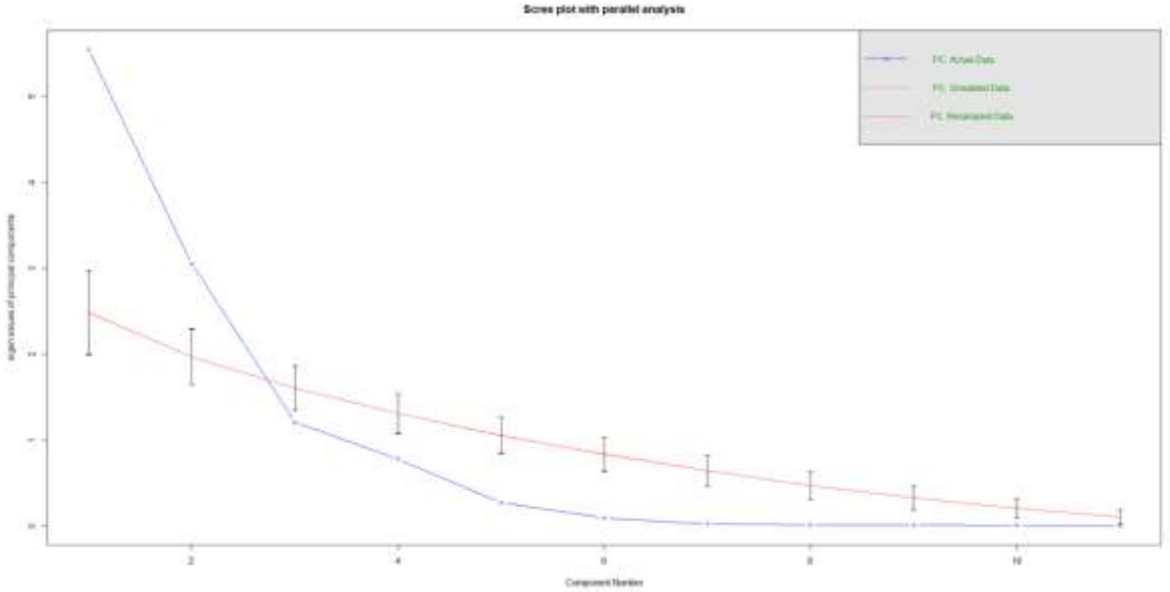

FIGURE S10.3.5 | Scree plot for the PCA of the eleven phenotypic traits studied at both sites. This figure revealed that two principal components should be retained.

TABLE S10.3.11 **|** Results from Principal Component Analysis applied to phenotypic traits data from 19 *Pinus halepensis* populations studied at the two experimental sites. Variables with loadings > |0.80| in bold case.

Variable	**PC1**	**PC2**
H	**0.94**	-0.04
DBH	**0.91**	0.20
H/DBH	**0.84**	-0.38
BT130	**0.83**	0.28
BT10	0.44	-0.26
SER	0.19	**0.82**
SERa	0.30	**0.81**
TFR	-0.47	0.71
fRA	**-0.82**	0.46
CCB	-0.35	**0.90**
CCBa	-0.59	0.78
Importance of components		
Proportion of variance explained	0.44	0.34
Cumulative proportion of variance	0.44	0.78

Notes: Abbreviations for phenotypic traits as in Table 5.

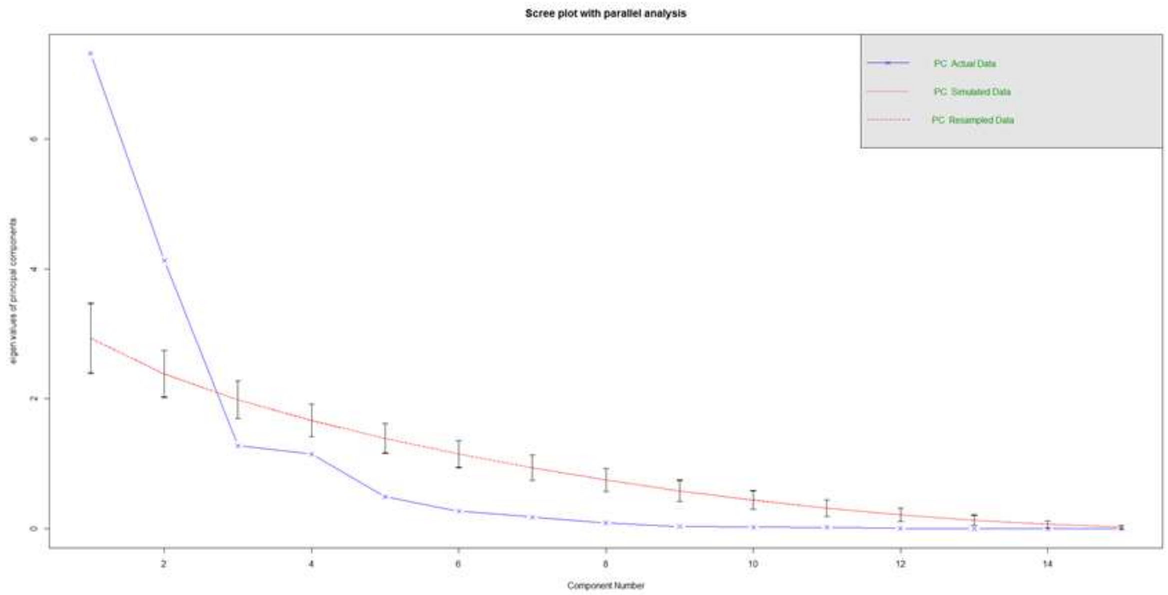

FIGURE S10.3.6 **|** Scree plot for the PCA of the fifteen phenotypic traits studied at the high-resource site (CUC). This figure revealed that two principal components should be retained.

TABLE S10.3.12 **|** Results from the PCA applied to phenotypic traits data from 19 *Pinus halepensis* populations studied at the high-resource site (CUC). Variables with loadings > **|**0.80**|** in bold case.

Variable	PC1	PC2
H	**0.94**	-0.03
DBH	**0.92**	0.21
H/DBH	**0.85**	-0.32
BT130	**0.85**	0.21
BT10	0.67	-0.08
SER	0.01	**0.83**
SERa	0.10	**0.84**
Aff	0.53	-0.60
Hff	**0.81**	-0.40
TFR	-0.01	**0.95**
fRA	-0.58	0.75
CCB	-0.02	**0.98**
CCBa	-0.36	**0.91**
QMR	-0.67	0.21
mRA	-0.74	0.12
Importance of components		
Proportion of variance explained	0.40	0.36
Cumulative proportion of variance	0.40	0.76

Notes: Abbreviations for phenotypic traits as in Table 5.

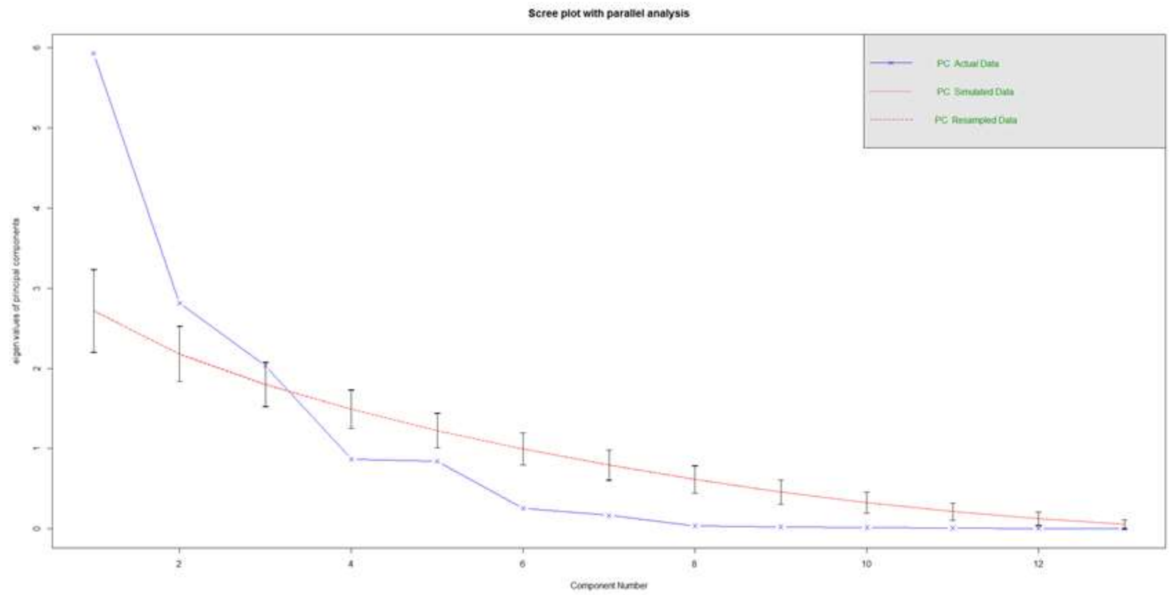

FIGURE S10.3.7 **|** Scree plot for the PCA of the thirteen phenotypic traits studied at the low-resource site (VED). This figure revealed that three principal components should be retained.

TABLE S10.3.13 **|** Results from the PCA applied to phenotypic traits data from 19 *Pinus halepensis* populations studied at the low-resource site. Variables with loadings > **|**0.80**|** in bold case.

Variable	**PC1**	**PC2**	**PC3**
H	-0.72	-0.35	0.22
DBH	**-0.89**	-0.24	0.15
H/DBH	0.19	-0.66	0.56
BT130	**-0.89**	-0.06	0.19
BT10	0.01	-0.06	-0.53
SER	-0.14	0.05	**0.94**
SERa	-0.22	0.02	**0.91**
TFR	0.22	**0.94**	-0.05
fRA	0.49	**0.84**	-0.14
CCB	0.16	**0.90**	0.36
CCBa	0.40	**0.87**	0.27
TMR	**0.90**	0.08	0.08
tmRA	**0.89**	0.30	0.05
Importance of components			
Proportion of variance explained	0.33	0.30	0.20
Cumulative proportion of variance	0.33	0.63	0.83

Notes: Abbreviations for phenotypic traits as in Table 5.

TABLE S10.3.14 **|** Correlations coefficients and significance of the correlation at the population level between raw data of the studied phenotypic traits at each experimental site and the Gower's distance to each site.

Trait	Coef. CUC	*P* value	Trait	Coef. VED	*P* value
H	-0.42	0.072	**H**	0.04	0.873
DBH	-0.30	0.210	**DBH**	-0.07	0.769
H/DBH	-0.39	0.101	**H/DBH**	0.04	0.873
BT130	-0.33	0.165	**BT130**	0.09	0.723
BT10	-0.11	0.647	**BT10**	-0.38	0.104
SER	0.10	0.686	**SER**	0.02	0.934
SERa	0.11	0.655	**SERa**	0.02	0.940
Aff	-0.34	0.161	**TFR**	0.07	0.761
Hff	-0.39	0.100	**fRA**	0.11	0.648
TFR	0.24	0.319	**CCB**	0.12	0.614
fRA	0.21	0.385	**CCBa**	0.09	0.727
CCB	0.29	0.233	**TMR**	0.35	0.138
CCBa	0.19	0.445	**tmRA**	0.29	0.223
QMR	0.38	0.108			
mRA	-0.14	0.558			

TABLE S10.3.15 | Correlations coefficients and significance of the correlation among the eight original environmental variables (before selection) and the three geographical variables from the origin of the 19 *P. halepensis* populations used in this thesis.

	TCM	TWM	Psp	Ps	Pa	PDM	P	T	Lat	Long	Alt
Cont.Index	__-0.76***__	__0.75***__	__-0.48*__	-0.24	__-0.55*__	-0.15	__-0.47*__	-0.40	__-0.65**__	0.02	__0.75***__
TCM	1	-0.14	-0.01	-0.24	__0.59**__	-0.28	0.24	__0.89***__	0.20	0.12	__-0.84***__
TWM	-0.14	1	__-0.73***__	__-0.61**__	-0.24	__-0.51*__	__-0.46*__	0.28	__-0.79***__	0.15	0.29
Psp	-0.01	-0.73	1	__0.94***__	-0.01	__0.83***__	__0.48*__	-0.29	__0.79***__	-0.02	-0.24
Ps	-0.24	-0.61	0.94	1	-0.09	__0.95***__	__0.48*__	__-0.46*__	__0.74***__	0.07	-0.08
Pa	0.59	-0.24	-0.01	-0.09	1	-0.10	__0.79***__	0.41	0.12	0.13	-0.49*
PDM	-0.28	-0.51	0.83	0.95	-0.10	1	__0.46*__	-0.45	__0.72***__	0.24	-0.08
P	0.24	-0.46	0.48	0.48	0.79	0.46	1	-0.02	0.42	0.09	-0.32
T	0.89	0.28	-0.29	-0.46	0.41	-0.45	-0.02	1	-0.10	0.23	__-0.73***__
Lat	0.20	-0.79	0.79	0.74	0.12	0.72	0.42	-0.10	1	0.25	__-0.55*__
Long	0.12	0.15	-0.02	0.07	0.13	0.24	0.09	0.23	0.25	1	__-0.46*__

Notes: ***P < 0.001, **P < 0.01, *P < 0.5

TABLE S10.3.16 | Global phenotypic differentiation among populations (Q_{ST}) with its confidence intervals (CI) for *Pinus halepensis* provenances grown in a high-resource site (CUC) of a common garden experiment, the narrow-sense heritability values (h^2) used for Q_{ST} calculation (see Table 7) and Q_{ST} - F_{ST} comparison. Q_{ST} - F_{ST} comparisons have been made comparing both the CIs and the distribution of values (always $P < 0.0001$ in the Kruskal-Wallis chi-squared).

Trait	Tree age	h^2	Q_{ST}	CI	Q_{ST}-F_{ST} **Comparison**
H	16	0.32	0.42	0.25-0.63	$Q_{ST} > F_{ST}$
		0.42	0.36	0.21-0.56	$Q_{ST} > F_{ST}$
		0.57	0.26	0.15-0.48	$Q_{ST} > F_{ST}$
		0.67	0.23	0.13-0.44	$Q_{ST} = F_{ST}$
DBH	16	0.16	0.42	0.22-0.64	$Q_{ST} > F_{ST}$
		0.26	0.31	0.13-0.50	$Q_{ST} = F_{ST}$
		0.48	0.20	0.08-0.35	$Q_{ST} = F_{ST}$
		0.58	0.17	0.06-0.31	$Q_{ST} = F_{ST}$
H/DBH	16	0.32	0.28	0.15-0.52	$Q_{ST} = F_{ST}$
		0.42	0.23	0.11-0.44	$Q_{ST} = F_{ST}$
		0.57	0.18	0.08-0.37	$Q_{ST} = F_{ST}$
		0.67	0.16	0.07-0.33	$Q_{ST} = F_{ST}$
TFR	16	0.47	0.06	0.00-0.20	$Q_{ST} = F_{ST}$
		0.57	0.05	0.00-0.17	$Q_{ST} = F_{ST}$
		0.68	0.05	0.00-0.15	$Q_{ST} = F_{ST}$
		0.78	0.04	0.00-0.13	$Q_{ST} = F_{ST}$
fRA	16	0.27	0.22	0.07-0.43	$Q_{ST} = F_{ST}$
		0.40	0.16	0.05-0.34	$Q_{ST} = F_{ST}$
		0.63	0.10	0.03-0.24	$Q_{ST} = F_{ST}$
SER	16	0.09	0.24	0.13-0.46	$Q_{ST} > F_{ST}$
		0.20	0.19	0.11-0.43	$Q_{ST} = F_{ST}$
		0.40	0.22	0.10-0.39	$Q_{ST} = F_{ST}$
SERa	16	0.09	0.78	0.63-0.93	$Q_{ST} > F_{ST}$
		0.20	0.62	0.42-0.84	$Q_{ST} > F_{ST}$
		0.40	0.45	0.25-0.70	$Q_{ST} > F_{ST}$
CCB	16	0.47	0.17	0.07-0.35	$Q_{ST} = F_{ST}$
		0.57	0.14	0.06-0.31	$Q_{ST} = F_{ST}$
		0.68	0.12	0.05-0.27	$Q_{ST} = F_{ST}$
		0.78	0.11	0.04-0.24	$Q_{ST} = F_{ST}$
CCBa	16	0.47	0.23	0.10-0.40	$Q_{ST} = F_{ST}$
		0.57	0.18	0.08-0.36	$Q_{ST} = F_{ST}$
		0.68	0.16	0.07-0.32	$Q_{ST} = F_{ST}$
		0.78	0.14	0.06-0.29	$Q_{ST} = F_{ST}$
BT130	18	0.05	0.79	0.56-0.88	$Q_{ST} > F_{ST}$
		0.15	0.45	0.29-0.71	$Q_{ST} > F_{ST}$
		0.24	0.34	0.19-0.59	$Q_{ST} > F_{ST}$
		0.34	0.26	0.14-0.50	$Q_{ST} = F_{ST}$
BT10	18	0.05	0.20	0.04-0.49	$Q_{ST} = F_{ST}$
		0.15	0.08	0.01-0.24	$Q_{ST} = F_{ST}$
		0.24	0.05	0.01-0.17	$Q_{ST} = F_{ST}$
		0.34	0.04	0.01-0.12	$Q_{ST} < F_{ST}$
QMR*	13	0.30	0.25	0.17-0.36	$Q_{ST} > F_{ST}$
		0.48	0.16	0.11-0.26	$Q_{ST} = F_{ST}$
		0.70	0.12	0.08-0.19	$Q_{ST} = F_{ST}$
mRA*	13	0.23	0.33	0.20-0.43	$Q_{ST} > F_{ST}$
		0.38	0.23	0.12-0.30	$Q_{ST} = F_{ST}$
		0.63	0.15	0.08-0.21	$Q_{ST} = F_{ST}$
Aff	18	0.27	0.16	0.03-0.38	$Q_{ST} = F_{ST}$
		0.43	0.11	0.02-0.28	$Q_{ST} = F_{ST}$
		0.67	0.07	0.01-0.20	$Q_{ST} = F_{ST}$
Hff	18	0.27	0.30	0.15-0.53	$Q_{ST} > F_{ST}$
		0.43	0.21	0.09-0.41	$Q_{ST} = F_{ST}$
		0.67	0.14	0.06-0.31	$Q_{ST} = F_{ST}$

Notes: *Qualitative data for male reproduction. Abbreviations for phenotypic traits as in Table 5.

TABLE S10.3.17 | Global phenotypic differentiation among populations (Q_{ST}) with its confidence intervals (CI) for *Pinus halepensis* provenances grown in a low-resource site (VED) of a common garden experiment, the narrow-sense heritability values (h^2) used for Q_{ST} calculation (see Table 7) and Q_{ST} - F_{ST} comparison. Q_{ST} - F_{ST} comparisons have been made comparing both the CIs and the distribution of values (always $P < 0.0001$ in the Kruskal-Wallis chi-squared).

Trait	Tree age	h^2	Q_{ST}	CI	Q_{ST}-F_{ST} Comparison
H	16	0.32	0.25	0.12-0.48	$Q_{ST} = F_{ST}$
		0.42	0.20	0.10-0.41	$Q_{ST} = F_{ST}$
		0.57	0.16	0.07-0.33	$Q_{ST} = F_{ST}$
		0.67	0.14	0.06-0.30	$Q_{ST} = F_{ST}$
DBH	16	0.16	0.38	0.16-0.60	$Q_{ST} > F_{ST}$
		0.26	0.27	0.10-0.48	$Q_{ST} = F_{ST}$
		0.48	0.17	0.05-0.32	$Q_{ST} = F_{ST}$
		0.58	0.14	0.04-0.28	$Q_{ST} = F_{ST}$
H/DBH	16	0.32	0.13	0.00-0.32	$Q_{ST} = F_{ST}$
		0.42	0.12	0.00-0.27	$Q_{ST} = F_{ST}$
		0.57	0.08	0.00-0.21	$Q_{ST} = F_{ST}$
		0.67	0.07	0.00-0.19	$Q_{ST} = F_{ST}$
TFR	16	0.47	0.27	0.11-0.42	$Q_{ST} = F_{ST}$
		0.57	0.23	0.09-0.37	$Q_{ST} = F_{ST}$
		0.68	0.20	0.07-0.33	$Q_{ST} = F_{ST}$
		0.78	0.18	0.06-0.30	$Q_{ST} = F_{ST}$
fRA	16	0.27	0.47	0.36-0.72	$Q_{ST} > F_{ST}$
		0.40	0.37	0.27-0.64	$Q_{ST} > F_{ST}$
		0.63	0.27	0.17-0.50	$Q_{ST} > F_{ST}$
SER	16	0.09	0.68	0.43-0.85	$Q_{ST} > F_{ST}$
		0.20	0.43	0.21-0.67	$Q_{ST} > F_{ST}$
		0.40	0.28	0.11-0.49	$Q_{ST} = F_{ST}$
SERa	16	0.09	0.73	0.48-0.87	$Q_{ST} > F_{ST}$
		0.20	0.47	0.28-0.73	$Q_{ST} > F_{ST}$
		0.40	0.31	0.15-0.55	$Q_{ST} > F_{ST}$
CCB	16	0.47	0.25	0.11-0.42	$Q_{ST} = F_{ST}$
		0.57	0.22	0.09-0.38	$Q_{ST} = F_{ST}$
		0.68	0.19	0.08-0.34	$Q_{ST} = F_{ST}$
		0.78	0.14	0.07-0.31	$Q_{ST} = F_{ST}$
CCBa	16	0.47	0.31	0.18-0.52	$Q_{ST} > F_{ST}$
		0.57	0.27	0.15-0.47	$Q_{ST} = F_{ST}$
		0.68	0.24	0.12-0.42	$Q_{ST} = F_{ST}$
		0.78	0.21	0.11-0.39	$Q_{ST} = F_{ST}$
BT130	18	0.05	0.88	0.74-0.94	$Q_{ST} > F_{ST}$
		0.15	0.65	0.48-0.82	$Q_{ST} > F_{ST}$
		0.24	0.54	0.36-0.74	$Q_{ST} > F_{ST}$
		0.34	0.45	0.27-0.65	$Q_{ST} > F_{ST}$
BT10	18	0.05	0.25	0.09-0.63	$Q_{ST} = F_{ST}$
		0.15	0.10	0.03-0.36	$Q_{ST} = F_{ST}$
		0.24	0.06	0.02-0.26	$Q_{ST} = F_{ST}$
		0.34	0.04	0.01-0.19	$Q_{ST} = F_{ST}$
TMR*	13	0.30	0.16	0.03-0.37	$Q_{ST} = F_{ST}$
		0.48	0.11	0.02-0.26	$Q_{ST} = F_{ST}$
		0.70	0.08	0.01-0.20	$Q_{ST} = F_{ST}$
tmRA*	13	0.23	0.31	0.17-0.59	$Q_{ST} > F_{ST}$
		0.38	0.20	0.09-0.44	$Q_{ST} = F_{ST}$
		0.63	0.13	0.06-0.32	$Q_{ST} = F_{ST}$

Notes: *Quantitative data for male reproduction. Abbreviations for phenotypic traits as in Table 5.