Conifer responses to environment at local and regional scales: the role of intraspecific phenotypic variation

Tesis Doctoral presentada por

Natalia Vizcaíno Palomar

2015

Universidad de Alcalá

Departamento de Ciencias de la Vida
Unidad Docente de Ecología

Miguel Ángel de Zavala Gironés, Profesor Titular del Departamento de Ciencias de la Vida de la Universidad de Alcalá y director de esta Tesis Doctoral,

Hace constar:

Que el trabajo descrito en la presente memoria, titulado "**Conifer responses to environment at local and regional scales: the role of intraspecific phenotypic variation**", ha sido realizado por Natalia Vizcaíno Palomar bajo su dirección en el Centro de Investigaciones Forestales (CIFOR-INIA) y en la Unidad Docente de Ecología del Departamento de Ciencias de la Vida de la Universidad de Alcalá, dentro del Programa de Doctorado "Ecología, Conservación y Restauración de Ecosistemas" (D330), reuniendo todos los requisitos necesarios para su aprobación como Tesis Doctoral.

Alcalá de Henares, 18 de noviembre de 2015

Dr. Miguel Ángel de Zavala Gironés

UNIVERSIDAD DE ALCALÁ, PATRIMONIO DE LA HUMANIDAD

Santiago César González Martínez, Director de Investigación en la Unidad INRA-BIOGECO en Cestas (Francia) y co-director de esta Tesis Doctoral,

Hace constar:

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Burdeos, 18 de noviembre de 2015

Dr. Santiago César González Martínez

Ricardo Alía Miranda, Científico Titular del Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria y co-director de esta Tesis Doctoral,

Hace constar:

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Madrid, 18 de noviembre de 2015

Dr. Ricardo Alía Miranda

</div>

Gonzalo Pérez Suárez, Director del Departamento de Ciencias de la Vida de la Universidad de Alcalá,

Hace constar:

Que el trabajo descrito en la presente memoria, titulado "**Conifer responses to environment at local and regional scales: the role of intraspecific phenotypic variation**", ha sido realizado por Natalia Vizcaíno Palomar en el Centro de Investigaciones Forestales (CIFOR-INIA) y en la Unidad Docente de Ecología del Departamento de Ciencias de la Vida de la Universidad de Alcalá, dentro del Programa de Doctorado "Ecología, Conservación y Restauración de Ecosistemas" (D330), reuniendo todos los requisitos necesarios para su aprobación como Tesis Doctoral, por acuerdo del Consejo de Departamento celebrado el día 30 de noviembre de 2015.

Alcalá de Henares, 30 de noviembre de 2015

Dr. Gonzalo Pérez Suárez

Conifer responses to environment
at local and regional scales:
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Memoria presentada para optar al grado de Doctora por la
Universidad de Alcalá

Programa de Doctorado:

"Ecología, Conservación y Restauración de Ecosistemas"
(D330)

Natalia Vizcaíno Palomar

Director: Dr. Miguel Ángel de Zavala Gironés
Co-director: Dr. Santiago César González Martínez
Co-director: Dr. Ricardo Alía Miranda

Alcalá de Henares, Noviembre de 2015

Agradecimientos

Y por fin, el final de la tesis llegó. Pasé por tantos momentos similares, que cuando éste se acercó no lo tomé en serio y seguí haciendo mi vida.

Empecemos. Agradezco a Miguel Ángel, mi director, la oportunidad que me brindó el día que me seleccionó como candidata firme en su proyecto. Aquel día significó para mí la apertura al inquietante y misterioso mundo de la ciencia. Durante muchos años había crecido en mí la idea de la pureza y poder de la ciencia para contribuir al cambio, si no de todo, de parte de los problemas en el mundo a través del conocimiento. Seguidamente, agradezco el apoyo de mis dos co-directores de tesis, Santi y Ricardo; actores que aparecieron en el segundo acto y que desarrollaron papeles principales. Los tres habéis formado parte de este proyecto. Cada uno de vosotros ha incidido en mí de una manera muy particular, y me habéis mostrado "el destape" de la ciencia. Además, quiero dar especialmente las gracias a Santi, porque estuvo en los momentos difíciles, acudió a mis mails de auxilio, leyó y contestó todos mis comentarios, y lo más importante, se tomó mi trabajo con la misma seriedad y preocupación que yo lo hacía. Y cómo no, agradecer a la Pequeña Tesis. No sólo me ha dado algún que otro dolor de cabeza y preocupación, sino que ha sido la excusa perfecta para conocer partes del mundo, y sobre todo, para aprender infinidad de cosas que de otra manera, muy probablemente, me las hubiese perdido.

Gracias a mis compañeros del despacho de becarios a ambos lados del microondas. Este tiempo ha sido, sin duda, mucho mejor contando con todos vosotros. En este despacho se han fraguado muchas risas, muchas conversaciones –de todo tipo: rosas, verdes, naranjas… ☺–, bailes, bizcochos, chocolates, y momentos en los que los unos a los otros nos hemos apoyado. Gracias por los mágicos desayunos en el INIA. ¡Sois geniales! Sois todos muy especiales, y cada uno tiene una parte que le hace genuino y hace que el despacho crezca. Desde aquí os mando ¡¡mucha, mucha fuerza y ánimos para terminar vuestras tesis!!. Por cierto, éste es el testigo oficial de: las llevas.

Gracias a Arianna, desde que te conozco he aprendido muchísimo; ahora sé que las utopías ayudan a soñar y las realidades guían el camino. Gracias a la

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Resumen

Durante las últimas décadas, la persistencia de nuestros bosques –naturales y artificiales– está amenazada por los efectos del cambio global, y particularmente, por el cambio climático. Los bosques nos proveen de una gran variedad de servicios ecosistémicos para el beneficio y bienestar de la sociedad, desde materias primas a bienes y servicios que en último lugar permiten el funcionamiento de la Tierra como ecosistema. Hoy en día, se ha reconocido internacionalmente el vital y destacado papel que juegan nuestros bosques en la mitigación de los efectos del cambio climático. Es por ello que existe una necesidad imperiosa de mejorar el conocimiento sobre la capacidad de nuestras especies forestales para adaptarse y aclimatarse a las nuevas condiciones del futuro. En este sentido, el **objetivo general** de la presente Tesis Doctoral ha sido el estudio de fenómenos que determinan en árboles forestales la variación fenotípica a nivel de especie, como son el género, la adaptación local y la plasticidad. Esta base de conocimiento pondrá de manifiesto cómo la variación fenotípica varía dentro de los árboles forestales, y por tanto, facilitará la inferencia de respuestas –p.ej. bajo escenarios de cambio climático– que a medio-largo plazo podrían alterar la demografía de la especie y su distribución. Finalmente, este conocimiento permitirá el desarrollo de políticas y prácticas forestales encaminadas a la adaptación de nuestros bosques al cambio climático, y así garantizar un adecuado funcionamiento y uso sostenible de los mismos.

La presente tesis doctoral la componen un total de siete capítulos dedicados a la contextualización, desarrollo y discusión de algunos temas principales en torno a la variabilidad fenotípica intraespecífica. El primer capítulo de la tesis, **capítulo 1**, recoge una introducción sobre los impactos y riesgos de los bosques ante el cambio climático. Además, se justifica la selección de las especies objeto de estudio –las coníferas, grupo de especies con un papel destacado en las fases iniciales de la sucesión ecológica–, así como también el área de estudio centrado en la Península Ibérica –región con un destacado papel como refugio para flora y fauna en la última glaciación–. En el **capítulo 2** nos centramos en el estudio de los factores bióticos subyacentes que generan la variación fenotípica en el crecimiento de

individuos de la especie dioica –i.e. los órganos reproductivos masculinos y femeninos se encuentran en individuos distintos– *Juniperus thurifera* L. El estudio se desarrolla en un rodal casi monoespecífico, situado en el centro de la Península Ibérica, con alta densidad de individuos, la mayoría juveniles. Este rodal puede ser el resultado del abandono de las prácticas agrícolas, lo cual constituye un escenario nuevo para la especie dentro de un contexto de cambio global. En general, nuestros resultados sugieren que rodales monoespecíficos de sabina albar en fases tempranas de desarrollo, donde los procesos de auto-clareo parecen aún no haberse producido, la dinámica de crecimiento de la masa está controlada por el tamaño de los individuos y la clase reproductora, mientras que la competencia intraespecífica –ni intra- ni inter-sexual– no tiene efectos en el crecimiento. En los siguientes capítulos de la tesis (capítulos 3, 4, 5 y 6), la plasticidad fenotípica y la adaptación local se consideran las principales fuentes generadoras de la variación fenotípica intraespecífica en un grupo de especies monoicas –i.e. los órganos reproductivos masculinos y femeninos se encuentran en los mismos individuos–. En el **capítulo 3** se estudia el fenómeno de la adaptación local – i.e. la población local presenta una mayor fitness en su hábitat respecto a poblaciones foráneas–. Centramos el estudio en dos poblaciones alejadas – Centro y Este de la Península Ibérica– y de ambientes contrastados – continental y mediterráneo– de pino negral (*Pinus pinaster* Ait.). El estudio se enmarca en la fase de germinación y reclutamiento que se experimenta durante el primer año. Este conocimiento es esencial para inferir la persistencia de las masas forestales en el futuro. Mediante un experimento *in situ* de siembra recíproca, en condiciones semi-naturales con control del componente genético a nivel de familia, testamos la existencia de adaptación local así como el efecto modulador de los factores abióticos de luz y temperatura, aproximados a través del nivel de cobertura del dosel arbóreo, sobre nuestras variables respuesta de estudio –emergencia y supervivencia estival–. Las poblaciones resultaron no estar localmente adaptadas ni en la fase de germinación ni en la fase de reclutamiento, y los microambientes testados aparentemente jugaron un papel favorecedor para aquella población con la estrategia vital más exitosa con independencia de su origen (local o no local). Este trabajo pone de manifiesto la gran estocasticidad existente en la fase de germinación y reclutamiento, así como la complejidad de los factores

que influyen en las primeras etapas de establecimiento del pino negral. La generalización de medidas, como la migración asistida, conducentes a la mitigación de los efectos del cambio climático pueden suponer un riesgo cuando las decisiones están basadas solamente en factores de similitud del clima medio de origen y destino. En el **capítulo 4** modelizamos la variación fenotípica intraespecífica del rasgo de la altura para la especie *Pinus sylvestris*. Para ello, empleamos de manera complementaria dos métodos caracterizados por ser o bien "universales", o bien demasiado específicos. En este estudio utilizamos datos derivados de ensayos de procedencia que permiten la separación de la componente genética –en nuestro caso, origen de la población– de la componente medioambiental. El objetivo principal de este estudio fue añadir evidencia experimental acerca de la conveniencia de considerar respuestas a nivel de población, en lugar de hacerlo a nivel de especie. Nuestros resultados pusieron de manifiesto que respuestas adaptativas y plásticas generaban variación fenotípica dentro de la especie, y que por tanto, es conveniente estudiar la dinámica de la especie, al menos, a nivel de población. Ambos métodos ofrecieron resultados similares; el más específico complementó al "universal" a la hora de profundizar en determinadas poblaciones. Este hecho los convierte en técnicas complementarias para nuestro caso de estudio. En el **capítulo 5** el estudio se centra en el análisis de la variación entre y dentro de especies del rasgo, en este caso compuesto, alométrico altura total vs diámetro a altura del pecho para cuatro de las seis especies de pino presentes en la Península Ibérica. Para ello empleamos –al igual que en el capítulo anterior– datos derivados de ensayos de procedencia. Por un lado, el análisis entre especies nos permite comparar las diferentes estrategias vitales. Por otro lado, el análisis intraespecífico complementa al anterior y nos permite distinguir si la variación observada es resultado de procesos de selección natural y/o procesos histórico-demográficos. Nuestros resultados mostraron marcadas diferencias en la respuesta alométrica, i.e. en las estrategias de asignación de recursos al crecimiento en altura o en anchura, entre la especie boreal, *Pinus sylvestris,* con aquéllas más mediterráneas, *P. halepensis, P. nigra* y *P. pinaster.* Además, a nivel intraespecífico, se detectó que los patrones alométricos identificados fueron causa de adaptaciones al clima, así como el resultado de procesos demográficos. En el **capítulo 6** analizamos cómo la

variación fenotípica intraespecífica en la alometría se vería afectada bajo un escenario de cambio climático. Nuestros resultados indicaron que, en general, la variación fenotípica intraespecífica bajo las condiciones de los nuevos climas se vería alterada. Además, los pinos de orígenes más xéricos podrían modificar significativamente sus alometrías resultando más esbeltos. Sin embargo, las especies de pinos con hábitats asociados a ambientes montañosos tenderían a mantener estrategias similares. Por último, nuestros resultados pusieron de relieve la falta de variación genética en la respuesta plástica del rasgo alométrico. Este resultado sugiere que la capacidad de aclimatación a nuevos ambientes es idéntica a nivel intraespecífico, y por tanto, se esperarían respuestas plásticas similares en las diferentes procedencias. Finalmente, el presente documento termina con una serie de conclusiones generales recogidas en el **capítulo 7**.

Palabras clave: cambio climático, rasgo compuesto, coníferas, supervivencia estival, dioecia, crecimiento, variación intraespecífica, adaptación local, plasticidad fenotípica, pinos, rasgo simple.

Summary

In recent decades, the effects of global change, particularly climate change, threaten the persistence of natural forests and tree plantations. Forests provide a variety of ecosystem services for the benefit and welfare of society, from raw materials to goods and services that ultimately allow the functioning of the Earth as an ecosystem. Today, the vital role of forests to mitigate the effects of climate change has been internationally recognized and highlighted. In this sense, the **general objective** of this Thesis is the study of phenomena that determine forest tree phenotypic variation at the species level, such as gender, local adaptation and plasticity. This base of knowledge will reveal how phenotypic variation varies within species, and thus will facilitate the inference of responses –e.g. under scenarios of climate change– that in the medium-long term could alter the demography and distribution of forest tree species. Finally, this knowledge will enable the development of forest policies aiming at managing and preserving forests under climate change, and thus ensure proper ecosystem functioning and sustainable use.

This Thesis is composed of seven chapters to contextualize, develop and discuss main issues with regards of forest tree intraspecific phenotypic variation. The first chapter, **chapter 1**, includes an introduction about the impacts and risks of forests under climate change. Moreover, we present the group of species studied –a set of conifer species with a distinguished role in the early stages of the ecological succession– and the Iberian Peninsula as study region –in the last glaciation, this region played a significant role as a refuge for flora and fauna–. In **chapter 2**, we targeted the underlying biotic factors leading phenotypic variation in tree growth rates of dioecious –i.e. male and female reproductive organs are in separate individuals– *Juniperus thurifera* L. The stand forest studied was located in the center of the Iberian Peninsula, it was almost monospecific and presented high density of individuals, most at the juvenile stage. This scenario is possibly the result of abandonment of agricultural practices in the context of global change. Overall, our results suggest that for monospecific stands of *J. thurifera* in early stages of development, when self-thinning seems not to have been produced yet, the growth dynamics is controlled by the size of individuals

and the reproductive class, while intraspecific competition has no effect on growth, either intra- or inter-sexual. In the following chapters of the thesis (chapters 3, 4, 5 and 6), phenotypic plasticity and local adaptation are considered as the main sources promoting intraspecific phenotypic variation in a number of monoecious species –i.e. both male and female reproductive organs are in the same individual–. In **chapter 3**, we studied the phenomenon of local adaptation –i.e. when the local population has a higher fitness in their habitat compared to foreign populations–. The study focused on two distant populations of maritime pine (*Pinus pinaster* Ait.) –Center and East of the Iberian Peninsula– with contrasting environments –continental and mediterranean–. The study targeted germination and recruitment during the first growing season. This knowledge is essential to forecast the persistence of forests in the future. Based upon the establishment of an *in situ* reciprocal sowing experiment in semi-natural conditions, we tested local adaptation and the effect of abiotic factors –such as light and temperature– approximated through the canopy effect on our study variables –emergence and early survival–. Maritime pine populations did not show evidence of local adaptation for germination and early recruitment. Furthermore, microenvironments apparently selected the populations with the most successful life strategy regardless of its origin (local or non-local). This study highlighted the high stochasticity in the recruitment phase and the complexity of factors that influences the early stages of establishing in maritime pine. The generalization of measures, such as assisted migration, suggested to mitigate the effects of climate change, can pose a risk when decisions are based only on average-climate similarity between source and destination. In **chapter 4**, we modeled intraspecific phenotypic variation of tree height in *Pinus sylvestris*. To do so, we used two complementary methods; one is characterized to be "universal" and the second as too specific. We used data derived from provenance common gardens that allow separating the genetic –in our case, the population origin– and the environmental components. The main objective of this study was to contribute with experimental evidence about the convenience of account for the study of tree species dynamics, at least, the population level instead of just the species level. Our results showed that adaptive and plastic responses generated partly from the observed phenotypic variation within species. Both

methods gave similar results; the too specific supplemented the more "universal" above all when focusing into a particularized population. This converts them as complementary techniques to our case study. In **chapter 5**, the study focused on the analysis among and within species of a composite trait, the tree height-diameter allometry trait –i.e. the allometric relationship between total height and diameter at breast height–. We developed this study in four out of the six species of pines found in the Iberian Peninsula. To do so, we used data derived from provenance common gardens, as in the previous chapter. On one hand, the analysis between species allows us to compare different life strategies. On the other hand, the intraspecific analysis complements the previous and permits us to distinguish whether genetic variation is the result of natural selection processes and/or historical-demographic processes. Our results showed marked differences in the allometric responses of pine species and populations, i.e. in the strategies for allocating resources to grow in height or width between the more cold-tolerant species, *Pinus sylvestris*, and those more Mediterranean, *P. halepensis*, *P. nigra* and *P. pinaster*. Moreover, at the intraspecific level, the identified allometric patterns took place because of adaptations to climate as well as because of demographic processes. In **chapter 6**, we analyzed how climate change could affect intraspecific variation in tree height-diameter allometric relationships. Our results indicated that, in general, intraspecific phenotypic variation would be altered. In addition, pines from xeric origins may significantly modify their allometries, resulting in much more slender trees. However, pine species associated with mountain environments would tend to keep similar strategies –not meaningful changes in their allometry–. Finally, our results highlighted the lack of genetic variation in plastic responses. This result suggests that the ability to acclimatize to new environments is identical across provenances, and therefore similar plastic responses would be expected from different genetic origins. Finally, in **chapter 7** we present the general conclusions.

Keywords: climate change, composite trait, conifer species, dioecy, early survival, growth, intraspecific variation, local adaptation, phenotypic plasticity, pinus, single trait.

Chapter 1

General Introduction and
Methodological Overview

<u>General Introduction</u>

Impacts and risks of climate change on forest functioning

Forests cover about 31% of the Earth's land surface, about 4,000 billion hectares (FAO 2010). Nowadays, the importance of forests is well recognized, but this conception has evolved along the human history. In the past, forest trees played a key role in the first human populations that took sustainable advantage from the natural resources to feed and to take shelter. Later in time, inhabitants over-exploited natural environments, causing forest degradation and deforestation, mostly to supply the industrial demand (reviewed in FAO 2012).

In the 21st century, the interaction between humans and forests has changed. Forests are now well recognized as (i) the world's largest repository of terrestrial biodiversity (harbouring two thirds of the world diversity, Millennium Ecosystem Assessment, 2005); (ii) contributors to soil and water conservation in many fragile ecosystems; (iii) vital players in global climate change mitigation –considered as one of the biggest threat today–; and (iv) suppliers of human well-being (employment, energy, nutritious foods and a wide range of other goods and ecosystem services) (FAO 2014). Moreover, forests make a meaningful contribution to country's welfare, which is about 1.2% of the global economy[1] or about US$730 billion (FAO 2014). However, the Earth planet has currently faced a loss of forest area of ca. 1.3% of the total forest area –data calculated over the ten-year period of 2000-2010–

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[1] This quantity includes the contribution of the formal forest sector and the incomes from woodfuel production and non-wood forest products to gross domestic product.

(FAO 2014). Altogether, there is no question that forests are of utmost importance, and that the persistence and functioning of Earth's ecosystem rely strongly on them.

The major threats and enemies of natural forests have also changed over time. For millennia, overexploitation has been the major thread on forest persistence (IPCC 2007a, 2014). However, today, climate change has amply surpassed overexploitation risks, as it has been insistently reported by the Intergovernmental Panel on Climate Change (IPCC 2007b, 2014).

Climate projections, according to the IPCC's Fifth Assessment Report (AR5, 2013), predict an increase of the global temperatures in the coming decades, particularly in the latter part of this century (Figure 1a) (IPCC 2007a, 2014). Future precipitation patterns are less consistent, with some parts of the globe showing net increases and others showing net decreases (Figure 1b). But what is consistently projected, is a more vigorous hydrologic cycle because of the greater heat energy in the atmosphere. To sum up, longer and more intense drought periods in many regions of the world are projected (Dai 2013), intermingled with heavy precipitation events. These storms will provide a larger proportion of the total annual precipitation, provoking more frequent runoffs and floods (Milly *et al.* 2002; Lenderink & van Meijgaard 2008). The hardness of these significant changes will be site dependent (Figure 1).
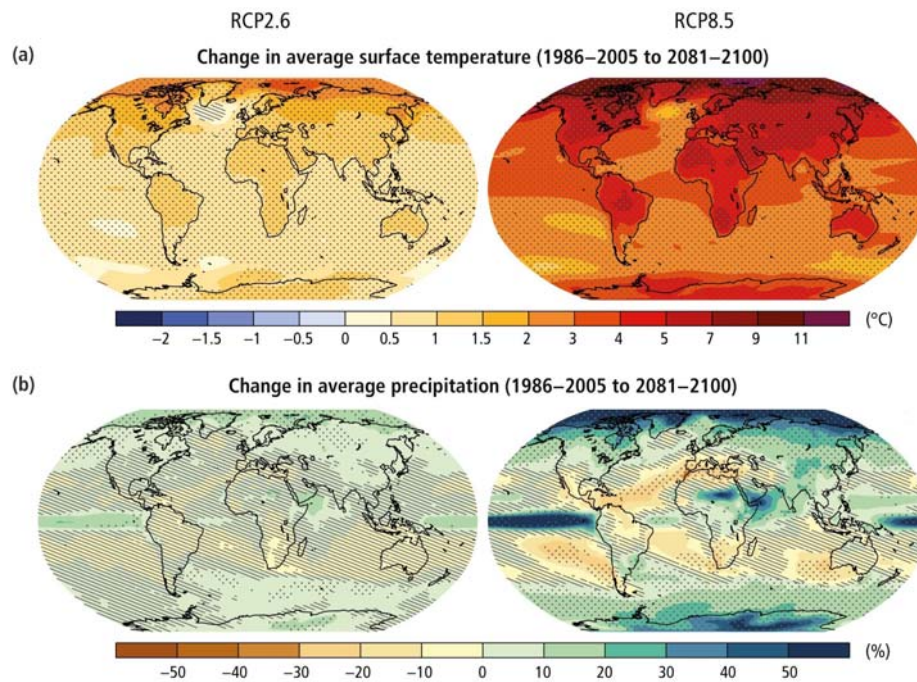
Figure 1 Predicted changes in average surface temperature **(a)** and precipitation **(b)** for the scenarios RCP2.6 and RCP8.5 in 2081-2100. Stippled regions mean large and confident change prediction; hatched regions mean small signal of change. All changes are relative to the baseline period 1986–2005. Source: (IPCC 2013) Assessment report WGI AR5.

Climate change will affect the entire natural system, ranging from physical systems –such as glaciers, snow, rivers, etc.– to biological systems –such as terrestrial and marine ecosytems–, and human and managed systems –such as food production, health, economics, etc.– (Figure 2). In general, climate change is predicted to alter biodiversity patterns and species distribution. In this sense, previous works have shown potential northward and upward range shifts of many species in the Northen hemisphere (Thuiller *et al.* 2005; Araújo & Rahbek 2006; Benito-Garzón, Sánchez de Dios & Sainz Ollero 2008); as well as phenological shifts –e.g. flowering time, bud burst and

fruiting– (Menzel *et al.* 2006; Parmesan 2006; Franks, Sim & Weis 2007), that could disrupt producer-herbivore dynamics (Visser and Holleman 2001). Climate change can also alter the frequency and intensity of forest disturbance mechanisms, such as wind, fire events or forest pests and pathogens (Logan, Régnière & Powell 2003; Allen *et al.* 2010). Overall, these climate change consequences could lead to local and massive extinctions, biodiversity losses, changes in major vegetation types and alterations in the atmosphere-vegetation interaction (Chapin *et al.* 2000).

Figure 2 Observed impacts in recent decades attributed to climate change on natural and human systems. Figure adapted from the Assessment report WGII AR5 in IPCC (2014).

Under climate change, trees, and in extent forest ecosystems, are especially endangered due to inherent characteristics that make them more sensitive. Trees are long-lived –with long lasting generations– and sessile

organisms –unable to escape from non-optimal living conditions–, which limit their capacity to adapt to rapid environmental change (Jump & Peñuelas 2005; Jump, Hunt & Peñuelas 2006; Lindner *et al.* 2010; IPCC 2014). However, forest trees and populations can offset, at least partially, these negative predicted impacts. In general, trees display (i) high levels of gene flow –which can act either positevely or negatively to adapt to new environmental conditions, depending on the source–; (ii) high fertility, which coupled with natural selection processess can result in microevolution; and (iii) a wide set of phenotypes in response to new environments –phenotypic plasticity–, which can promote rapid acclimatation. All these characteristics can help forest trees to overcome current and predicted environmental stress. However, if trees are not able to genetically adapt to changes, they will be forced to either migrate or go extinct (Aitken *et al.* 2008).

In the present climate change context, understanding the forest tree abilities to offset environmental changes are key to (i) preserve the global Earth ecosystem functioning; and (ii) develop forest policies and practices promoting sustainable forest management ensuring the full range of forests' contributions to economy and social well-being (FAO 2011, 2014). To do so, it is mandatory to start by understanding well the processes that constrain or enhance the persistence of species and populations (Davis, Shaw & Etterson 2005; Aitken *et al.* 2008), making essential the study of intraspecific phenotypic variation.

Intraspecific phenotypic variation and its role to overcome changes in environmental conditions

In forest trees, intraspecific phenotypic variation[2] results from a combination of abiotic –climate, soil types, stand structure, elevation, slope, etc.– and biotic –interaction effects between conspecific individuals, and with other plants, animals, etc.– factors and its genetic potential (Figure 3). In fact, this variation makes species capable to occupy large distribution ranges and contribute to maintenance of biodiversity (Albert *et al.* 2011; Bolnick *et al.* 2011). During decades, ecologists were devoted to unveil the general principles of plant world diversity, complexity and functioning (Calow 1987). Consequently, intraspecific phenotypic variation and promoting sources were neglected, and hence are less understood compared to interspecific phenotypic variation (Bolnick *et al.* 2011).
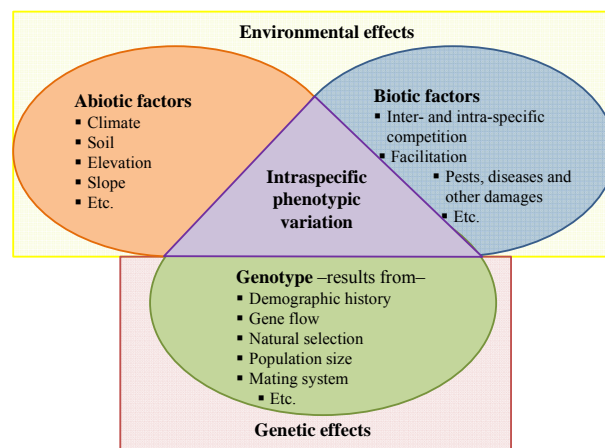
Figure 3 Conceptual diagram of interacting factors giving rise to intraspecific phenotypic variation.

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[2] Variation is here understood as the variation within species from either individual to individual, or population to population.

A key step to study intraspecific phenotypic variation is to consider populations living in distinct local environments. In this thesis, we have focused into local adaptation and plastic responses as sources of intraspecific phenotypic variation from population to population, i.e. within genetic structured groups in terms of seed source origin. Nonewithstanding, the particular reproductive system of woody species interacting with their surrounding environment can be considered as another source of intraspecific phenotypic variation. Understanding the full potential of intraspecific phenotypic variability could be decisive for increasing resilience of natural and planted forests, as well as for the maintenance of productivity in the context of climate change (Hamrick 2004; Chmura *et al.* 2011; Benito-Garzón *et al.* 2011).

a) Dioecy

Dioecious species present male and female reproductive structures on separate plant individuals. Here, gender differences can explain a meaningful part of their intraspecific phenotypic variation. Male and female individuals behave differently and thus present differentiated life history strategies and dynamics (Delph & Meagher 1995; Obeso 2002). For instance, male and female individuals can differ in vegetative and reproductive strategies giving rise to differences in population structure and variation in their relative frequency and abundance along environmental gradients (Freeman, Klikoff & Harper 1976). In general, males show relatively higher vegetative growth (Lloyd & Webb 1977; Cipollini & Whigham 1994; but see Gimeno *et al.* 2012) and survival rates than females (Doust, Brien & Doust 1987; Allen & Antons 1993). Although the study of dioecious plant species has received considerable attention, some species –such as *Juniperus thurifera*– are now

experiencing a range expansion (Olano, Zavala & Rozas 2011) as a result of the abandonment of traditional agricultural practices. Thus, new grow dynamics and interactions are expected to occur. Revisiting these likely new dynamics in these novel environments, accounting for intraspecific phenotypic variation, is of vital importance to manage these new forest stands in the context of climate change.

b) Local adaptation

Genetic adaptation takes place when natural selection (e.g. due to abiotic or biotic drivers) overcomes other evolutionary forces (genetic drift, mutation, migration, recombination), causing genetic differentiation among populations, and hence promoting intraspecific phenotypic variation (Linhart & Grant 1996). Formally, a population is considered locally adapted, sensu Kawecki and Ebert (2004), when local populations always overperform non-local ones. In both plants and animals, patterns of phenotypic variation are often arrayed in clines; frequently, these phenotypic clines have resulted from a balance between local adaptation and gene flow (Endler 1977; Barton 1999).

Many studies have shown that forest trees present substantial potential for local adaptation, despite the intense gene flow which is particularly high in conifer species (Kawecki & Ebert 2004; Notivol *et al.* 2007; Savolainen, Pyhäjärvi & Knürr 2007; Alberto *et al.* 2013). Great potential for genetic adaptation help forest trees to display broad distributions across heterogenous environments. Differential local conditions –driven by temperature, light, soil, water availability, or other factors– can led to significant clinal variation in growth, phenology, and physiological

traits. For instance, in many conifers, nothern populations set buds earlier than the southern ones and present lower growth rates (Mikola 1982; Morgenstern 1996; Hurme *et al.* 1997; Howe *et al.* 2003; Savolainen *et al.* 2007; Vitasse *et al.* 2009).

While the ability to adapt to local conditions is perceived positevely, the current concern is whether populations will be able to respond to warming climate at the pace required. In fact, after generalised postglacial migrations, many populations are inhabiting suboptimal climates still today, e.g. *Pinus contorta:* Rehfeldt *et al.* 1999; Rehfeldt *et al.* 2001; *Pinus sylvestris:* Rehfeldt *et al.* 2003. This suggests that present populations are still adapted to past conditions and that the process of adaptation can be slow. In addition, it is unclear if locally adapted populations have enough standing genetic variation –as a result of past selective forces guiding to adaptation– to adapt to new climates.

On the whole, local adaptation outcomes contribute to delineate a map of intraspecific phenotypic variation across environments –with optima reached in different climates, soils, etc.–. Thus, studies targeting population dynamics –chiefly, regeneration, growth and mortality– and future species' distributions need to account for this variation contained within species. Clearly, the use of a unique mean response representing the whole-species spectrum does not match the reality.

c) Phenotypic plasticity

Phenotypic plasticity, the capacity of a tree genotype[3] to render different phenotypes under diverse environmental conditions (Bradshaw 1965; Sultan 2000), contributes together with genetic variation to generate intraspecific phenotypic variation in response to environmental cues.

Many studies have demonstrated that forest tree species can display plastic responses in morphological, phenological, physiological and/or reproduction traits in response to a variety of drivers. For instance, plastic responses have been described –at seedling and juvenile stages– in response to drought stress (Irvine *et al.* 1998; Aranda *et al.* 2009; Sánchez-Gómez *et al.* 2013; Taeger, Sparks & Menzel 2014), and differentiated light, temperature, CO_2 gradients (Sánchez-Gómez, Valladares & Zavala 2006).

In the context of climate change, phenotypic plasticiy has gained in importance in research to explore the limits and ability of natural and planted forest populations to acclimate to the new climates in the short-term. However, it is not clear yet if plastic responses can play a long-term role (see review in Matesanz *et al.* 2010). The existence of genetic variation in plastic responses, i.e. interactions between genotype and environment, reflects that distinct genetic material can present different abilities to acclimate to the local environment. In fact, this is the case for many conifer populations when evaluated for vegetative traits –such as total height, diameter and survival– (e.g. Rehfeldt *et al.* 2001; Wang *et al.* 2010; McLane *et al.* 2011; Leites *et al.* 2012). These findings make think that evolution may act upon these new modified phenotypes (Sultan 1987; Schlichting 2002; Ghalambor *et al.* 2007). Therefore, the ability to display plastic responses

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[3] In this thesis, genotype refers to individuals with common origin, e.g. provenance.

increase the probability to face succesfully environmental changes. Thus, high adaptive plastic phenotypes are depicted as more favourable to survive in a broader range of environmental conditions against those that are less plastic (Rehfeldt *et al.* 2001; Sultan 2001; Benito-Garzón *et al.* 2011), though this is not always the case (Valladares, Gianoli & Gómez 2007).

13

<u>Methodological Overview</u>

Study region: the Iberian Peninsula

The research studies of this thesis were developed in the Iberian Peninsula. This region has a set of intrinsic characteristics and challenges that makes it an interesting site of study.

Regarding to the inherent features of the region, we highlight the following. First, the region, despite relatively small, is highly heterogeneous in topography and climate. For instance, great differences can be found in altitude (from 0 to 3,500 m a.s.l.); in climates (from continental Mediterranean to Atlantic ones), and in soil types (western area is mainly acidic, while eastern area is basic) (Costa, Morla & Sáinz 1997). Second, the region has played an influential role during past glaciations acting as refugia for European flora and fauna (Petit *et al.* 2003; Médail & Diadema 2009), and now harbouring high genetic diversity in plants –particularly in trees– (Hampe & Petit 2005; Fady & Conord 2010). Third, many forest tree species with ample and continuous distributions across Europe have their southern margin in the Iberian Peninsula (e.g. *Pinus sylvestris*), or in the Mediterranean region (e.g. *Pinus nigra, P. pinaster*). Climate is expected to be the primary limiting factor for tree species persistence along latitudinal clines (Parmesan 2006). At the southern part of these clines, this is augmented by greater abiotic stresses, more intense interspecific competition, lower relative fecundity and lower local population densities than in the core species distribution (Case & Taper 2000; Aitken *et al.* 2008).

Overall, it is expected local adaptation to have contributed to high levels of phenotypic variation among and within populations in the Iberian

Peninsula because of both the postglacial demographic history and the current environmental heterogeneity, as well as the stronger selection pressure in southern distribution limits.

Furthermore, we have identified in the region the following two facts that we believe are challenges for present and future forest management. First, ca. 36% of the Iberian Peninsula is covered by forests from which a 15% corresponds to forest plantations –i.e. with an artificial origin–. This extension is growing today –at a rate ca. of 1% (FAO 2010)– mainly due to agricultural land abandonment, forest plantation campaigns, coppice conversion, etc. (FAO 2013). Second, this region is considered one of the most vulnerable areas in the world under the predicted scenarios of climate change (Brunet *et al.* 2007; Giorgi & Lionello 2008). Therefore, here forest ecosystems –either naturally or artificially regenerated– are under a higher risk and need urgent action. On the whole, there is a real need to manage and preserve both natural forests and new and current plantations, within the climate change context.

Plant material: Iberian Conifers

In this thesis, we have selected five conifer species as case studies. The five of them had glacial refugia in the Iberian Peninsula and are keystone species in many forest ecosystems. Four out of the five species are pine species: *Pinus halepensis, P. pinaster, P. nigra and P. sylvestris*; and the fifth conifer species is *Juniperus thurifera* (Figure 4).

a)   *P. halepensis* – Aleppo pine

b)   *P. pinaster* – Maritime pine

c)   *P. nigra* – Black pine

d)   *P. sylvestris* – Scots pine

e)   *Juniperus thurifera* – Spanish juniper
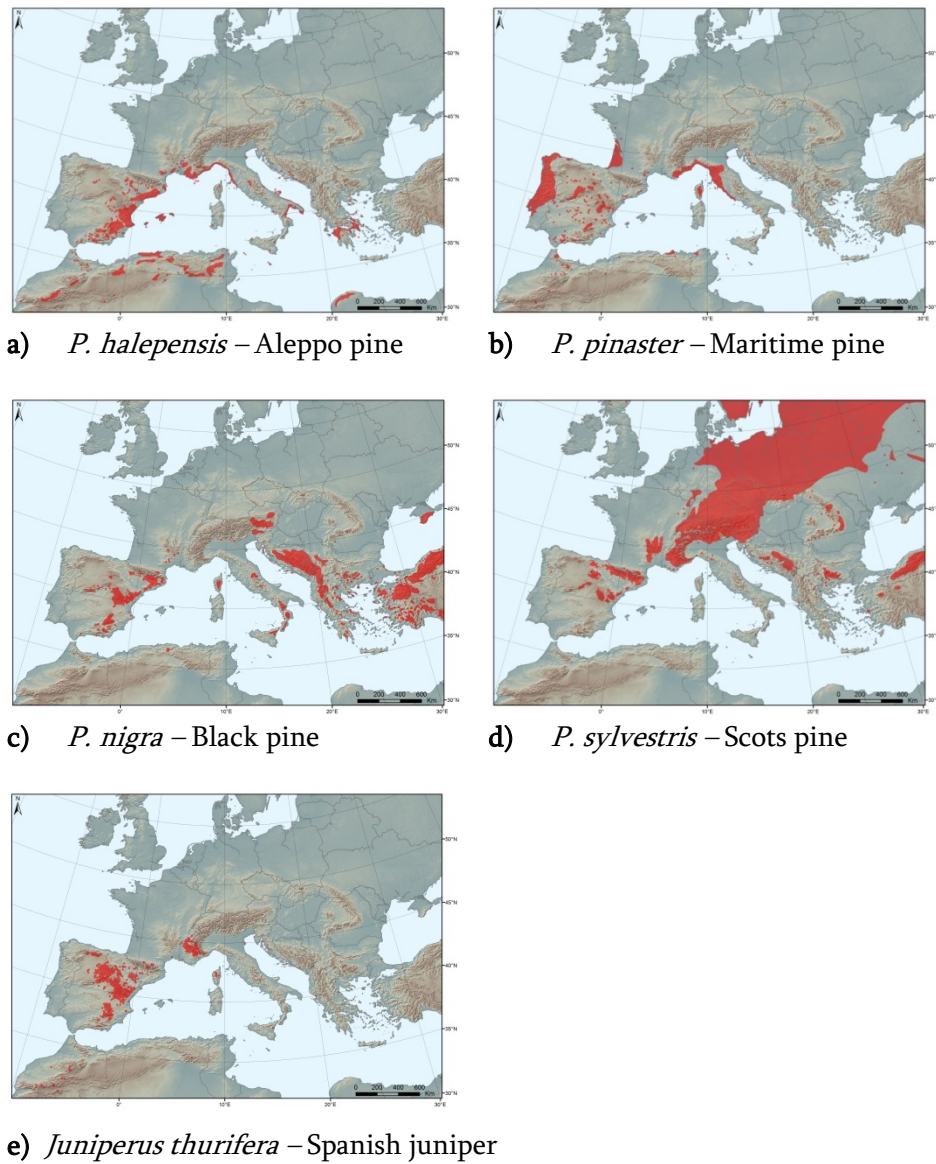
Figure 4 Natural distribution area of the five conifer species used in the present thesis. From **a)** to **d)** data have been compiled by members of the EUFORGEN Networks (2009). Data of figure **e)** has been compiled by GBIF.org (24th October 2015) GBIF Occurrence Download http://doi.org/10.15468/dl.zl4ur6

Pinus halepensis is distributed all around the Mediterranean basin, mainly along the coast and, exceptionally, inland in Spain, Tunisia and Italy. *Pinus nigra* is often associated with Mediterranean mountain areas. *Pinus pinaster* occurs naturally from southwestern Europe to north-western Africa, showing several adaptations to local conditions in vegetative traits (height, diameter and survival) (Alía, Gil & Pardos 1995); in fire-related traits (Tapias *et al.* 2004; Budde *et al.* 2013); etc. Finally, *P. sylvestris* displays the largest tree distribution across the Eurasian region, and reaches its southern limit in southwestern Europe predominantly confined to mountainous areas.

Juniperus thurifera is mainly found in high plateaus and mountain regions of the Iberian central-east at a variety of altitudes (140-1,800 m a.s.l.) in continental and cold Mediterranean climatic conditions (Gauquelin *et al.* 1999; Terrab *et al.* 2008; Figure 4e). It is usually the dominant species in low-density woodlands on poor, shallow, or rocky soils (both acidic and calcareous, although more abundant in the latter) (Gauquelin *et al.* 1999).

Natural populations and common gardens

In this thesis, we have used two types of data. Data collected in natural populations in the field –observational studies–, and data derived from multisite common gardens –experimental studies–. A natural population was sampled in the framework of chapter 2. The population was located in Center of the Iberian Peninsula. In this research study, dimeter, height and position were taken from each individual in the forest stand sampled.

The second type of data is derived from 'historical provenance common garden experiments' installed in Spain. Research using common

gardens is becoming popular worldwide because of the valuable information contained –e.g. among population genetic variation, degree of locally adapted populations, etc.– and the numerous applications that can be given to the data collected in common environments –e.g. predict the performance of a population at a planting site under future circumstances, predict spatially explicit estimates of fitness traits, etc.– (Savolainen *et al.* 2007; Alberto *et al.* 2013). In short, they are currently considered as climate change experimental designs. This is the case for the data analysed in chapters 3, 4, 5 and 6. Chapter 3 relies on the data generated in a short-term experiment specifically developed for this thesis, while the rest of the chapters took advantage from an extensive database stored in the GENFORED network – www.genfored.es– (Figure 5). This network is handled by the Forest Research Centre of INIA –CIFOR– and the Agrifood Research and Technology Centre of Aragón –CITA, and aims to facilitate the exchange of information about the Spanish forest genetic trials.

In provenance commmon gardens, plant material is grown from open-pollinated seed material collected from wild stands of the species –it is desirable that parent trees are separated a distance of a least 50 m to avoid inbreeding, González-Martínez et al (2006)–, often covering the full natural range. These seeds are grown under similar nursery conditions to finally constitute the plant material to be transplanted, after one or two years, to the planting site location. Provenance tests are normally long-term experiments, with an average duration of 30 years or even more, and are normally measured every 5 years or less.
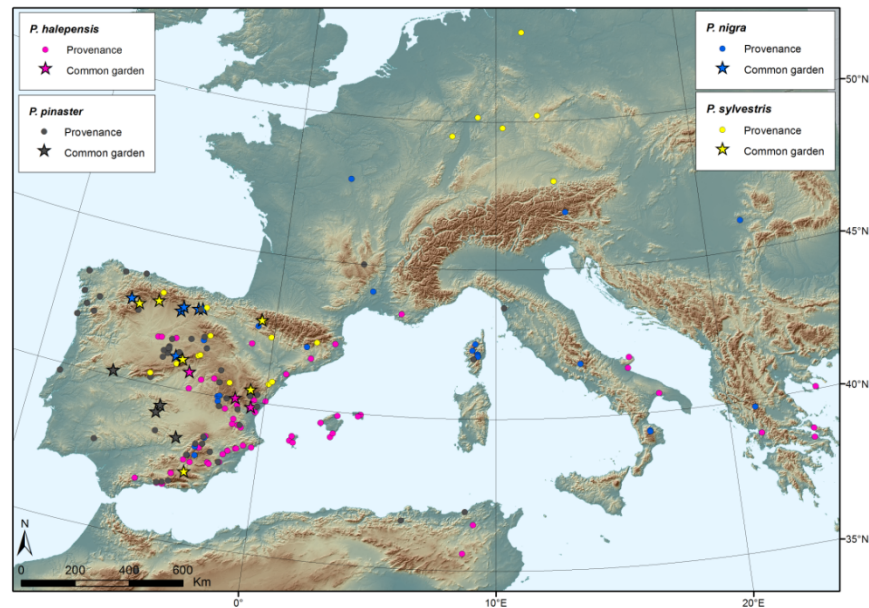
Figure 5 Example of provenance common gardens installed in Spain and stored in the GENFORED network. In particular, this figure shows those common gardens analysed in the present thesis and the provenances tested.

The most common experimental design layout is a randomized block design which can be either complete –all provenances are planted throughout all blocks (repetitions)– or incomplete –where the blocks contain an incomplete set of provenances–. Moreover, these experimental designs can be replicated across different environments –sites– and then getting a multisite common garden. This layout allows for separating the environmental and genetic effects on the tree phenotype and hence, assessing intraspecific phenotypic variation at different levels among the set of provenances tested. Also, this permits to assess properly plastic and genetic plastic responses –i.e. genotype×environment interaction–. A particular case of provenance common gardens are reciprocal transplants. These experiments have a long history dating back to the end of the XIX century (Von Marilaun & Oliver)

and allowed Turesson (1922) to demonstrate that the spatial heterogeneity in populations' traits was adaptive and genetically based. Here, the provenances included in the experimental desing are tested in their local site of origin. Reciprocal common gardens are also interesting because they can incorporate the microenvironmental variation of the natural environments where populations have evolved (see chapter 3).

Statistical analysis

In this thesis, we have employed the set of different statistical analysis that better suited to our specific questions, and also that were relevant for the learning process associated to the development of a PhD Thesis.

Mixed-effect models

Mixed-effect models provide the ability to analyze data with dependence structures, imbalances and heterogeneous variances (Bolker *et al.* 2009; Zuur *et al.* 2009). Also, the inference space offered is wider and can better understand the data structure compared to non-mixed effect models. The different levels in a fixed effect are of interest and need to be estimated, while the levels of a random effect are a sample from a bigger universe and the interest relies on variation. Mixed-effect models can have multiple and diverse purposes, and this will lead the way the model is constructed and to even find opposite model structures regarding the standpoint of different disciplines.

In our research studies, we have employed mixed-effect models to account: (i) the hierarchy structure of data originated from experimental designs; and (ii) unknown sources of variation which can affect the response

variable as well. These models can be easily implemented with specific packages developed within R environment (R Core Team 2012), such as lme4 (Bates *et al.* 2015) and nlme (Pinheiro *et al.* 2013).

Assessment of local adaptation

We have assessed local adaptation based on different approximations. A first approach uses a reciprocal transplant –in our case, sowing– experiment described in chapter 3. This is a powerful tool to detect and test local adaptation –sensu Kawecki and Ebert 2004–. The basis relies on measuring the fitness of populations in their own habitat and when transplanted to other habitats. Local adaption is only recongnised when the local populations always outperform foreign ones at their origin.
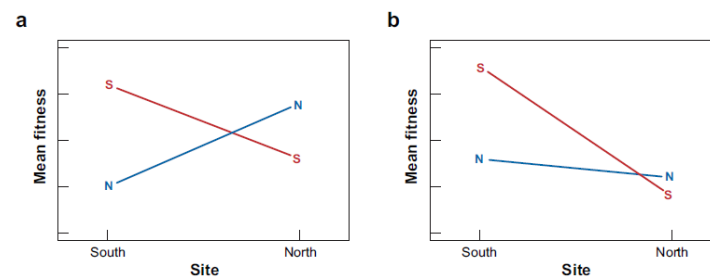
Figure 6 Definition of local adaptation (Kawecki & Ebert 2004). The fitnesses of the northern and southern populations show a genotype by environment interaction. In (**a**) each has highest absolute and relative fitness at its local site, in (**b**) both have highest absolute fitness in the south, but each has highest relative fitness at its site. Figure taken from Savolainen *et al.* (2007).

A second approach is based upon the universal response function –URF– proposed by Wang et al (2010), and it does permit to assess local adaptation sensu lato. This approach has been developed in chapter 4. This function combines at the same data information from planting sites and

population origin –normally climate information–. A first order partial derivate of the URF is solved for the environmental parametes associated to the population origin, and the maximum value is provided. This approach relies on the idea that this maximum is reached when a population is well adapted. A third approach permits to indicate the existence of local adaptation, sensu lato, but not to confirm whether the local population has always the highest fitness in its local environment. This approach is based on correlations; these correlations can be done between environmental variables and phenotypic traits or between estimated model parameters –such as in chapter 5–.

Evaluation of plasticity

Assessment of plastic responses needs to account for the effect of environmental forces on genetically structured groups –e.g. provenance, full-sib or half-sib families and clones- to avoid confusion between genetic and plastic responses. Therefore, the appropriate way to explore plastic responses is through the setting of experimental designs –such as the provenance common gardens previously explained or by developing ad hoc experiments– that permit to isolate the desired effects. In this thesis, we have used provenance common gardens to assess plastic responses along climatic or geographical gradients. There is a variety of statistical approaches to assess plastic responses. For example, we can use climate transfer functions (Leites *et al.* 2012), or population response functions that depict the so called reaction norms (e.g. Rehfeldt *et al.* 1999; Rehfeldt *et al.* 2003; Wang *et al.* 2006; McLane *et al.* 2011); or even the universal response –URF–functions described above (e.g. Wang *et al.* 2010; Chakraborty *et al.* 2015). In our case,

we have drawn reaction norms in chapter 4, 5 and 6, and we based upon population response functions and URFs.

Thesis Outline and Objectives

The main goal of this thesis is to assess distinct sources of intraspecific phenotypic variation –chiefly, local adaptation, phenotypic plasticity and gender– along different demographic stages –regeneration, growth and maturity (resources allocation)– and across different conifer species. A better understanding of intraspecific phenotypic variation sources would confer a full understanding of species potential for acclimation to new environments, and this is critical for making reliable forecasts, in particular within the climate change context. The role of other types of environmentally induced variation –which could facilitate adaptive evolution, such as maternal effects (Räsänen & Kruuk 2007) and epigenetic inheritance (e.g. True *et al.* (2004); Sáez-Laguna *et al.* (2014))– are out of the scope of this thesis.

The present PhD thesis is structured in seven chapters, including the General Introduction and Methodological Overview –chapter 1– developed here. Chapters 2 to 6 are research studies, including each the usual sections of Summary, Introduction, Material and Methods, Results and Discussion. Chapter 7 presents a set of general conclusions derived from the present thesis. Finally, at the end of the dissertation an updated curriculum vitae is attached.

A general overview of the specific objectives of each research study is presented below and summarized in Table 2:

Table 2 General overview of research studies with their objectives, material and methods and results in form of publications and manuscripts included in the present thesis.

Chapter	Objectives	Material and methods		Results
		Study sites	Statistical method	
2	Disentangling the biotic factors driving growth patterns in a dioecious species	Central plateau of the Iberian peninsula	- Non–linear models estimated by maximum likelihood statistic	Vizcaíno-Palomar et al. (2014). *European Journal of Forest Research*
3	Test local adaptation in emergence and early survival in two contrasted populations. Test whether natural selection acts equally on all families within populations	Central and East part of the Iberian peninsula	- Survival analysis. - Discrete-time logistic models. - Fractional paternity analysis	Vizcaíno-Palomar et al. (2014). *PLoS ONE*
4	Use of two complementary techniques to model height population responses to climate	Iberian peninsula	- Linear mixed models	Vizcaíno-Palomar et al. (In preparation)
5	To analyse climate effects for tree height–diameter allometry in four pine species	Iberian peninsula	- Linear mixed models	Vizcaíno-Palomar et al. (Under review) *New Forests*
6	To investigate inter an intraspecific differences for tree height–diameter allometry in four pine species	Iberian peninsula	- Non–linear models estimated by Bayesian statistics	Vizcaíno-Palomar et al. (Under review) *Ecol and Evol*

Chapter 2 - Main drivers of tree growth in a developing *Juniperus thurifera* stand in Central Spain

In this chapter, we studied phenotypic variation in tree growth rates of the dioecious *Juniperus thurifera* species. To achieve this main goal, we addressed three underlying factors potentially controlling demographic growth in this species: gender, size and competition. A better understanding of these factors will help to predict the future dynamics of this species in novel environments –such as new stands resulting from the abandonment of the traditional management practices–. We specifically tested the following hypotheses:

- In a high density forest stand and at early stages of development, tree size is expected to be an important factor determining tree growth

- Intraspecific competition affects negatively tree growth

- Tree growth rates differ among different reproductive classes. Specifically, non-reproductive individuals would grow faster than males and these in turn faster than females

Chapter 3 - The role of population origin and microenvironment in seedling emergence and early survival in Mediterranean maritime pine (*Pinus pinaster* Aiton)

In this chapter, we focused on intraspecific variation found in emergence and early survival stages in *Pinus pinaster* (Aiton). We hypothesized the existence of genetic-based differences in recruitment among populations and families of the same species, probably led by strong selective forces at early

establishment stages. We specifically aimed to answer the following questions:

- Is there local adaptation for emergence and early survival in maritime pine populations of contrasted origin?

- Is early survival affected by time of emergence? And what is the role of canopy cover (i.e. light regime)?

- Can we observe a transition in emergence and survival from open- to closed-canopy environments along the summer season?

- Is natural selection acting equally on all families within populations or, alternatively, is natural selection favouring some families, resulting in genetic change over generations for heritable traits?

Chapter 4 - Modelling population responses to climate: a case study in of *Pinus sylvestris* from in the Iberian Peninsula

In this chapter, we assessed the observed intraspecific phenotypic variation in tree height for *Pinus sylvestris* by using two complementary methods, the universal response function and population response functions.

We specifically aimed to assess:

- The suitability of the complementary use of two methods to model intraspecific phenotypic variation in tree height

- Contribute with experimental evidence to support the increasingly recognized necessity of including intraspecific phenotypic variation to better forecast the effects of changing climates on forest dynamics

Chapter 5 - Adaptation and plasticity in aboveground allometry of four contrasting pine species along environmental gradients

In this chapter, we have developed a multi-species study enabling species comparison under identical statistical framework. We also assessed the advantages of modelling a composite trait –to obtain a more complete view– instead of a single trait. Inter and intraspecific variation in tree height-diameter allometry were studied in four out of the six pine species present in the Iberian Peninsula. We used data derived from provenance common gardens that allow separating the genetic –gauged by the population origin in our case– and the environmental components.

We specifically aimed to test the following hypothesis:

- Height-diameter allometry patterns in pines are constrained by both the species and the intraspecific variation related to population origin

- Phenotypic variation in height-diameter allometry reflects adaptive patterns along climatic and geographic gradients

- Phenotypic plasticity modulates species and populations response to different environments

Chapter 6 - Climate and population origin shape pine tree height-diameter allometry: implications for forest management

In this chapter, we were interested in assessing the potential effects of new expected climates on tree height-diameter allometry using the models developed in chapter 5.

We specifically aimed to assess the following questions:

- What are the species-specific phenotypic changes expected for tree height-diameter allometry under future climates?

- What is the relative importance of inter- and intra- specific phenotypic plasticity in this context?

- What are the specific consequences of plastic allometric changes for the species' management, and their ecology and potential risks?

Funding

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Chapter 2

Main drivers of tree growth in a developing *Juniperus thurifera* stand in Central Spain

Resumen

A lo largo de las últimas décadas, el abandono de las prácticas tradicionales de gestión en España ha favorecido la expansión y densificación de las masas forestales de ciertas especies que en el pasado exhibían rangos de distribución más restringidos. Las superficies de sabina albar (*Juniperus thurifera* L.) están experimentando este fenómeno debido tanto al abandono de las tierras agrícolas como a la disminución de la presión ganadera en las mismas. A día de hoy, desconocemos cuáles son los factores que subyacen en la estructura y dinámica de estos nuevos paisajes. En este trabajo, se investigaron los principales factores bióticos que determinan el crecimiento de los individuos de sabina en un rodal caracterizado por una alta densidad y una edad temprana de desarrollo de sus individuos (edad media 32 años, 50 años después de abandono de la tierra). El crecimiento del rodal se caracterizó con 299 muestras procedentes de canutillos de crecimiento, diferenciándose los individuos en tres clases reproductoras: masculina, femenina y no-reproductor. Se utilizaron modelos de vecindad para evaluar la importancia relativa del tamaño del árbol y la competencia de los vecinos en el crecimiento en cada una de las clases reproductoras de la parcela de estudio. Se encontró que el tamaño del individuo presentó el mayor efecto sobre el crecimiento del árbol, mientras que el efecto de la competencia intraespecífica fue insignificante. Se observaron diferencias en los patrones de crecimiento entre las clases reproductoras a lo largo de tamaños de diámetro del tronco. Las tres clases reproductoras presentaron patrones idénticos de crecimiento cuando los tamaños de diámetros son pequeños. Sin embargo, en tamaños más grandes, los individuos femeninos presentaron mayor crecimiento, seguidos de los individuos masculinos y de los no-

reproductores. En general, nuestros resultados sugieren que en los rodales monoespecíficos de sabina albar en fases tempranas de desarrollo, donde los procesos de auto-clareo aún no se han producido, el tamaño de los individuos y la clase reproductora podrían desempeñar un papel relativamente más importante que la competencia intraespecífica como motores de crecimiento. Estos resultados constituyen nueva información que contribuye a la comprensión de la dinámica de crecimiento en etapas tempranas del desarrollo de esta especie dioica. Por otra parte, nuestros resultados proporcionan directrices para la gestión selvícola; aparentemente operaciones de clareo en fases tempranas no se traducirían en un mayor crecimiento de los individuos remanentes.

Palabras clave: dioico, competencia intraespecífica, abandono de los usos del suelo, bosques mediterráneos, modelos de vecindad, clases reproductivas, crecimiento de los árboles.

Abstract

Over the last few decades, abandonment of traditional management practices in Spain has led to widespread stand densification and has favored the expansion of some forest species that previously exhibited more restricted ranges. Spanish juniper (*Juniperus thurifera* L.) woodlands are experiencing this phenomenon due to agricultural land abandonment and a decrease in the livestock pressure. Yet the main drivers underlying stand structure and dynamics at this novel scenario are poorly understood. In this study, we investigate the main biotic drivers of tree growth in a high-density stand of the dioecious *J. thurifera* at an early developmental stage (mean tree age of 32 years, 50 years after land abandonment). Tree growth was measured by coring 299 individuals of different reproductive classes (male, female and non-reproductive). Neighbourhood models were used to assess the relative importance of tree size and neighbourhood competition on tree growth of each reproductive class in the study plot. We found that tree size had the strongest effect on tree growth, whereas the effect of intraspecific competition was negligible. We observed differences in growth patterns among reproductive classes along trunk diameter sizes. Thus, at smaller sizes, the three reproductive classes presented identical patterns of growth. However, at bigger sizes, females were the fastest growing individuals, followed by males and non-reproductive individuals. Overall, our results suggest that in young *J. thurifera* monospecific forests, where self-thinning processes may have not undergone yet, tree size and the reproductive class could play a relatively more important role than competition as drivers of tree growth. These findings constitute new information which contributes to understanding growth dynamics at early developmental stages in this

dioecious species. Furthermore, our results provide guidelines for silvicultural managing; suggesting that at these young juniper stands thinning would likely not translate into enhanced growth on remnant trees.

Keywords: dioecious, intraspecific competition, land use abandonment, Mediterranean forests, neighbourhood models, reproductive class, tree growth.

Introduction

In Mediterranean countries human activities have shaped the structure and function of the forest ecosystems for ages (e.g. Thirgood 1981; Blondel & Aronson 1995; Urbieta, Zavala & Marañón 2008). Over the last few decades, abandonment of traditional management practices has led to a general stand densification and has favoured forest expansion (Matesanz, Escudero & Valladares 2009; Olano, Zavala & Rozas 2011; Vayreda *et al.* 2012). These relative novel conditions mostly appear confined either to unproductive marginal lands or to mountain areas, where abandonment of traditional agriculture and the decrease in livestock pressure have happened as a consequence of depopulation of rural areas (Thompson 2005). Several studies have reported forest expansion and densification in different tree species (Poyatos, Latron & Llorens 2003; Gehrig-Fasel, Guisan & Zimmermann 2007). For example, Améztegui *et al.* (2010) reported that *Pinus uncinata*, a mountain pine species, has increased its surface coverage in the Catalan Pyrenees (north-east Spain) by more than 16% in a period of 50 years.

Likewise, Spanish juniper (*Juniperus thurifera* L.) woodlands are experiencing a similar phenomenon (Blanco *et al.* 2005; Olano *et al.* 2011; Gimeno *et al.* 2012c). They are one of the dominant plant communities in the scarcely-populated mountain regions of the central Iberian Peninsula. In the past, juniper woodlands were largely shaped by livestock grazing and wood extraction pressures by the inhabitants of the area. As a consequence, recruitment, survival and growth rates of the species were arrested (Olano *et al.* 2008; DeSoto *et al.* 2010). However, nowadays a process of densification is described due to the decline of these traditional activities. As a result of these

novel conditions, these forests may undergo very different stand dynamics in relation to past forest dynamics.

Over the last decade, several studies have focused on understanding the patterns of juniper growth in Spanish woodlands either as a consequence of land use changes (Olano *et al.* 2008, 2011; DeSoto *et al.* 2010) or as a gender-specific response (e.g. Montesinos *et al.* 2006, 2012; Rozas, DeSoto & Olano 2009; Gimeno *et al.* 2012a). Nevertheless, most of these studies have been conducted in mature stands, where average individual age was ~ 70-100 years (e.g. Rozas *et al.* 2008; Olano *et al.* 2008; DeSoto *et al.* 2010; but see Gimeno *et al.* 2012a,c). In contrast, the mechanisms driving population dynamics at earlier stages (age ~ 40 years), which are representative of these new juniper forests, have hardly been explored. In particular, we aimed to understand the role of intraspecific and intersexual competition in these new stands resulting from the release of traditional practices. Overall, this information could be crucial for establishing new scientific based management options for the future in this species.

Population structure results from a combination of abiotic and biotic factors, ranging from environmental conditions to inter- and intra-specific interactions (e.g. Hara 1984; Stoll, Weiner & Schmid 1994; Coomes & Allen 2007). We hypothesize that three potentially important biotic factors might explain inter-individual differences in tree growth in high-density novel stands: (i) the reproductive class of the individual tree, (ii) tree size and (iii) neighbourhood competition. According to allocation theory (Chapin *et al.* 1987), resources acquired by individual plants must be distributed among several competing functions, chiefly growth, maintenance and reproduction (Harper 1977). Consequently, the growth of non-reproductive individuals

might be expected to differ from that of reproductive ones (Delph 1999). Specifically, we expect that non-reproductive individuals might invest a null amount of resources in reproduction, and hence show greater growth rates than reproductive individuals (Bazzaz 1997). Moreover, in dioecious plant species, male and female individuals are expected to differ in their vegetative and reproductive strategies leading to differences in population structure and dynamics (Freeman, Klikoff & Harper 1976). In general, males show relatively higher vegetative growth (Lloyd & Webb 1977; Cipollini & Whigham 1994; Gimeno *et al.* 2012a) and survival rates than females (Doust, Brien & Doust 1987; Allen & Antons 1993).

Size is one of the main determinants of a plant's interaction with its environment (Schulze 1982) and a common factor used to predict tree growth (Coomes & Allen 2007; Gómez-Aparicio *et al.* 2011). The relationship between size and growth rate arise both from internal and physiological causes (Gower, McMurtrie & Murty 1996), and because increasing size affects a tree's ability to acquire resources. The most common pattern for this relationship is a rapid increase of growth at small tree sizes until a maximum growth is reached at some intermediate size, and then a more or less sharp decline (depending on the species) in growth in larger size classes (Muller-Landau *et al.* 2006; Russo, Wiser & Coomes 2007; Gómez-Aparicio *et al.* 2011). Finally, competition at early stages of forest stand development constitutes a third critical factor influencing individual tree growth and determining future stand development patterns (Harper 1977; Kobe 1996; Coomes & Allen 2007). Furthermore, intraspecific competition is usually stronger than interspecific competition (Tilman 1982; Stoll & Newbery 2005). This may be due to a higher competitive equivalence among

individuals of the same species than among individuals of different species (Silvertown & Charlesworth 2001). Within species, differences in resource allocation between reproductive classes might turn into dissimilarities in the competitive ability of males, females and non-reproductive individuals. Specifically, we expect females to compete less strongly than males and non-reproductive individuals.

In this study, we aimed to explore tree growth patterns of the dioecious species *J. thurifera* L. in a forest stand which is representative of new juniper forests resulting from agricultural and livestock farming abandonment (i.e. ca. 50 years) in the Iberian Peninsula. We used neighborhood models to predict tree growth for different reproductive classes (males, females and non-reproductive) as a function of size and intraspecific competition from neighbours (including interactions within and among the different reproductive classes). We specifically addressed the following hypotheses: (i) in a high density forest stand and at early stages of development, tree size is expected to be an important factor determining tree growth, (ii) intraspecific competition is expected to have a negative effect on tree growth, and (iii) tree growth rates are expected to differ among different reproductive classes. Specifically, non-reproductive individuals are expected to grow faster than males and these in turn faster than females.

Material and methods

Study species and study area

Juniperus thurifera L. (Cupressaceae) is mainly a dioecious tree, though infrequently monoecious trees are found (Borel & Polidori 1983; Lathuillière 1994). In the Iberian Peninsula, this species is mainly found in high plateaus and mountain regions of the central-east at a variety of altitudes (140-1,800 m a.s.l.) in continental and cold Mediterranean climatic conditions (Gauquelin *et al.* 1999; Terrab *et al.* 2008, Figure S1). It is usually the dominant species in low-density woodlands on poor, shallow, rocky soils (both acidic and calcareous, although more abundant in the latter) (Gauquelin *et al.* 1999). Juniper males and females flower at the end of the winter and wind-pollinated female cones mature during 20 months. It is a masting species, that is, individuals present low or null reproduction for several years, but every few years most individuals in a population present a massive reproduction event (Montesinos 2007). It can also be considered a slow-growing species (Gómez-Aparicio *et al.* 2011).

The study area was located in Monte Pradenilla (Segovia, north-central Spain), near the Sierra of Guadarrama, at 1,120 m a.s.l. Soils are calcareous cambisols developed on Cretaceous dolomitic substrates. Climate in this region is continental Mediterranean, with hot and dry summers and cold and long winters. Mean annual rainfall is 572.41 mm (1957-1990, data from a close meteorological station Prádena C.F.: 41°08'20" N, 3°41'17" W, 1,110 m a.s.l.). Mean annual temperature, mean minimum temperature and mean maximum temperature are 10.6 ℃, 4.3 ℃ and 16.83 ℃, respectively (1988-1992; all data provided by the Spanish Agencia Estatal de Meteorología). The study area was dominated by the species *J. thurifera* L.,

which forms even-aged monospecific forests with scatter presence of *J. communis* L. subsp. *hemisphaerica* (K. Presl.) and a sparse understory of small calcicolous shrubs. Traditional management (livestock grazing and wood harvest) was abandoned in the late 1970s; the stand is currently in an early development stage. Within this study area, we selected a rectangular study plot of approximately 2,250 m^2, with a high density of trees (0.71 trees m^{-2}), fairly flat topography and homogenous in rockiness (J. Pavón-García, personal observation).

Data collection and reproductive class identification

Data collection was conducted during the summer and fall of 1993. We selected this time of the year because it was adequate to visually identify the reproductive structures of individuals (when existing). We tagged all the individuals presented in the study plot, a total of 1,604. The study plot was composed of 447 reproductive individuals (225 males, 215 females and 7 monoecious individuals) and 1,157 non-reproductive individuals. Within reproductive individuals (males and females), 17% presented multi-caulis structure, i.e. more than one stem. Within the non-reproductive class, 19% presented multicaulis structure. In the study plot, the reproductive/non-reproductive ratio was 0.39:1 and the sex ratio (male/female) was 1.05: 1. For each individual in the study plot, we recorded tree size by measuring the trunk perimeter at 10 cm from the ground, total height and the reproductive class (males, females and non-reproductive). Reproductive class assignation was based on the presence of reproductive structures. Accordingly to this criterion, the male class was composed of individuals with male cones; the female class was composed of individuals with either female cones or the

existence of fruits; and the non-reproductive class was composed of those individuals without any reproductive structure in their branches yet. Reproductive class assignation was verified two more times, in 1994 and 1998. This verification helped us to assure that non-reproductive individuals were not a consequence of a non-masting year.

Wood cores were taken from a subsample of the individuals in each reproductive class (hereafter target trees) for characterization of growth rates and tree age. Target trees were selected following three main criteria (Pavón-García 2005): (i) trees should have a unique trunk in order to facilitate growth rate estimates, (ii) trees should have a minimum diameter of 25 mm (measured at 10 cm from the ground) in order to be able to core the trunk and to minimize serious damage after coring, and (iii) overlapping among neighbourhood areas should be avoided or minimized as much as possible. Overall, 115 males, 105 females and 79 non-reproductive individuals were selected (Table 1, Figure 1). Wood cores were taken as close as possible to the ground, at approximately 10 cm, perpendicularly to the trunk and heading north-east and reaching central trunk section by using a Pressler's increment borer. Cores were mechanically surfaced and then manually polished with a series of successively finer grades of sandpaper until the xylem cellular structure was clearly visible. In order to correctly visualize tree rings, a dissolvent (toluene) was added to remove traces or wood resins. Tree rings were visually dated following a standard procedure (Stokes & Smiley 1968) using a binocular regulated glass which helps to measure and count the tree rings. The double rings were scarce and easily detected. Radial growth of each target tree (mm yr^{-1}) was calculated by dividing the total length of the last ten tree-ring growth (mm) by the number of 10 years. Finally, we

characterized the neighborhood of each target tree by recording the number, size and reproductive class of each neighbour tree within three different radii (R_i: 1 m, 2 m, 3 m) from the target tree (Table 2).

Table 1 Summary data from target trees (t.). Number of individuals, age, growth rate, trunk diameter and height for each reproductive class are shown. Statistical differences between reproductive classes were tested using one way ANOVA test in: age, growth rate*, trunk diameter** and height. SE means standard error. Different letters indicate significant differences between reproductive classes for the studied variables at $\alpha = 0.05$.

		Male t.	Female t.	Non-reproductive t.
Number of individuals		115	105	79
Age (yr)	range	23-41	26-42	23-41
	mean	33.14 a	34.19 a	31.27 b
	SE	0.33	0.35	0.40
Growth rate (mm yr^{-1})	range	0.17-1.85	0.26-2.78	0.22-1.43
	mean	0.77 a	0.84 a	0.50 b
	SE	0.05	0.05	0.04
Trunk diameter (mm)	range	28.97-165.50	31.19-176.70	25.46-84.35
	mean	71.86 a	81.04 a	45.02 b
	SE	3.37	3.43	2.59
Height (cm)	range	150-470	150-480	70-260
	mean	258.17 a	275.52 a	156.89 b
	SE	8.94	9.11	6.88

* Estimated at 10 cm from the ground
** Measured at 10 cm from the ground

Table 2 Summary data of neighbourhood conditions for target trees (t.) in each reproductive class. Range, mean and standard error (SE) are shown for each variable. NCI (Neighborhood Competition Index) is the total basal area (cm^2) from neighbours contained in circumferences at different distances (1, 2 and 3 m) from the target tree; and Aver. nº ind. is the average number of individuals contained in circumferences at different distances from the target tree. Differences between reproductive classes were tested using one way ANOVA test in NCI and with generalize linear models (family= Poisson distribution) in Aver. nº ind. Different letters indicate significant differences between reproductive classes for the studied variables at $\alpha = 0.05$.

		Male t.	Female t.	Non-reproductive t.
NCI (1m) (cm^2)	range	0 - 281.62	0 - 266.00	0 - 358.10
	mean	52.44 a	47.47 a	57.35 a
	SE	6.34	8.24	8.39
NCI (2m) (cm^2)	range	32.56 - 644.04	6.44 - 504.34	47.80 - 1045.13
	mean	164.65 a	172.87 a	203.58 a
	SE	14.10	18.31	18.66
NCI (3m) (cm^2)	range	35.43 - 914.65	140.89 - 1191.94	158.38 - 1495.60
	mean	273.87 a	245.45 ab	293.64 b
	SE	19.62	25.48	25.97
Aver. nº ind. (1 m)	range	0 - 10	0 - 10	0 - 8
	mean	2.10 b	2.33 a	2.71 a
	SE	0.09	0.09	0.07
Aver. nº ind. (2 m)	range	2 - 24	1 - 19	3 - 24
	mean	8 b	9 c	10 a
	SE	0.05	0.05	0.03
Aver. nº ind. (3 m)	range	4 - 36	7 - 38	8 - 37
	mean	18 b	19 c	20 a
	SE	0.03	0.03	0.02
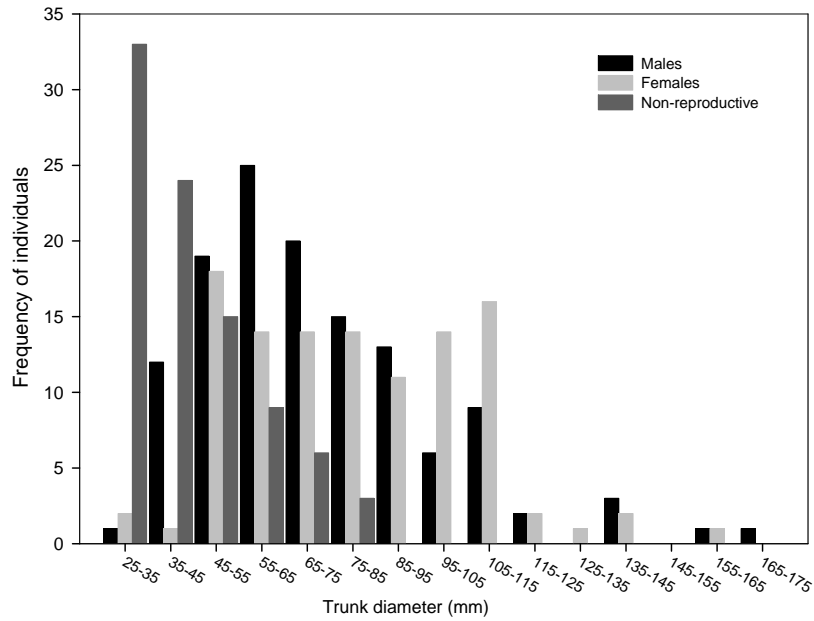
Figure 1 Observed frequency distribution of trunk diameter (stem diameter at 10 cm from the ground) for male, female and non-reproductive *J. thurifera* target individuals. Diameters are separated into 10 mm classes.

Statistical analysis of growth models

We used a neighbourhood approach based on similar studies of tree growth (Canham & Uriarte 2006; Gómez-Aparicio & Canham 2008) to predict recent tree growth (last ten years) of the 299 target trees. Growth data were grouped in four subsets: one including all individuals (i.e. without differentiation regarding the reproductive class); a second one including only male individuals; a third one including only female individuals; and the fourth one including only non-reproductive individuals. We predicted radial tree growth in each subset (RG, mm yr^{-1}) as a function of: (i) the potential growth of a hypothetical 'free growing' tree, i.e. without any competition

effect (Pot RG, mm yr^{-1}), (ii) the size (trunk diameter) of the target tree and (iii) competition from neighbouring trees. The model takes the form:

$$RG = Pot\ RG \times Size\ effect \times Competition\ effect \qquad [Eq.\ 1]$$

where Pot RG is the parameter estimated from the data. The size and competition effects are scalars ranging from 0 to 1, which act to reduce potential growth of a hypothetical 'free growing' tree. In this model, at a Competition effect of 0 (intense competition), growth is 0, and at a Competition effect of 1, growth is no longer limited by this interaction. Similarly to other studies (Canham, Lepage & Coates 2004; Coates, Canham & LePage 2009; Gómez-Aparicio *et al.* 2011), we used a lognormal function to shape the size effect [Eq. 2].

$$Size\ effect = \exp\left[-\frac{1}{2}\left(\frac{ln(\,D\,/\,X_0\,)}{X_b} \right)^2 \right] \qquad [Eq.\ 2]$$

where D is the trunk diameter (mm) of the target tree, X_0 is a parameter that represents the trunk diameter (mm) of the target tree at which Pot RG occurs (i.e. the peak of the lognormal shape), and X_b is a parameter that determines the breadth of the function. The lognormal function is flexible enough to be monotonically increasing (i.e. when X_0 is very large), decreasing (i.e. when X_0 is very small), or to have a single 'hump' and a skew to the left when X_0 is within the normal range of trunk diameter.

The competition effect was modelled using a Neighbourhood Competition Index (NCI). This index takes into account the total basal area from neighbours contained in circumferences at different distances from the target tree. This type of indices has been shown to be generally sufficient to predict competition effects in relatively uniform even-aged stands (Lorimer

1983). Neighbours were defined as individuals growing within three different radii (R_i: 1 m, 2 m, 3 m) from target trees (Weiner 1984; Pacala & Silander 1985) within the study plot. We tried three different radii because the effects of local crowding can potentially vary depending on the radius used to define the local neighbourhood (Peterson & Squiers 1995; He & Duncan 2000). The NCI took the form:

$$\text{NCI}_{Ri} = \sum_{j=0}^{n} BA_j \qquad \text{[Eq. 3]}$$

where BA_j is the basal area of the neighbour trees (cm²) within one of the three influence areas tested, and R_i is the distance to the target tree (either 1 m, 2 m or 3 m).

We tested two forms of the NCI: (i) all neighbours were considered to be equivalent [Eq. 3], and (ii) the effect of neighbours was a function of their reproductive class (male, female and non-reproductive). The reproductive condition of the neighbour was included into the NCI by a new parameter (λ_k) that ranges from 0 to 1 and allows for differences among sexual condition in their competitive effect on the target tree [Eq. 4]:

$$\text{NCI}_{Ri} = \sum_{i=0}^{n} \lambda_k \times BA_j \qquad \text{[Eq. 4]}$$

The competition effect was assumed to decrease exponentially as a function of the NCI:

$$\text{Competition effect} = \exp\left[-C \times (NCI_{R_i})^{\alpha}\right] \qquad \text{[Eq. 5]}$$

where α and C are parameters estimated by the analyses that determine the shape of the neighbour effect on NCI and the intensity of competition, respectively.

We also tested whether the sensitivity of the target tree to competition decreased or increased with tree size. This allowed us to test whether a given level of competition had a greater effect depending on the size of the target trees (Gómez-Aparicio & Canham 2008). To this end, the exponential decay term (C, [Eq. 5]) was allowed to vary as a function of target tree size, following the functional form:

$$C = C' \times D^{\gamma} \qquad\qquad [Eq. 6]$$

If $\gamma = 0$, then sensitivity to competition does not vary as a function of target tree size. If $\gamma < 0$, then sensitivity to competition declines as target tree trunk diameter increases, and if $\gamma > 0$ then larger trees are more sensitive to a given level of crowding than smaller trees.

Parameter estimation and comparison of alternate models

The modelling process followed two steps. First, we ran univariate models for each effect (size and competition) independently and compared them to the null model which assumes constant growth in the stand. By doing this, we assessed whether including any of these effects into a model significantly improved its explanatory power. Second, bivariate models were fitted when both size and competition were found to have an effect on growth when evaluated alone. The models were done separately for each of the four subsets of data (i.e. all target individuals, males, females and non-reproductive individuals).

We used simulated annealing, a global optimization procedure, to determine the most likely parameters (i.e. the parameters that maximize the log-likelihood) given our observed data (Goffe, Ferrier & Rogers 1994). We

used information criteria as an indicator of parsimony and likelihood (the Akaike Information Criterion corrected for small sample sizes, AIC_c) to select the best growth model (Burnham & Anderson 2002). The absolute magnitude of the differences in AIC between alternate models (ΔAIC) provides an objective measure of the strength of empirical support for the competing models. The model with the strongest empirical support has the minimum AIC (Akaike 1992). Models with ΔAIC between 0 and 2 were considered to have equivalent and substantial empirical support, ΔAIC between 4 and 7 indicated less support, and models with $\Delta AIC > 10$ were dismissed, as they had negligible empirical support (Burnham & Anderson 2002). As a measure of goodness-of-fit, we used the R^2 of the regression (1 - SSE/SST, SSE: sum of squares error, SST: sum of squares total); and as a measure of bias, we used the slope of the regression with a zero intercept between observed and predicted radial growth (with an unbiased model having a slope of 1). We used asymptotic two-unit support intervals to assess the strength of evidence for individual maximum likelihood parameter estimates (Edwards 1992), which is roughly equivalent to a 95% support limit defined using a likelihood ratio test (Hilborn & Mangel 1997). A support interval is defined as the range of the parameter value that results in less than a two-unit difference in AIC. Residuals were normally distributed $N \sim (0, 1)$. All the analyses were done in the R environment (R Core Team 2012) using the likelihood package version 1.5 (Murphy 2008).

Results

Biotic factors of tree growth assessed by model comparison and evaluation

All of the best models produced unbiased estimates of growth (i.e. slopes of predicted versus observed growth were all very close to 1) and the percentage of variance explained by the best models ranged from 35% to 49% (Table 3, Figure S2). On one hand, models that included the effect of target tree size on growth had in all cases a better fit to the data than the null model (Table 3). On the other hand, models that included the effect of competition on tree growth had larger support than the null model (i.e. lower AIC_c) for all the trees together at R = 2 m and for female target trees at all the radii, but not for male or non-reproductive trees. However, bivariate models including size and competition were never a better fit to the data that univariate models considering only size (Table 3).

Table 3 Comparison of alternate growth models analysing the effect of size and competition at three different distances for the whole population together and for each reproductive class of the target trees (t.). The most parsimonious model (indicated in bold) is the one with the lowest AICc. NP means the number of parameters of the model. Slope and R^2 (the goodness of t) is given for the best model.

		NP	AIC_c	ΔAIC	Slope	R^2
All target t.	Null	2	219.36	198.12		
	Size	**4**	**21.25**	**0.00**	**1.00**	**0.49**
	Competition (R=1 m)	4	223.34	202.09		
	Competition (R=2 m)	4	216.18	194.93		
	Competition (R=3m)	4	221.46	200.21		
	Gender competition (R=1 m)	10	223.40	202.15		
	Gender competition (R=2 m)	10	228.32	207.07		
	Gender competition (R=3 m)	10	228.59	207.34		
	Size + competition (R= 2 m)	6	25.28	4.03		
Male t.	Null	2	68.40	61.47		
	Size	**4**	**6.93**	**0.00**	**1.00**	**0.44**
	Competition (R=1 m)	4	71.08	64.16		
	Competition (R=2 m)	4	71.82	64.89		
	Competition (R=3 m)	4	70.40	63.48		
	Gender competition (R=1 m)	10	81.36	74.43		
	Gender competition (R=2 m)	10	75.55	68.62		
	Gender competition (R=3 m)	10	76.72	69.79		
Female t.	Null	2	109.08	56.79		
	Size	**4**	**52.28**	**0.00**	**1.00**	**0.35**
	Competition (R=1 m)	4	97.53	45.25		
	Competition (R=2 m)	4	94.98	42.69		
	Competition (R=3 m)	4	97.02	44.73		
	Gender competition (R=1 m)	10	109.94	57.66		
	Gender competition (R=2 m)	10	96.26	43.97		
	Gender competition (R=3 m)	10	105.07	52.78		
	Size + competition (R=1 m)	6	56.95	4.66		
	Size + competition (R=2 m)	6	57.00	4.71		
	Size + competition (R=3m)	6	57.02	4.73		
	Size + gender competition (R=1 m)	10	381.08	328.80		
	Size + gender competition (R=2 m)	10	380.43	328.15		
	Size + gender competition (R=3 m)	10	377.65	325.37		
Non-reproductive t.	Null	2	363.19	46.82		
	Size	**4**	**316.37**	**0.00**	**0.99**	**0.48**
	Competition (R=1 m)	4	366.07	49.70		
	Competition (R=2 m)	4	367.57	51.20		
	Competition (R=3 m)	4	367.56	51.18		
	Gender competition (R=1 m)	10	381.08	64.71		
	Gender competition (R=2 m)	10	380.43	64.06		
	Gender competition (R=3 m)	10	377.65	61.28		

Differential growth patterns between reproductive classes

The three reproductive classes showed different patterns of variation in predicted radial growth as a function of target tree size (Figure 2). At small sizes, predicted radial growth of the three reproductive classes was similar and increased rapidly with trunk diameter. However, at a certain size, non-reproductive individuals grew much more slowly than non-reproductive individuals. Non-reproductive individuals reached a growth peak at an intermediate size of trunk diameter and a slight decline afterwards. In contrast, reproductive individuals kept a monotonic growth curve, with female individuals of large size growing faster than males (Figure 2).
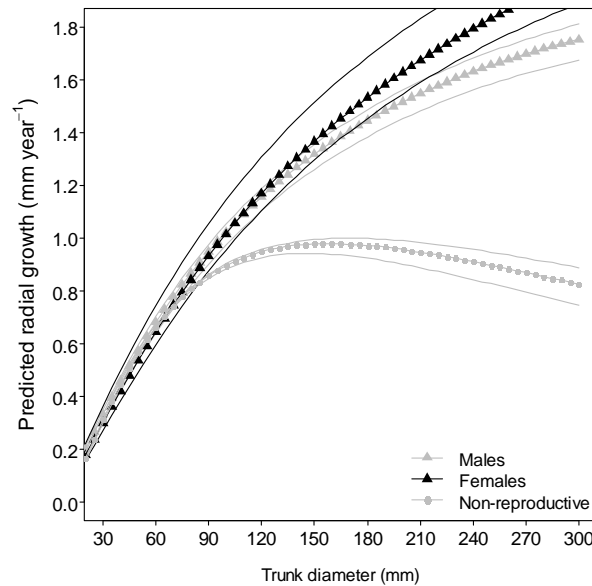
Figure 2 Predicted radial growth (mm yr^{-1}) as a function of size (trunk diameter in mm) for each reproductive class in the absence of competition effects. See Table 4 for the estimated parameters of the fitted Eq. 1. Confident intervals are represented by continuous lines.

Differences in growth rates among reproductive classes were also supported by the different values of the potential radial growth parameter (Pot RG), which measures the growth rate of a hypothetical 'free growing tree' of optimal size (i.e. a target tree with $D = X_0$, Eq. 1). Predicted potential growth rates (Pot RG) were highest in females (2.56 [2.39-2.73] mm yr^{-1}, mean [support interval]), followed by males (1.93 [1.83-2.03] mm yr^{-1}) and non-reproductive individuals (0.99 [0.94-1.00] mm yr^{-1}) (Table 4).

Table 4 Maximum likelihood parameter values and 2-unit support intervals [in brackets] for the selected best models. Pot RG: maximum potential radial growth mm $year^{-1}$; X_0: trunk diameter (mm) of the target tree at which Pot RG occurs; X_b: breadth of the function; sd: standard deviation.

	Model	Pot RG	X_0	X_b	sd
All t.	Size	2.40	1,000	1.76	0.25
		[2.33-2.47]	[960.40-1,000]	[1.74-1.77]	[0.23-0.27]
Male t.	Size	1.93	616.34	1.61	0.24
		[1.83-2.03]	[579.96-662.38]	[1.56-1.65]	[0.22-0.27]
Female t.	Size	2.56	996.27	1.69	0.30
		[2.39-2.73]	[927.13-1,000]	[1.64-1.74]	[0.26-0.34]
Non-reproductive t.	Size	0.99	158.11	1.10	1.70
		[0.94-1.00]	[146.30-169.90]	[1.05-1.17]	[1.47-2.00]

Discussion

Biotic factors influencing tree growth patterns

Our results showed that *Juniperus* growth at early stages of stand development was largely governed by tree size and the reproductive class rather than by neighbourhood competition. Specifically, in agreement with our first hypothesis, size of the target tree was the main factor driving tree growth (e.g. Lee *et al.* 2003; Mencuccini *et al.* 2007; Gimeno *et al.* 2012a). The size-growth curve showed a rapid increase of growth with size for the three reproductive classes (see also Gómez-Aparicio *et al.* 2011 for a similar result). However, the shape of the curves indicated that non-reproductive individuals have a lower growth potential than reproductive individuals, reaching a growth peak much faster than reproductive individuals. In fact, the shape of the size-growth curve for reproductive individuals suggests that such a peak would occur at larger sizes than those found in our study site. A plausible explanation for this would be the fact that our study forest is relatively young and the trees are relatively small, so our study population might not include reproductive individuals large enough to have reached their maximum growth yet.

Intraspecific competition did not constrain tree growth, neither when all neighbours were considered as equal competitors nor when they were separated into different reproductive classes. This result is contrary to our second hypothesis, which stated that neighbourhood competition may become a key driver of individual tree growth as previously reported for *J. thurifera* (Gimeno *et al.* 2012c) and other dioecious tree species (Herrera 1988; Vasiliuskas & Aarssen 1992; Houle & Duchesne 1999; Zhang *et al.* 2009). These studies have addressed the existence of intraspecific

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competition, and moreover, they have described inter- and intra-specific interactions between reproductive classes, although without finding consistent trends. For example, Vasiliuskas & Aarssen (1992) showed for *J. virginiana* that the presence of neighbours decreased tree growth, independently of the reproductive class of the nearest neighbours. Meanwhile, Houle & Duchesne (1999) in *J. communis* suggested the existence of a moderate intraspecific competition only between males. These studies, however, focused on uneven aged populations that might already be experiencing self-thinning processes which could explain the existence of the observed competition interactions. On the contrary, in our young forest of study, the lack of canopy overlapping among young *Juniperus* (J. Pavón-García, personal observation), and the inherent slow-growing resource-conservative strategy of the species (Gómez-Aparicio *et al.* 2011; García-Morote *et al.* 2012) would help to explain the lack of competitive interactions among *Juniperus* individuals despite the high stand density.

Differential growth rates between reproductive classes

Our results suggested that females may grow faster than males. This finding is confirmed by both the size-growth curves and the potential growth rates (Pot RG) estimated in the models. This result is in disagreement with our third hypothesis based on the fact that differences in resource investments to reproduction could result in different patterns of growth, with females showing slower growth rates than males and non-reproductive individuals (Herrera 1988; Vasiliuskas & Aarssen 1992; Allen & Antons 1993). Previous studies with *J. thurifera* suggest the lack of a consensus about which gender grows faster than the other and why. For example, higher growth rates in

males have been interpreted as the delayed cost of reproduction in females (Gauquelin *et al.* 2002; Montesinos *et al.* 2006). However, other studies have found no differences in growth rates between males and females of *J. thurifera* (Gimeno *et al.* 2012a,b), even in situations where the female reproductive effort was much greater than that of males (Ortiz 2002). Finally, some authors have found females to grow faster than males in individuals larger than those from our study plot (Rozas *et al.* 2009). Our results are in agreement with this last line of evidence. A higher female growth rate might be explained by the underlying mechanisms which offset female reproduction costs, e.g. photosynthetic reproductive structures, delayed reproduction, nutrient resorption from senescing organs or fallen fruits under the female's canopy, module specialization or higher photosynthetic rates in females (Delph 1990, 1999; Obeso 2002). In particular, Montesinos *et al.* (2012) suggested that female *J. thurifera* seem to have a long-term strategy which allows them to store and use their resources for future requirements, while males seem to have a short-term strategy which makes them adjust their growth and reproduction as a function of the current resource availability.

Lastly, and also contrary to our third hypothesis, our results showed that non-reproductive individuals had lower growth rates than male and female individuals. Our expectation was based on the idea that a lack of reproduction costs incurred by non-reproductive individuals would result in greater growth rates than reproductive individuals, as found in previous studies for the same species (Gimeno *et al.* 2012c). A plausible explanation to this finding could be related to the potential existence of small-scale spatial heterogeneity in environmental conditions (e.g. soil fertility), with non-

reproductive individuals being located in sub-optimal microsites that could limit their capacity to invest in both growth and reproduction. In fact, mean size of non-reproductive individuals was smaller than that of reproductive individuals despite having similar ages. Moreover, small junipers present higher sensitivity than large ones to stressful environmental conditions (Rozas *et al.* 2009), which might further limit their performance (i.e. lower growth rates and delayed reproduction). However, we cannot discard other alternative causes, such as the existence of genetic variability or seed size effects, that might have prevented non-reproductive individuals from having a better performance.

Overall, our results suggest that in young *J. thurifera* monospecific forests, where self-thinning processes may have not undergone yet, tree size and the reproductive class could play a relatively more important role than competition as drivers of tree growth and stand dynamics. Female *Juniperus* apparently make a much more efficient use of their available resources allowing them to grow faster than males and non-reproductive individuals. The non-reproductive condition of individuals could be linked to specific microsite conditions or genetic variability effects which could hamper their development. Although competition is considered a major determinant of tree performance and population dynamics (Weiner 1984), intraspecific differences in growth patterns alone can also be of paramount importance in explaining population structure and dynamics (e.g. Zavala *et al.* 2007)

In conclusion, this study contributes to understanding growth dynamics at early developmental stages in a dioecious tree species such as *J. thurifera*. Additionally, our study provides guidelines to develop silvicultural recommendations in order to manage these new woodlands. Recently,

juniper wood has aroused interest due to its quality for constructions (Crespo *et al.* 2006). Therefore, managers aim to seek both quantity and quality wood by controlling stand density throughout thinning (Nyland 1996). Our results suggest that at this early successional stage, thinning would have little influence on the growth of remnant trees, as competition did not seem to limit growth of relatively young junipers.

Acknowledgments

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Supplementary material
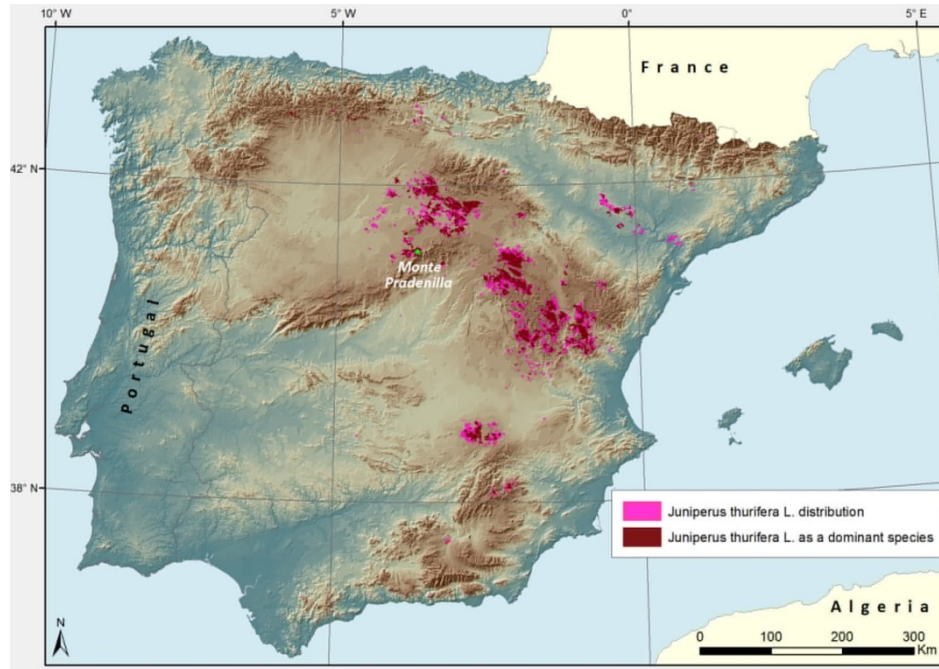
Figure S1 Geographic distribution of *Juniperus thurifera*, indicating those areas where the species appears as the dominant. This map has been drawn based on information from the Mapa Forestal de España 1:50,000 (Ministerio de Medio Ambiente and Banco de Datos de la Biodiversidad).
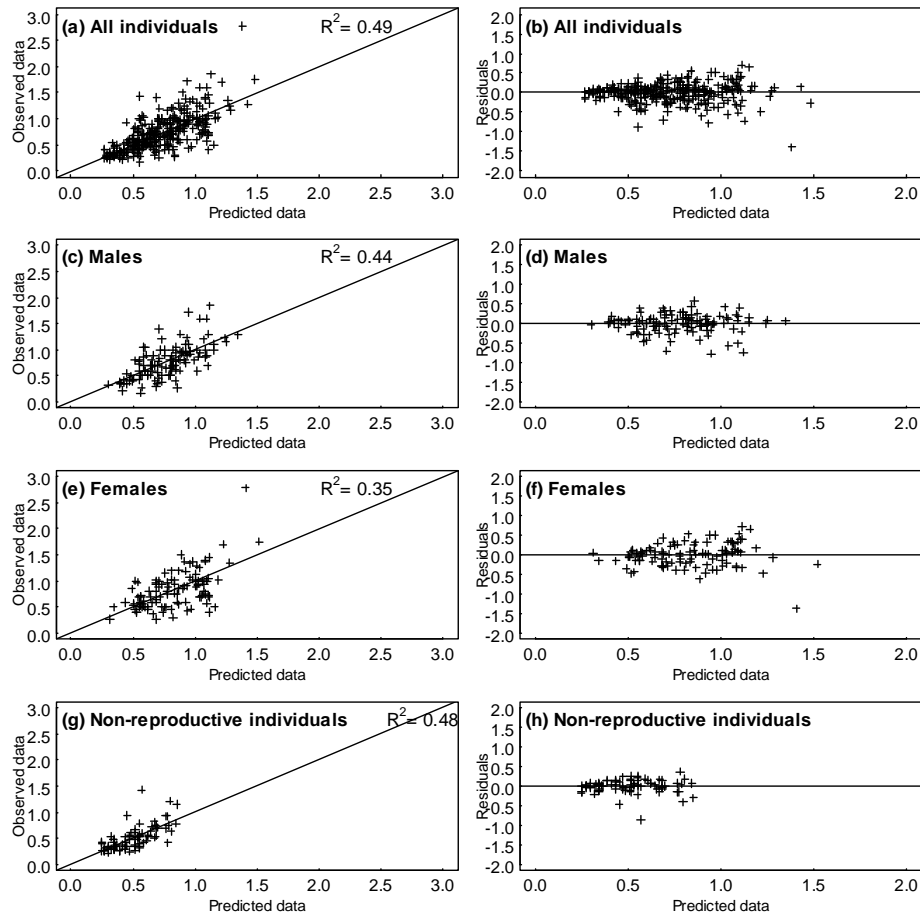
Figure S2 On the left panel, predicted vs observed growth data and the R^2 (percentage of variance explained of the best models). The solid lines represent linear regressions with a zero intercept and slope of one. On the right panel, residuals vs predicted data for the different reproductive classes and the whole dataset.

Chapter 3

The role of population origin and microenvironment in seedling emergence and early survival in Mediterranean maritime pine (*Pinus pinaster* Aiton)

Resumen

Un apropiado entendimiento del proceso de regeneración de los bosques es necesario para predecir la distribución de los mismos en el futuro. Son muchos los estudios que han descrito los factores ecológicos más relevantes que afectan al éxito de reclutamiento en los árboles. Sin embargo, la importancia del componente genético en dicho proceso ha sido escasamente estudiada. En este estudio, se estableció un transplante (en concreto, siembra) recíproco en condiciones semi-naturales con el objetivo de testar tanto la adaptación local de las dos poblaciones de estudio, como el efecto del microambiente (evaluada por la fracción de cabida cubierta de copas) en la emergencia y supervivencia estival de pino negral (*Pinus pinaster* Aiton), un árbol emblemático del bosque mediterráneo. Además, se aplicó una novedosa técnica basada en marcadores moleculares con el objetivo de testar la existencia selección familiar en el proceso, y por tanto, el consiguiente potencial genético de cambio a lo largo de las generaciones. En general, no se encontraron evidencias que confirmaran la existencia de adaptación local en las fases tempranas de reclutamiento. Además, se encontró un efecto débil de selección familiar, lo cual sugiere que en ambientes estresantes caracterizados por una baja supervivencia (estival), los procesos estocásticos y la variabilidad inter-anual del clima parecen jugar un papel importante durante la germinación y el reclutamiento. Sin embargo, nuestro estudio puso de manifiesto que, en las primeras etapas de reclutamiento, los microambientes pueden favorecer a aquella población que presente la estrategia vital más exitosa con independencia de su origen (local o no local). Asimismo, el tiempo de germinación resultó ser un factor clave para la supervivencia de las plántulas en ambientes mediterráneos estresantes. Finalmente, nuestro

estudio puso de manifiesto la complejidad de los factores que influyen en las primeras etapas de establecimiento del pino negral, y se proporcionan medidas de gestión encaminadas a la mitigación de los efectos del cambio ambiental. En particular, el diseño de planes de migración asistida resulta difícil debido a la alta estocasticidad asociada al proceso de reclutamiento en ambientes estresantes, así como a las diferentes estrategias adaptativas de cada población.

Palabras clave: emergencia, variabilidad intraespecífica, adaptación local, pino negral, proceso de reclutamiento, sombra parcial, sequía estival, supervivencia estival, tiempo de emergencia.

Abstract

Understanding tree recruitment is needed to forecast future forest distribution. Many studies have reported the relevant ecological factors that affect recruitment success in trees, but the potential for genetic-based differences in recruitment has often been neglected. In this study, we established a semi-natural reciprocal sowing experiment to test for local adaptation and microenvironment effects (evaluated here by canopy cover) in the emergence and early survival of maritime pine (*Pinus pinaster* Aiton), an emblematic Mediterranean forest tree. A novel application of molecular markers was also developed to test for family selection and, thus, for potential genetic change over generations. Overall, we did not find evidence to support local adaptation at the recruitment stage in our semi-natural experiment. Moreover, only weak family selection (if any) was found, suggesting that in stressful environments with low survival, stochastic processes and among-year climate variability may drive recruitment. Nevertheless, our study revealed that, at early stages of recruitment, microenvironments may favor the population with the best adapted life strategy, irrespectively of its (local or non-local) origin. We also found that emergence time is a key factor for seedling survival in stressful Mediterranean environments. Our study highlights the complexity of the factors influencing the early stages of establishment of maritime pine and provides insights into possible management actions aimed at environmental change impact mitigation. In particular, we found that the high stochasticity of the recruitment process in stressful environments and the differences in population-specific adaptive strategies may difficult assisted migration schemes.

Introduction

Sustained tree recruitment is fundamental to ensure forest persistence under global climate change (Gómez-Aparicio 2008; Pérez-Ramos *et al.* 2013). Understanding tree recruitment processes and potential biotic and abiotic interactions can also help to realistically forecast the distribution of future forests. Tree recruitment involves multiple life-history stages –from seed to adult tree– that are connected by transitional processes: seed maturation, dispersal, germination, emergence and survival. Demographic collapse at any of these stages can doom recruitment (Clark *et al.* 1999; Muller-Landau *et al.* 2002), jeopardizing a long-term preservation of the population. Many studies have reported relevant ecological factors that affect recruitment success in trees –light, microtopography, physical and chemical soil characteristics, herbivory, pathogens and competition with herbs (Augspurger 1984; Facelli & Pickett 1991; Kobe *et al.* 1995)– but the potential for genetic-based differences in recruitment (among populations and families of the same species) has often been neglected in experimental or modeling studies. For example, in Mediterranean environments, summer drought and the shortness of favorable periods (i.e. when temperature and water availability are suitable for plant growth) are main abiotic factors constraining tree establishment (Pigott & Pigott 1993; Castro *et al.* 2005; Pérez-Ramos *et al.* 2013). It is also well-known that Mediterranean tree populations and families have large genetic differences in drought response and growth (Voltas *et al.* 2008; Aranda *et al.* 2009; Klein *et al.* 2013; Sánchez-Gómez *et al.* 2013). However, genetic factors are rarely considered in Mediterranean tree recruitment studies.

From a demographic standpoint, the seed and seedling stages are probably the most vulnerable (Harper 1977; Gómez-Aparicio 2008). This is typically reflected in a low early survival, with one-season mortality rates up to 70-100% (Harper 1977; Herrera *et al.* 1994; Gómez-Aparicio 2008; Rodríguez-García, Ordóñez & Bravo 2011c; Pérez-Ramos *et al.* 2013). High mortality rates in seedlings are mainly related to their small size, which makes them susceptible to factors that generally affect larger plants only to a lesser extent, such as competition with neighboring vegetation (including intraspecific competition), browsing, extreme climatic events, and insect or disease infestation (Harper 1977; Peet & Christensen 1987) in turn influencing adult traits and fitness (Reich *et al.* 2003; Donohue *et al.* 2010).

Natural selection can cause evolutionary change on contemporary time scales (e.g. Franks, Sim & Weis 2007) and, by filtering the better adapted genotypes, natural selection can result in local adaptation (Davis & Shaw 2001). Many forest tree species are thought to be locally adapted (reviewed in Savolainen et al 2007), in particular following environmental clines along large geographical scales (Endler 1977; Barton 1999; Aitken *et al.* 2008). The existence of past local adaptation in forest trees at the population level may indicate some capacity to face new pressures arising from climate change (e.g. bark beetle outbreaks caused by warming conditions (Klenner & Arsenault 2009)), although, at the same time, those locally-adapted genotypes may not be able to respond to unprecedented rapid warming due to genetic constrains (Rehfeldt *et al.* 1999; Savolainen *et al.* 2004; Aitken *et al.* 2008).

Reciprocal transplant experiments are a powerful way of testing for local adaptation (sensu Kawecki and Ebert, i.e. the relative fitness of local

and foreign populations between sites or habitats (Primack, Kang & Primarck 1989; Kawecki & Ebert 2004)). There is an ample literature on local adaptation using reciprocal transplant experiments –see reviews in Leimu and Fischer (2008) and Hereford (2009)– that has shown that local populations often have a higher fitness than non-local ones, with more significant differences detected when population sizes are big (Leimu & Fischer 2008). Typically, local adaptation studies have focused on fitness-related traits such as reproductive success, plant size and survival rates, usually under controlled conditions across contrasted sites or along gradients. However, they have rarely considered long-lived plant species such as forest trees, or the early stages of establishment when natural selection is expected to be stronger.

The early stages of plant life are time- and microenvironment-dependent (Schupp 1995; Kollmann 2000; Chambers 2001). Temporal and environmental heterogeneity is large in Mediterranean woodlands, even within the same forest (Gómez-Aparicio 2008). Plant establishment success largely depends on the timing of key regeneration processes, such as dispersal and germination. With respect to microenvironmental factors, the light regime, governed by tree canopy cover, is probably the most important in Mediterranean forests (Gómez 2004; Rodríguez-García, Gratzer & Bravo 2011b). A partial canopy cover may protect seedlings from high radiation and temperatures and from losses of soil moisture in stressful sites, thereby increasing early survival (Gómez 2004; Castro *et al.* 2005). This is particularly relevant in species that recruit in summer, such as Mediterranean pines. Additionally, as summer develops, heat cumulative degrees make open-canopy areas less favorable for emergence in comparison to sites with partial

shade. Therefore, a shift in emergence from open-canopy to shaded areas is expected.

Maritime pine is a typical component of Mediterranean landscapes. This emblematic pine is characterized by a scattered distribution that may have both limited gene flow among different groups of populations, and high levels of genetic divergence promoted as a result of combining genetic drift and natural selection (Q_{ST} = 0.29–0.46 for height growth and survival, Isabel Rodríguez-Quilón per. comm.; see also González-Martínez, Alía & Gil (2002a)). At the phenotypic level, water availability and forest fires are considered to be major drivers of local adaptation in maritime pine (Verdú & Pausas 2007; Aranda *et al.* 2009). However, among its literature (e.g. Ruano, Pando & Bravo 2009; Rodríguez-García *et al.* 2011b), there are no studies considering the species' genetic make-up at the early stages of establishment in natural populations. The aim of this work is to assess the factors controlling emergence and early seedling survival in natural maritime pine forests and, in particular, the role of local adaptation and microenvironment. To accomplish this general goal, we established a semi-natural reciprocal sowing experiment in the field under distinct canopy-cover (i.e. light regime) conditions using two contrasted maritime pine origins that have evolved under different selective pressures associated with drought response and fire regime: Coca (central Spain), a non-fire adapted, continental-dry population living in sandy soils, and Calderona (eastern Spain), a population that is subjected to milder climate and adapted to fire (Tapias *et al.* 2004).

Most reciprocal transplantation experiments are based on spaced plantings of seedlings previously produced in greenhouse conditions. This may limit some of the effects of natural selection at the germination and

early establishment stages (e.g. Giménez-Benavides, Escudero & Iriondo 2007; Ishizuka & Goto 2012). To circumvent this issue, we developed a novel approach that avoided greenhouse manipulation and used molecular markers to identify the genetic origin (i.e. the family) of the surviving seedlings. We tested for local adaptation (following Kawecki and Ebert (2004) and Leimu and Fischer (2008)), considering two distinct microenvironments relevant for tree recruitment and forest colonization: open-canopy (i.e. under light exposition) vs. closed-canopy (i.e. under partial shade by dominant trees). The interactions between local adaptation at different genetic levels (population and family) and microenvironment (i.e. environmental heterogeneity in light regime within populations) have never been studied before in semi-natural conditions and may provide new insights into how genetic adaptation takes place in natural plant populations.

Using our semi-natural reciprocal sowing experiment, we specifically addressed the following questions: (i) Is there local adaptation for emergence and early survival in maritime pine populations of contrasted origin?; (ii) Is early survival affected by time of emergence?; (iii) What is the role of canopy cover (i.e. light regime) in this species' emergence and early survival?; iv) Can we observe a transition in emergence and survival from open- to closed-canopy environments along the summer season?; and (v) Is natural selection acting equally on all families within populations or, alternatively, is natural selection favoring some families, resulting in genetic change over generation for heritable traits? Our expectation is that, despite range fragmentation, maritime pine populations may have maintained enough standing genetic variation for local adaptation and family selection to have taken place, which

would explain the large phenotypic variability observed across natural populations of this Mediterranean conifer.

Material and methods

Ethics Statement

Permits to conduct research (including plant material collection) in Coca (latitude: 41°15'17''N, longitude 4°29'52''W) and Calderona (latitude: 39°44'56''N, longitude 0°29'44''W) sites were obtained from the Forest Services of the Autonomous Communities of 'Castilla y León' (Contact Person: Ing. María Bragado) and 'Valencia' (Contact Person: Mr. Antoni Marzo), respectively. These field studies did not involve endangered or protected species or have any long-term consequences for the forests studied.

Study sites and experimental design

Our reciprocal sowing experiment was conducted in two Mediterranean maritime pine (*Pinus pinaster* Aiton) sites separated by a distance of 535 km (Figure 1). The first site was located in Coca, in the Spanish province of Segovia, situated in the Castilian central plateau (from now on "Coca *site*"). Its mean and maximum daily air temperatures over the course of the experiment, from March to September 2011, were 17.15 °C and 25.09 °C, with a range of 8.20-22.32 °C and 13.69-30.86 °C, and coefficient of variation, C.V. of 0.32 and 0.28, respectively (data from Migueláñez meteorological station, c. 18 km away from the study site, corrected using Gonzalo's phitoclimatic model for Spain (Gonzalo-Jiménez 2008).
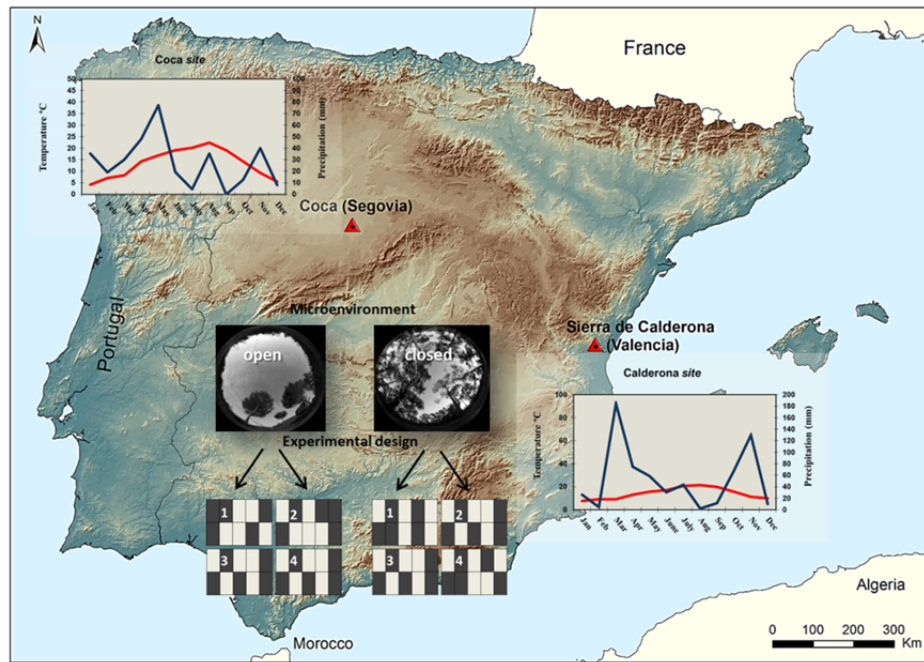
Figure 1 Semi-natural reciprocal sowing experiment established in maritime pine to study local adaptation at early stages of establishment. Experimental sites and population's origin are shown in red triangles. Climodiagrams (mean monthly temperature in red and monthly precipitation in blue) of both sites are also given (data from the Spanish National Meteorological Agency, after corrections using Gonzalo's phitoclimatic model for Spain (Gonzalo-Jiménez 2008). The experimental design (in the center of the figure) consisted in a split-plot with four replicates in each microenvironment (open canopy, exemplified in the photo on the left side; and closed canopy, the photo on the right side); dark grey and light grey boxes represent the two origins tested (Coca and Calderona).

Annual precipitation and precipitation during the study period (March to September) were 329.01 mm and 214.36 mm, respectively. Partial records in a climate logger from May to September (HOBO Weather Station Data Logger Massachusetts, USA) showed similar mean soil daily temperatures, but much lower C.V. (0.11) than air temperatures (Figure S1), and relative humidity of 49.44 % (range of 17.25-86.53 %, C.V. of 0.19). The

second site was located in Sierra de Calderona in the Spanish province of Valencia (from now on "Calderona *site*"), in the eastern Iberian coastal mountains. Its mean and maximum daily air temperatures over the course of the experiment were 16.77 °C and 22.83 °C, with a range of 9.14-21.58°C and 13.97-28.16 °C, and C.V. of 0.27 and 0.24, respectively (data from Segorbe meteorological station, c. 6 km away from the study site, corrected using Gonzalo-Jiménez's (2008) phitoclimatic model for Spain). Annual precipitation and precipitation during the study period (March to September) were 643.80 mm and 405.70 mm, respectively. As in the case of Coca, an in situ climate logger (HOBO Weather Station Data Logger Massachusetts, USA) recorded similar but less variable (C.V. of 0.08) soil than air temperatures (Figure S1). Relative humidity was 63.87 % (range of 37.92-87.18 %, C.V. of 0.26). A comparison of climatic data from the study period with historical data recorded between 1983 and 2013 indicated that the study year was not far from historical records, except for a somewhat warmer April (see details in Figure S2). Overall, Calderona *site*, with slightly lower temperatures (in particular maximum temperatures) but much higher precipitation (both annual and during the duration of the experiment) and relative humidity, presented a less extreme climate when compared to Coca *site.* Temperature variability (evaluated by C.V.) was also higher in Coca *site*. Besides, the two study sites differed in soil substrate. Calderona *site* presented a developed substrate composed by litter and leaves from typical Mediterranean shrubs; whereas Coca *site* presented a sandy soil, with c. 90% of sand (see soil analysis in Rodríguez-García *et al.* (2011b)), which limits its capacity to retain rain water.

The experimental design was a complete randomized and balanced split-plot, where each plot was composed by 10 subplots, five for each population origin (Figure 1). Dimensions of plots were 6 m × 5 m, and dimensions of subplots 1 m × 2.5 m. A narrow corridor of 20 cm was left between subplots to ease sowing during experiment establishment, and to allow both monitoring of seedling emergence and death events without disturbing natural recruitment during experiment duration. Plots were physically divided using a thin rope. To avoid predation by birds and small mammals, plots were protected and covered with 2.5 × 3 cm gardening mesh (PROJAR S.A., Spain). This large mesh size was chosen to avoid any significant modification of microenvironmental conditions out and under the mesh. To ensure similar initial conditions in both experimental sites and avoid facilitation/competition effects, shrubs (mainly *Quercus coccifera* and *Ulex* sp.) were removed using pruning scissors before sowing when needed (Figure S3). We tried not to manipulate the soil surface or to remove litter in any of the two field sites not to change soil conditions for emergence.

To test for differences in local adaptation related to environmental heterogeneity within each experimental site, plots were placed under either open or partial shade canopy (i.e. 'closed canopy'), as light regime is a major factor determining soil microhabitats and has a relevant impact on pine survival and establishment (Ruano *et al.* 2009). Light availability was estimated by means of hemispherical canopy photography. Photographs were taken at the seedling level in the centre of each experimental unit, using a horizontally levelled digital camera (Coolpix 4500, Nikon, Tokyo, Japan) with a fish-eye lens of 180° view field (FCE8, Nikon). We used Hemiview Canopy Analysis v. 2.1 software (Delta-T Devices Ltd, Burwell,

UK) to analyze the images and calculate Global Site Factor (GSF), which represents the fraction of total radiation above the canopy that penetrates below the canopy (Anderson 1964). Open-canopy plots, GSF values of 0.72 ± 0.05 (mean $\pm$ SE), represented areas where future seedlings would be more free from the influence of adult trees, in particular shadowing, on bare soil with areas of sparse herbaceous vegetation (mainly annuals and short-lived perennials), whereas closed-canopy plots, less accessible for light with GSF values of 0.51 ± 0.04, were placed in areas under much more direct adult-tree influence. Four replicates were established on each of the two canopy cover classes (see Figure 1).

Plant material

Unlike most reciprocal transplant experiments, ours was based on random sowing of seeds and not on planting greenhouse-raised seedlings or regularly-spaced seeds either on the soil or in pots. This strategy was chosen for two reasons: (i) to fully consider selection pressure taking place at germination, emergence and early establishment, including intraspecific competition, and (ii) to avoid seedling mortality due to transplant shock that may be confused with natural selection.

Needles and mature female cones from 25 individuals (i.e. maternal trees) were collected from each population (Coca *origin* and Calderona *origin*) at about the same date in February 2011. Needles were kept in individual paper bags inside a bigger plastic bag filled with silica gel. This material was used to obtain mother-tree genotypes using molecular markers (see below) and to be able to assign each offspring to its family at the end of the experiment. In this way, seeds from different families could be pooled

without risk of losing their family identity or modifying their natural emergence and first establishment conditions. Mature female cones were collected to extract the 1,600 seeds per family (see below) needed for our experiments. To open the cones and extract the seeds, they were placed in a forced-air oven at 60 °C for approximately 48 h. Then, seed wings were removed from seeds manually with a winnow. We prepared 80 samples (5 subplots × 8 replicates × 2 experimental sites) of 20 seeds per family from each of the two sites (hereafter Coca *origin* and Calderona *origin*), i.e. a total of 160 seed lots. Average seed weight did not significantly differ between populations (Calderona *origin:* 0.054 ± 0.002 g (mean ± SE) and Coca *origin:* 0.062 ± 0.003 g). The whole experiment involved 80,000 seeds, 40,000 (1,600 seeds per family × 25 families) from each origin. Finally, at the end of the experiment, surviving seedlings were collected and kept in collection microtubes with silica gel until DNA extraction.

Data collection

In maritime pine, dispersal begins in the spring and peaks during the summer months (July and August), often associated to dry episodes or storms (Juez *et al.* 2014). Sowing took place in 2011, on March 10th in Coca and April 7th in Calderona, therefore it approximately coincided with the beginning of the year's dispersal. Seeds were thrown randomly along the longest side of each subplot simulating natural dispersion as much as possible. The experiment was visited approximately every 20 days. On each visit, the date of every new emergence was individually tagged and recorded as well as any mortality events. We did not to register new emergences from July 1st onwards to avoid the monitoring of naturally-dispersed seeds from local

pines. Competition intensity was not strong, as judged by relatively sparse germination (see Figure S4), and most seedling mortality apparently took place due to seasonal drought. The end of the experiment took place at September 20th.

Molecular markers

Needles from the mother trees (N=50) and all surviving offspring in the reciprocal sowing experiment (N=56, see Results) were collected and dried in silica gel for subsequent DNA extraction using the Invisorb® DNA Plant HTS 96 Kit/C kit (Invitek GmbH, Berlin, Germany). Four high-resolution (Polymorphic Information Content, PIC, of 0.488-0.822, and combined Exclusion Probability, EP, of 0.91, see González-Martínez *et al.* (2002b) for a definition of EP) nuclear microsatellites (nuSSRs) were scored in all samples (total N of 106), except mother trees from Coca *origin* that were scored with only three (NZPR1078 did not amplify; combined EP of 0.87), following protocols in González-Martínez *et al.* (2002b): ITPH4516 and FRPP94, and Chagné *et al.* (2004): NZPR1078 and NZPR413. PCR fragments were resolved on a Li-Cor 4300 DNA analyser (Li-Cor Biosciences, Nebraska, USA). To reduce the probability of scoring errors, a selection of samples that covered the fragment size range was included as internal standard in each gel. SAGA^{GT} vs. 3.3. was used for gel calibration and scoring (Li-Cor Biosciences, Nebraska, USA).

Temporal patterns of emergence and early survival

We first examined the evolution of seedling emergence and early survival with time for each of the maritime pine origins, considering the two

experimental sites (Coca vs. Calderona) and microenvironments (open canopy vs. closed canopy), as well as the interactions between these factors. For this purpose, we conducted a non-parametric survival analysis appropriate for duration data (see review in McNair, Sunkara & Frobish (2012)). Our survival analysis can handle right censored data properly (avoiding biased results) and does not require normally distributed data (Kaplan & Meier 1958; Onofri *et al.* 2010). Emergence and survival data are right censored because many individuals either did not emerge or were still alive at the end of the observation. We computed the non-emergence and survivor functions, NE(t) and S(t) to measure, respectively, the probability of a seed of staying in the ground or a seedling of surviving (among those that emerged) beyond time t: NE(t) or S(t) = P[$T \geq t$]. We used the Kaplan-Meier (KM) estimator, which provides empirically constructed and discrete step functions over time in the different cases considered. The Kaplan–Meier estimator, $\hat{S}_{KM}$, for non-emergence and survival is given by:

$$\hat{S}_{KM} = \prod_{t_i < t} \frac{r(t_i) - d(t_i)}{r(t_i)} \qquad \text{[Eq. 1]}$$

where $d(t_i)$ is the number of emergences or deaths at time t_i and $r(t_i)$ is the number of individuals at risk at time t_i (i.e. those that have not emerged or died yet). We computed Kaplan-Meier estimators over time for each combination of sites and microenvironment (i.e. canopy-cover class). To assess statistical differences between functions we used log-rank tests that are suitable when censored data is present (Fox 2001). All analyses were performed with the *survival* package ver. 2.37-4 (Terry 2012) in the R environment (R Core Team 2012).

Local adaptation and the role of the microenvironment

To provide insights into local adaptation patterns at the early stages of establishment in maritime pine, we used discrete-time logistic models within the survival analysis framework. This approximation has been seldom used to address ecological problems but it is widespread in biomedical and sociological sciences (Kalbfleisch & Prentice 1980; Allison 1982). Alternative approaches, such as accelerate failure models (AFT) or proportional hazard models (Cox), treat time as continuous, which in our case would have been incorrect since we observed recruitment processes in discrete units of time. Moreover, taking time as continuous would have forced us to assume a determinate shape for the emergence and survival time distributions (i.e. exponential, gamma, lognormal, etc.), which, given few observation points along time, could have led to variable results depending on function specification (Allison 1982). Finally, continuous-time approaches are not convenient when there is a large amount of ties (i.e. simultaneous occurrence of an event for many individuals), as in our case.

Therefore, instead of measuring the time to failure, we modeled each interval between censuses individually, by creating a binary variable, y_i (0, 1), which indicates whether the event (emergence or survival) occurred in the interval [t, t+1]. With this method, one record for each interval time for each individual is obtained. In practical terms, this increases the number of observations but it does not produce any bias (Allison 1982). The probability that individual i emerges or survives during interval t, p_{ti}, given that no event has occurred before the start of t, can then be computed as:

$$p_{ti} = Pr\left(y_{ti} = 1 / y_{t-1,i} = 0\right) \qquad \text{[Eq. 2]}$$

$$\text{logit}\left(p_{ti}\right) = \log\left[\frac{p_{ti}}{1 - p_{ti}}\right] = \alpha D_{ti} + \beta X_{ti} + \varepsilon \qquad\qquad [\text{Eq. 3}]$$

where D_{ti} is the vector of coefficients α of the step function that captures the baseline hazard function, i.e. the risk per interval time that an event happens (as it is called in continuous time analysis). Four and five intervals were considered in the emergence and survival models, respectively, according to visit number to the field sites. The X_{ti} is a vector of explanatory covariates and β the corresponding estimated coefficients.

In our analysis of emergence and early survival, we assessed the following variables: (i) experimental site (Calderona and Coca), (ii) population's origin (Calderona and Coca), (iii) microenvironment (open and closed canopy), (iv) all two-way possible interactions of the latter (in particular interaction between site and population origin, which can be considered a formal test of local adaptation), and (v) census (time) interval. In addition, in the early survival analysis, time to emergence was included as another independent covariate. In both models, Calderona *origin*, Calderona *site* and closed-canopy microenvironment were chosen as the reference level for the odds ratios. For census intervals, odds ratios offer a picture of the probability of emerging or surviving for each of the intervals between censuses, controlling for the rest of the effects. To obtain robust standard errors despite repetitive measures of the same individuals (mainly associated with the D_{ti} vector), the discrete-time logistic analyses were clustered around individuals (Singer & Willett 2003). Coefficients in emergence and survival models were estimated using maximum likelihood. Statistical significance was assessed by Wald χ^2 test (Hosmer & Lemeshow 1989).

Analyses were performed using Stata vs. 11 (StataCorp. Stata Statistical Software: Release 11. 2009).

Finally, as a complementary method to discrete-time logistic models, we also computed effect sizes (Hedges' g*, a standardized and unbiased estimate of mean differences (Hedges 1981; Cohen 1988)) for local and microenvironmental adaptation (see Supporting Information File 2).

Genetic change over generations (family selection)

Natural family selection can result in genetic changes over generations for heritable traits. To test whether natural selection affected differently offspring from different families in Calderona (there was no survivors in Coca, see Results), we used a fractional paternity approach (similar to Devlin, Roeder & Ellstrand (1988)). Mother trees with incomplete genotypes were removed from the analyses (one mother from Calderona and six from Coca). Missing mothers may have reduced the power of the test, but fractional paternity approaches perform well with moderate numbers of unsampled potential parents (Devlin *et al.* 1988; Jones & Ardren 2003). They also perform better than direct parent assignment when many ties are expected in parent-offspring links. First, LOD scores for each mother-offspring relationship, i.e. the probability to assign survivor offspring to candidate mothers based only on genotypes, were calculated using, respectively, three highly-discriminant nuSSRs for Coca mothers and four for Calderona's (see Materials and Methods). Second, LOD scores were summed up across all offspring for each mother and divided by the sum of LOD scores across all mothers, to produce a relative measure of female reproductive success for each mother tree. This step was done, respectively, for 32 and 24 survivors

from Coca and Calderona *origin*. Third, we tested for over-representation of specific families among surviving offspring by using a *G*-test to compare observed with expected (i.e. equal) female reproductive success. Unfortunately, low overall offspring survival (see Results) did not allow more detailed quantitative genetics analyses.

Results

Temporal patterns of emergence and early survival

Emergence was generally low, ~2.73%, irrespectively of population origin. Total number of emergences (both experimental sites combined) was 985 (2.46%) and 1,203 (3.01%) seedlings from Coca and Calderona origins, respectively. Early survival was also very low. Only 32 (3.25 %) and 24 (1.99 %) seedlings from Coca and Calderona origins, respectively, remained at the end of the experiment, all of them found in the milder Calderona site.

Emergence temporal patterns were very similar for both origins in the two experimental sites (Figure 2, top: here displayed as the probability of a seed of staying in the ground, i.e. the 'non-emergence'), although there were significant differences ($p < 0.001$, Table S1) across sites and local environments along the temporal axis (higher overall emergence in Calderona and under closed canopy). Around 40 days after seeds were sown, there was a big pulse of seedling emergences in Calderona, specifically under open canopy, but not in Coca. Moreover, around day 70 a shift in the suitable local environment to emerge took place, from open-canopy to closed-canopy areas, although, once again, only in Calderona.

Non-emergence
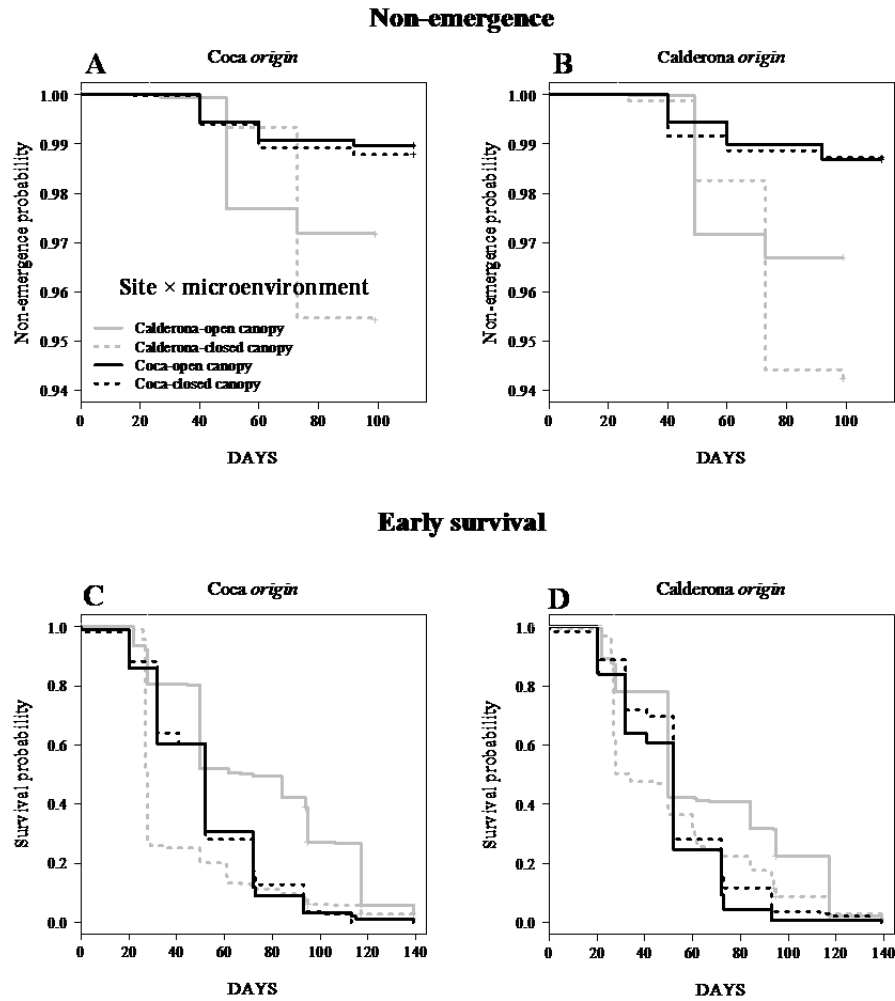
Early survival

Figure 2 Kaplan-Meier estimators for non-emergence (A–B) and early survival (C–D) probability in the reciprocal sowing experiment. Four functions are shown, each corresponding to a combination of site and microenvironment (i.e. open- or closed-canopy cover, see legend in the figure). Differences across sites-microenvironments were highly significant ($p < 0.001$), as shown by log-rank tests (see Table S1).

With respect to early survival, the two populations assayed showed a different temporal pattern (Figure 2, bottom). Calderona *origin* did not show marked differences across sites and canopy cover (i.e. low plasticity) while survival curves along time for Coca *origin* ran more distanced among them,

evidencing a higher survival under open canopy in Calderona and a lower one under closed canopy in this same site. Nevertheless, in both cases log-rank tests were significant for both origins ($p < 0.001$, Table S1).

Local adaptation and the role of the microenvironment

Discrete-time logistic models were fitted to unveil ecological and genetic factors related to emergence and early survival in maritime pine, in particular in the context of local adaptation. The emergence process was driven by the following highly-significant factors ($p < 0.001$): study site, population origin, microenvironment (i.e. the canopy-cover class) and the interaction term of site × microenvironment (Table 1). Neither the interaction term of site × population's origin (the standard test of local adaptation) nor the local environment × population's origin one were statistically significant. The significant interaction indicated a different effect of microenvironment across sites: Coca *site* had an overall lower emergence risk than Calderona *site* but it was lower under closed canopy (0.23) than in open canopy (0.36), despite the overall lower emergence risk under open canopy (0.59). Seedlings from Coca *origin* had a lower emergence risk (0.81) than Calderona *origin*, the reference level. Finally, considering emergence time intervals, the probability to emerge was higher between the second and third field census, which corresponded to about between 22 and 85 days after seeds were sown (Table 1).

Table 1 Odds ratios (OR) for the discrete-time logistic emergence model. Calderona *site*, Calderona *origin*, closed canopy, and the second-time interval (between census one and two, c. 20-50 days after sowing) are the reference levels (given also between parentheses for each factor/model). SE: standard error; CI: confidence intervals.

Factor	OR	SE	z	$P > z$	95% CI	
Site (Calderona)						
Coca	0.23	0.02	-17.24	0.00	0.19	0.27
Origin (Calderona)						
Coca	0.81	0.05	-3.57	0.00	0.72	0.82
Microenvironment (Closed canopy)						
Open canopy	0.59	0.04	-8.11	0.00	0.52	0.67
Site × origin	1.07	0.11	0.66	0.51	0.87	1.32
Site × microenvironment	1.59	0.17	4.38	0.00	1.29	1.95
Origin × microenvironment	1.01	0.09	0.16	0.87	0.85	1.21
Time intervals						
First	0.03	0.01	-18.20	0.00	0.02	0.04
Third	1.06	0.05	1.39	0.17	0.97	1.16
Fourth	0.09	0.01	-21.47	0.00	0.08	0.12

Number of observations generated: 316,935 (80,000 seeds)

Emergence time, study site, population origin and the two interaction terms of population's origin × microenvironment and site × microenvironment were highly significant ($p < 0.01$) in early survival models, while the interaction term of population's origin × site, i.e. the standard test of local adaptation, was not significant ($p = 0.94$) (Table 2). Date of emergence played an important role for early survival, with each additional day that a seed had taken to emerge increasing c. 8 % death probability. Considered together, the interaction terms pointed to a lack of local adaptation but a high effect of microenvironment. Overall, the best

environment for survival was Calderona *site* (Calderona was the only site with survivors) under open canopy (Table 2).

Table 2 Odds ratios (OR) for the discrete-time logistic survival model. Calderona *site*, Calderona *origin*, closed canopy, and the second-time interval (between census one and two, c. 20-52 days after seedlings emerged) are the reference levels (given also between parentheses for each factor/model). SE: standard error; CI: confidence intervals.

Factor	OR	SE	z	*P* > z	95% CI	
Time to emergence	0.93	0.00	21.18	0.00	0.93	0.94
Site (Calderona)						
Coca	0.45	0.31	5.54	0.00	0.34	0.60
Origin (Calderona)						
Coca	0.62	0.16	4.81	0.00	0.51	0.75
Microenvironment (Closed canopy)						
Open canopy	1.09	0.09	-0.83	0.41	0.89	1.33
Site × origin	1.01	0.16	-0.08	0.94	0.74	1.39
Site × microenvironment	0.63	0.27	2.78	0.01	0.45	0.87
Origin × microenvironment	2.04	0.07	-5.29	0.00	1.56	2.70
Time intervals						
First	2.33	0.04	-9.95	0.00	2.00	2.78
Third	2.00	0.06	-6.24	0.00	1.61	2.50
Fourth	1.16	0.11	-1.23	0.22	0.92	1.49
Fifth	0.12	1.58	11.64	0.00	0.08	0.17

Survival of Coca *origin* seedlings (i.e. the foreign population) was lower (OR of 0.62) than Calderona's (i.e. the local population) but only under closed canopy, Coca *origin* performing better under open canopy (OR of 1.26; see also Figure 3 and Table S1). Finally, probability of surviving was similar all along the experiment, except for an accused increase of mortality towards its end (between days 93 and 117 after sowing).

Analyses based on Hedges' g^* effect sizes for both germination and survival showed similar results to discrete-time logistic models (see Supplementary material, Complementary analysis).
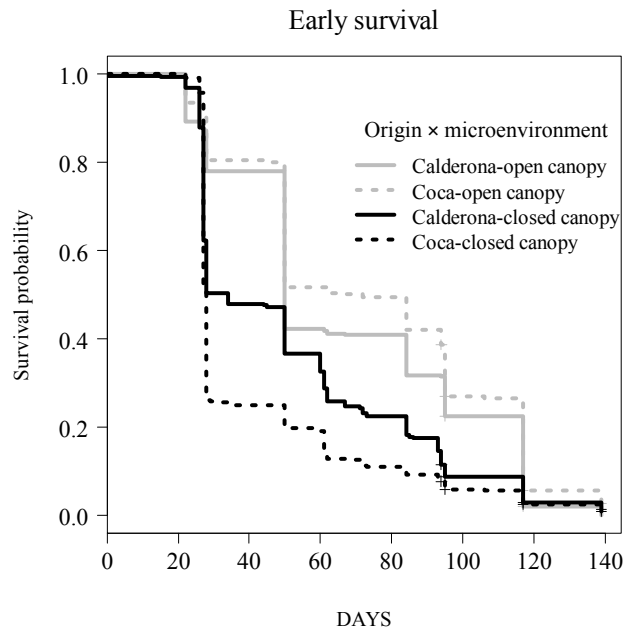
Figure 3 Kaplan-Meier estimators for early survival in Calderona, for two geographically-distant maritime pine origins. Four functions are shown, each one corresponding to a combination of origin and microenvironment (i.e. open- or closed-canopy cover, see legend in the figure). Differences across origins-microenvironments were highly significant ($p < 0.001$), as shown by log-rank tests (see Table S1).

Genetic change over generations (family selection)

There were only 56 survivors at the end of the experiment and all of them were found in milder-climate Calderona *site*. Fractional paternity analyses showed relative mother reproductive success from 0.054 to 4.195 for Coca's

mothers and from 0.231 to 2.035 for Calderona's (Table S2). *G*-tests testing the hypothesis of equal effective number of offspring across mothers showed that survivors in this site were a random representation of the families included in the reciprocal sowing experiment (*G*-test = 33.659, d.f. = 42, *p*-value = 0.817), with selection not specifically favoring any family. However, significant differences were found when only mother-trees from Coca's origin were considered (*G*-test = 29.074, d.f. = 18, *p*-value = 0.047).

Discussion

Successful recruitment was low in Mediterranean maritime pine, with just a few survivors in the milder Calderona *site*. This high level of mortality during the first growing season has often been reported in previous studies (Ruano *et al.* 2009; Rodríguez-García, Juez & Bravo 2010). Using a semi-natural reciprocal sowing experiment and discrete-time logistic models, we found that Calderona *origin* outperformed Coca *origin* in both emergence and survival, regardless of the study site. Thus, despite of the moderate-high level of genetic diversity reported for maritime pine in the Iberian Peninsula and high selection pressure at early stages of establishment, we did not find evidence supporting locally adapted populations at this stage. Furthermore, microenvironment heterogeneity (as exemplified here by canopy cover, a relevant ecological factor affecting soil microhabitat and radiation intensity) played a key role driving emergence and early survival differences when site conditions were not too harsh (i.e. in the milder Calderona *site*). Thus, some microenvironments within populations may favor foreign origins that otherwise would perform worse than local ones. Finally, we identified time

of emergence as a key factor for seedling survival in stressful Mediterranean environments.

Optimal environment for maritime pine early recruitment

Water availability is the major limiting factor for maritime pine recruitment (Rodríguez-García *et al.* 2011b). We found an overall better performance in both emergence and early survival in Calderona *site*. These results clearly reflected the harsher (warmer and drier) climatic conditions in Coca *site* compared to those in Calderona *site*. Apart from the climatic conditions during recruitment, the spring and autumn precipitation from the previous year also affect natural regeneration success in this species (Rodríguez-García *et al.* 2011b). Calderona *site* had c. 1.46 times more precipitation the year before and during our experiment than Coca *site*. Moreover, sandy soils in Coca further limit water availability, are a poor source of nutrients, and can result in extreme soil temperatures (as high as 60 °C) (Gordo *et al.* 2012). The importance of soil surface and horizon characteristics, and thus soil water and nutrient availability, has been highlighted for pine recruitment (Rojo, Montero & Orgeta 1994; Juez *et al.* 2014). Extreme environmental conditions in Coca would also explain the relative low importance of microenvironment (evaluated as open- or closed- canopy cover) in this site. In contrast, less stressful conditions for seedlings in Calderona *site* allowed microenvironment to play a key role for emergence and early survival processes. This is in agreement with previous research in Mediterranean plants (Gómez-Aparicio 2008).

Effect of time of emergence in early survival

Date of emergence played an important role in early seedling survival, with a daily decrease in the probability of survival of c. 8%. This result is in agreement with previous studies in different environments and plants that found that seedlings that emerge earlier have also higher survival rates (e.g. in annual species in an early old-field plant community (Miller 1987), in a temperate deciduous forest with *Fagus crenata* (Abe *et al.* 2007), and in Mediterranean forests with *Pinus sylvestris* (Castro 2006)). Early emergence gives advantages for plant success in future life-history stages in different fitness components (survival, growth and/or fecundity) (Ross & Harper 1972; Verdu & Traveset 2005). For example, plants may develop a better root system, better competitive hierarchies or better access to light. Especially, in Mediterranean and arid environments, an early emergence enables seedlings to grow sufficiently during spring –benefiting from better environmental conditions (e.g. in terms of soil moisture, temperature and water regime)– so as to go on to survive the summer drought (Castro 2006). However, the critical period in the establishment phase takes place when the seedling changes its dependence from cotyledon reserves to its own photosynthesis (Harper & White 1974). Short stressful events at this time, such as a few days with extreme temperatures, can be critical. In our study, the benefits of early emergence seemed to overcome the additional mortality risks entailed by Mediterranean climate heterogeneity.

Lack of local adaptation at early recruitment stages

Considering a strict definition of local adaptation (Kawecki & Ebert 2004), we did not find evidence for local adaptation at early stages of establishment

in the two population assayed, as we did not detect an overall better performance of local populations with respect to foreign ones using two complementary approaches (discrete-time logistic models and Hedges' g^* effect sizes). In fact, Calderona *origin* outperformed the local origin in Coca at the emergence stage whereas microenvironment was more relevant than population's origin for survival performance in Calderona *site* (the only one with survivors).

In contrast with our study, local adaptation has often been reported for contrasted plant populations (see examples in reviews by Leimu & Fischer (2008) and Hereford (2009); but notice that publication bias is expected to be large). Evidence for local adaptation in forest trees is also growing, thanks in part to the existence of large common gardens, in which more precise phenotypic evaluation is possible in comparison with semi-natural experiments. In general, these studies reflected a correlation of phenotypic variation with environmental factors (reviewed in Savolainen *et al.* 2007; Alberto *et al.* 2013). However, evidence of local adaptation in forest trees is more limited when strict tests associated to reciprocal transplants experiments are used. In long-lived species, such as forest trees, selection pressure can vary along life stages, which would blur local adaptation patterns measured at any given life stage. In addition, local adaptation in the populations studied may be associated with adult traits not expressed in seedlings (e.g. those related to adaptation to wildfire). Finally, given the high climatic heterogeneity across years of Mediterranean environments, our results may not be representative of the long-term evolution of the population, with years of better recruitment probably having more importance than those with low seedling survival (as the one studied).

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The lack of local adaptation in our study could also be related to the traits measured. Hereford's meta-analysis (Hereford 2009) found a greater magnitude of local adaptation when fecundity or composite measures of fitness (i.e. measures combining viability, usually survival and fecundity) were computed instead of just raw survival measurements. It was also suggested that a measure of viability, such as survival, might have less variance than fecundity or other fitness components, which may result into less accurate estimates of selection and local adaptation (Kingsolver *et al.* 2001). This would be exacerbated by the typically low power in semi-natural experiments (due to lesser environmental control). Another possible explanation could be related to population-specific recruitment strategies that confer higher overall fitness to some populations. For example, emergence outperformance by Calderona *origin* (1.23 times that of Coca) could be related to adaptation to high fire recurrence, which might have selected faster emergence relative to other populations within the species. In contrast, the greater climatic heterogeneity and sparse tree density in Coca might have selected for more moderate rates of emergence in this site.

Role of microenvironmental (canopy cover) variation

Microenvironment affected seedling's performance and modulated local adaptation patterns. In particular, Calderona's *origin* outperformed Coca's for survival at its home site but only under closed canopy. These results highlight two fundamental aspects affecting recruitment success in forest trees. On the one hand, they reflect variation in fitness-related performance traits due to specific microenvironment properties. Previous studies in *Pinus pinaster* have shown that successful recruitment depends on local climate

and stand characteristics (Ruano *et al.* 2009; Rodríguez-García *et al.* 2010). In American oaks (*Quercus rubra*), Sork, Kirk & Hochwender (1993) found local adaptation to herbivory in five year old seedlings from adjacent populations. On the other hand, better performance in particular microenvironments could be associated to population-specific life-history strategies. For example, Coca population, originated in the harsh environment of the Castilian central plateau, could present a life-history strategy better adapted to drier environments, which could have promoted its better performance under open canopy in milder sites. Drought-tolerant maritime pine populations have higher root biomass allocation (Joslin, Wolfe & Hanson 2000; Benito-Garzón *et al.* 2011) and this seems a plausible mechanism for Coca *origin*'s better performance than local origins in open-canopy microenvironment. However, this hypothesis remains untested and new experiments would be needed to elucidate population-specific adaptive strategies. Overall, our results suggest the necessity to include microenvironment variation within populations, as well as climate variation across years, to test for local adaptation, in particular in systems where environment (and, consequently, selection pressure) may vary at the fine temporal and spatial scales.

Expected shifts from open to closed microenvironments were observed during the emergence stage but not during the early survival stage. A plausible explanation for the latter could be that, as the growing season progresses, closed-canopy areas develop more suitable conditions to emerge, e.g. higher soil water retention. Greater emergence under canopy cover has been reported for maritime pine in previous studies (Ruano *et al.* 2009;

Rodríguez-García, Bravo & Spies 2011a). Nevertheless, these differences tend to disappear with time due to widespread mortality.

Family selection and potential for genetic change

The differential action of natural selection at the family level would, in principle, allow for microevolutionary change, given heritable adaptive traits (Pommel *et al.* 2002) (i.e. traits that respond to selection). In our study, some families of Coca *origin* produced a higher number of offspring than expected, assuming an equal survival in Calderona (the only site with survivors). These results suggest that Coca *origin* might contain more genetic variance available for selection than the local Calderona *origin*. However, apart from family selection, other factors, such as differences in seed quality among families, may have caused these differences. Moreover, our test combines absence of germination and seedling mortality, and thus family differences respond to multiple, not exclusive biological processes. Lack of family differences in the local (Calderona) origin could result from reduced genetic variance due to demographical history, past episodes of local adaptation, and/or absence of relevant microevolution drivers at early stages of establishment (e.g. wildfire). More detailed quantitative genetic field experiments including multiple populations would be needed to further evaluate genetic responses of contrasted maritime pine origins in the wild.

Conclusions

In this study under semi-natural conditions, we did not find evidence for local adaptation in Mediterranean maritime pine at the early stages of its life

cycle, with one of the studied origins outperforming the other in both sites. Apart from population origin, our study suggests that under stressful conditions with low survival, stochastic factors could be more important than natural selection at early stages of recruitment in forest trees. Moreover, if confirmed at adult stages, our study provides information to develop mitigation strategies in the face of climate change. For example, for assisted migration applications, i.e. the transplantation of populations following climate change, our results suggest that naturally pre-adapted foreign populations (or those containing higher levels of genetic variance) may perform better than local ones. However, we have shown that this process is modulated by microenvironmental variation, and that origin selection needs to account not only for climatic suitability, but also for population life-history strategies and the high stochasticity found for recruitment in stressful Mediterranean environments.

Acknowledgments

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Supplementary material
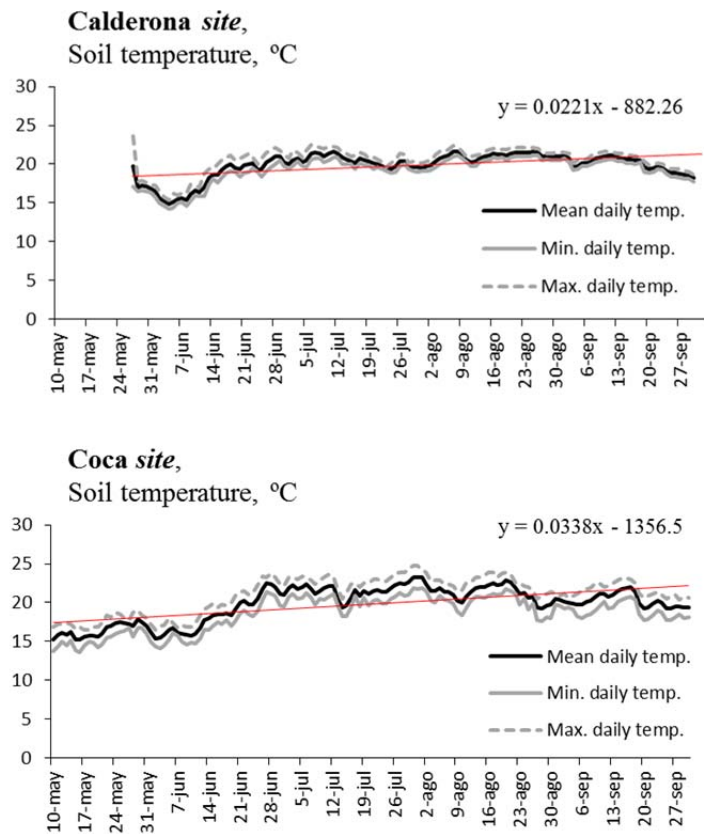
Figure S1 Soil temperature in Calderona and Coca experimental *sites*. Data recorded with a HOBO data logger from May to September. A trend line is represented in red color.

Coca *site*
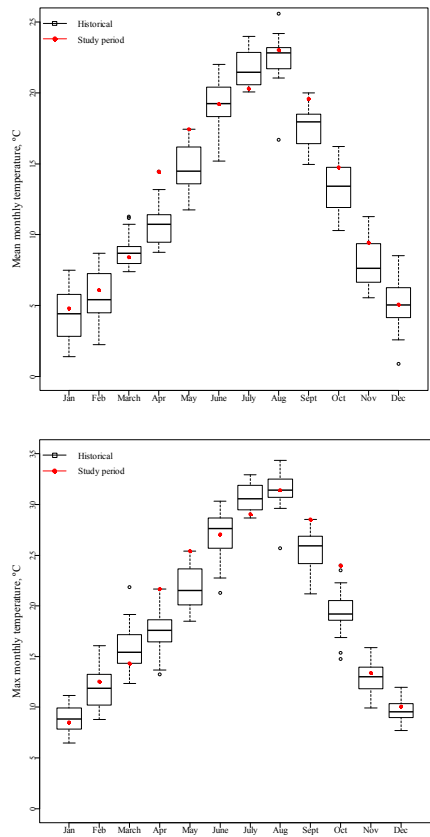
Calderona *site*

Continued

Coca site   *Calderona site*
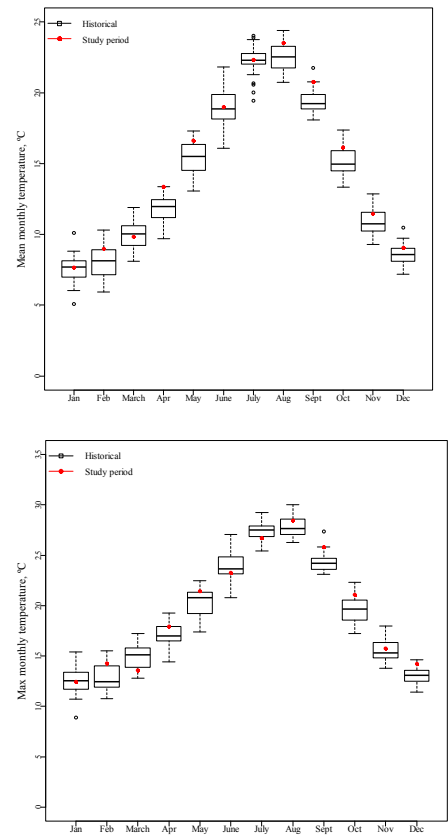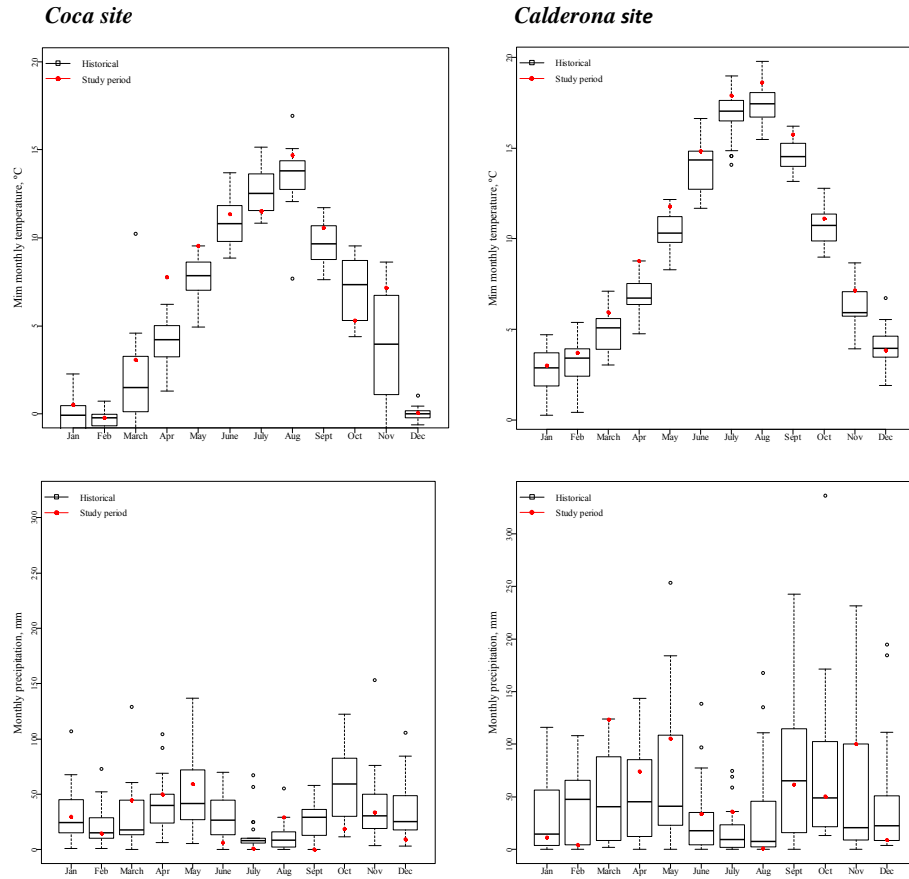
Figure S2 Mean, maximum and minimum monthly air temperature (°C) and monthly precipitation in Coca and Calderona experimental sites. Red circles represent data over the study period and boxes historical data between 1983 and 2013 from the closest meteorological stations with long climate series (Olmedo, c. 17 km from Coca, and Beteta, c. 13 km from Calderona) corrected using Gonzalo's phitoclimatic model for Spain (Gonzalo-Jiménez 2008).

Figure S3 Pictures showing initial soil conditions in the two experimental sites. Top, Coca *site*, where small shrubs are rare. Bottom, Calderona *site*; on the left side, an experimental plot is under preparation by removing small shrubs using pruning-scissors; the right side shows the initial soil conditions.

Figure S4 Pictures showing our semi-natural sowing reciprocal experiment. The bigger picture presents a general overview, centered in one experimental plot. This picture is complemented with four insects in its corners. Each insect shows different growing conditions for seedlings. On the left side, Coca *site*; and on the right side, Calderona *site*.

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Table S1 Log-rank tests to assess heterogeneous patterns in Kaplan-Meier (KM) functions for non-emergence and early survival probability: (i) site-microenvironment combinations for each origin, Coca and Calderona (see Figure 2); and (ii) origin-microenvironment combinations in Calderona (only for survival, see Figure 3).

Recruitment stage	KM functions	χ^2	df	*P* values
(i)				
Emergence	Coca *origin*	343	3	< 0.001
	Calderona *origin*	473	3	< 0.001
Survival	Coca *origin*	172	3	< 0.001
	Calderona *origin*	52.9	3	< 0.001
(ii)				
Survival	Calderona *site*	208	3	< 0.001

Table S2 Female reproductive success measures for each mother tree. a) Mother trees from Calderona *origin*. b) Mother trees from Coca *origin*. NA: Missing data.

a) Calderona *origin*

N	Candidate mother ID	Relative fertility success
1	M-CA1631	1.166
2	M-CA1632	0.637
3	M-CA1633	1.106
4	M-CA1649	1.575
5	M-CA1650	0.895
6	M-CA1766	0.617
7	M-CA1767	1.219
8	M-CA1768	0.951
9	M-CA1769	0.546
10	M-CA1770	0.231
11	M-CA1771	1.209
12	M-CA1782	1.107
13	M-CA1783	2.035
14	M-CA1785	0.809
15	M-CA1787	1.435
16	M-CA957	0.852
17	M-CA959	1.182
18	M-CA960	1.635

N	Candidate mother ID	Relative fertility success
19	M-CA961	0.835
20	M-CA964	0.518
21	M-CA966	1.115
22	M-CA992	1.368
23	M-CA993	0.271
24	M-CA998	0.686
25	M-CA000	NA

b) Coca *origin*

N	Candidate mother ID	Relative fertility success
1	M-CO10	0.633
2	M-CO105	1.042
3	M-CO11	0.813
4	M-CO111	3.400
5	M-CO165	0.243
6	M-CO169	0.269
7	M-CO21	4.195
8	M-CO224	0.054
9	M-CO230	2.439
10	M-CO262	2.204
11	M-CO301	2.233
12	M-CO353	0.906
13	M-CO45	1.501
14	M-CO57	4.171
15	M-CO73	1.490
16	M-CO84	0.469
17	M-COE	1.380
18	M-COI	0.557
19	M-COH	NA
20	M-CO88	NA
21	M-COX1	NA
22	M-COX4	NA
23	M-COX3	NA
24	M-CO110	NA
25	M-COX2	NA

Complementary analysis

Methods:

Effect sizes (Hedges'g*) for local and microenvironmental adaptation

We computed a Hedges' g^* index (Hedges 1981; Cohen 1988), a standardized and unbiased estimate of mean differences (see Eq. 1 and 3 below), to assess the magnitude of local adaptation across sites and microenvironments for both emergence and early survival. In our implementation, positive values indicate a better performance of local origin when compared to non-local. Briefly, the difference of average frequencies (d) of emergences and/or early survival cases for seedlings with local or foreign origin was calculated along the course of the experiment, i, for each combination of site and microenvironment as follows:

$$d_i = \frac{\bar{x}_1 - \bar{x}_2}{s^*} \qquad \text{[Eq. 1]}$$

where d_i is the effect size at the i time; $\bar{x}_1$ and $\bar{x}_2$ are the average frequency of emergence or early survival cases by experimental plot for, respectively, local and foreign seedlings; and s* is the adjusted standard deviation:

$$s^* = \sqrt{\frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2}} \qquad \text{[Eq. 2]}$$

where n_1 and n_2 are the samples sizes for local and foreign origins, and s_i the standard deviation of each origin within a specific environment and site. The effect size estimate d_i has a small upward bias that is relatively large when sample sizes are less than 20. This can be removed using a correction formula (which includes the correction factor, J) to derive the unbiased estimate of Hedges' g^*:

$$g^* = J(\,n_1 + n_2 - 2\,) \times d_i \approx 1 - \left(\frac{3}{4(\,n_1 + n_2\,) - 9}\right) \times d_i \qquad \text{[Eq. 4]}$$

and the variance of g^*:

$$v_{g^*} = J^2 v_{di} \qquad \text{[Eq. 5]}$$

with the variance of d_i being:

$$v_{di} = \frac{n_1 + n_2}{n_1 \times n_2} + \frac{d_i^{\,2}}{2(\,n_1 + n_2\,)} \qquad \text{[Eq. 6]}$$

Hedges' g^* index was computed using the *compute.es* package (del Re 2013) in the R environment (R Core Team 2012).

Results:

Hedges' g* effect sizes for local and microenvironmental adaptation

Overall, Calderona *origin* clearly outperformed Coca *origin* for emergence in both sites, and also under different light regimes along the whole course of the experiment. However, Hedges' g^* effect sizes were small (maximum of 0.311), with Hedges' g^* (variance) at the end of the study period of -0.256 (2.016×10^{-4}) and -0.239 (2.014×10^{-4}) for open and closed canopy in Coca, respectively, and of 0.080 (2.002×10^{-4}) and 0.311 (2.024×10^{-4}) in Calderona (see Table below). For survival in Calderona (the only site with survivors), Calderona *origin* outperformed Coca *origin* under closed canopy but not in open environments, as also evidenced in the survival models (see main text). In this case, Hedges' g^* (variance) at the end of the study period was

substantially larger than for emergence: -0.442 (6.473×10^{-3}) for open canopy and 0.363 (3.853×10^{-3}) for closed canopy.

Table Size effects (unbiased Hedges' g^* and its variance) for local vs. non-local performance by site (Coca and Calderona) and microenvironment (open- and close- canopy cover) along the course of the experiment.

	Open canopy		Closed canopy	
	Hedges' g^*	σ^2	Hedges' g^*	σ^2
Days from sowing				
Emergence in Coca				
18	0.321	2.026×10^{-4}	0	0
40	-0.007	2.00×10^{-4}	-0.394	2.039×10^{-4}
60	-0.081	2.001×10^{-4}	-0.276	2.019×10^{-4}
92	-0.265	2.017×10^{-4}	-0.239	2.014×10^{-4}
112	-0.256	2.016×10^{-4}	-0.239	2.014×10^{-4}
Emergence in Calderona				
27	-0.485	2.059×10^{-4}	0.379	2.036×10^{-4}
49	0.107	2.003×10^{-4}	0.787	2.155×10^{-4}
73	0.084	2.002×10^{-4}	0.208	2.011×10^{-4}
99	0.0800	2.001×10^{-4}	0.311	2.024×10^{-4}
Days of survival				
Early survival in Coca				
20	-0.257	1.785×10^{-2}	-0.245	1.671×10^{-2}
52	0	1.771×10^{-2}	-0.142	1.663×10^{-2}
90	0.165	1.664×10^{-2}	-0.071	1.660×10^{-2}
140	0	0	0	0
Early survival in Calderona				
27	0.047	6.321×10^{-3}	0.433	3.879×10^{-3}
45	0.057	6.322×10^{-3}	0.682	4.009×10^{-3}
85	-0.091	6.326×10^{-3}	0.618	3.969×10^{-3}
120	-0.436	3.862×10^{-3}	0.288	3.830×10^{-3}
140	-0.442	6.473×10^{-3}	0.363	3.853×10^{-3}

A positive effect size indicates better performance of local population compared to foreign population in a specific microenvironment at a given site.

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Chapter 4

Modelling population responses to climate: a case study of *Pinus sylvestris* in the Iberian Peninsula

This chapter is based upon the following manuscript:

Vizcaíno-Palomar, N., Benito-Garzón, M., González-Muñoz, N., Alía. R., González-Martínez, S.C. Modelling population responses to climate: a case study of *Pinus sylvestris* in the Iberian Peninsula. (In Preparation)

Resumen

Los pinos son elementos clave para el funcionamiento de los ecosistemas mediterráneos y producen múltiples beneficios para la sociedad. El tamaño del árbol, la competencia, la estructura del rodal y el clima –principalmente, la precipitación y la temperatura– determinan la dinámica, coexistencia y distribución de las especies forestales. Además, normalmente las especies forestales se encuentran adaptadas a su entorno local, y por tanto, es esperable que las respuestas de las poblaciones de una misma especie al medio ambiente puedan diferir. En este estudio hemos aplicado de manera complementaria dos métodos dentro del marco de los modelos mixtos – Universal Response Function, URF, y funciones de respuesta de las poblaciones– con el objetivo de modelizar la variación fenotípica intraespecífica, bien sea generada por respuestas plásticas, adaptativas, o por una combinación de las dos. Para ello hemos empleado los datos de altura de *Pinus sylvestris* a la edad 15 años, generados en un ensayo de procedencias replicado en múltiples sitios en España, cubriendo la variabilidad ambiental del rango de distribución de la especie. En general, encontramos que ambos métodos se complementan y proporcionan buenas aproximaciones para explicar la variación fenotípica entre poblaciones. Por tanto, los dos métodos juntos constituyen un compromiso adecuado entre métodos demasiado específicos o generalistas. Por otra parte, nuestros resultados ponen de manifiesto que las poblaciones presentan diferentes capacidades para aclimatarse a los nuevos climas; esto puede ser el resultado de fuerzas de selección natural y/o de la historia demográfica de la especie. Por ello, con el fin de pronosticar el crecimiento en altura dentro del contexto de nuevos

climas, resulta imprescindible tener en cuenta las respuestas a nivel de población, en lugar de hacerlo solamente a nivel de especie.

Palabras clave: Cambio climático, adaptación local, modelos mixtos, funciones de respuesta de las poblaciones, función universal de respuesta, plasticidad fenotípica, variación fenotípica.

Abstract

Pine trees are key elements for the functioning of Mediterranean ecosystems and provide multiples benefits to society. Tree size, competition, stand structure and climate –chiefly, precipitation and temperature– drive forest species dynamics, coexistence and distribution. In addition, forest tree species are often locally adapted to their surrounding environment, and hence it is expected for populations within species to differ in their responses to environment. In this study, we applied complementarily two methods within the linear-mixed modelling framework –Universal Response Function, URF, and Population Response Functions– to model intraspecific phenotypic variation generated by either plastic, adaptive responses or a combination of both. We used tree height data for *Pinus sylvestris* at 15 years old from a multisite provenance trial installed in Spain that covers the environmental variability of the species distribution in the Iberian Peninsula. In general, we found that both methods provide good approximations to explain tree phenotypic variation among populations, and that the two together provide a good compromise between too specific and more generalizable models. Furthermore, our findings highlight that populations have different abilities to acclimate to new climates, as a result of natural selection forces and/or past demographic history. Hence, in order to forecast height growth under new climates, it is necessary to account for population responses instead of species mean responses.

Keywords: Climate change, local adaptation, mixed-models, phenotypic plasticity, phenotypic variation, population response function, universal response function.

Introduction

Recent studies using Spanish forests as a study case have shown that forest tree species demography, distribution and assemblage are driven by the interaction between biotic –tree size, competition effects and stand structure– and climatic variables –chiefly, temperature and precipitation– (Vilà-Cabrera *et al.* 2011; Gómez-Aparicio *et al.* 2011; Ruiz-Benito, Gómez-Aparicio & Zavala 2012; Ruiz-Benito *et al.* 2013). However, in these works, neither local adaptation neither plastic responses were considered as sources of the observed phenotypic variation. Indeed, it is well known that forest tree species are locally adapted to their surrounding environment, despite extensive gene flow (reviewed in Savolainen, Pyhäjärvi & Knürr 2007; Alberto *et al.* 2013), harboring large amounts of phenotypic plasticity (e.g. Vizcaíno-Palomar *et al.* (under review); Chevin, Collins & Lefèvre 2012; Santos-Del-Blanco *et al.* 2013; Franks, Weber & Aitken 2013). Therefore, disentangling the contribution of local adaptation and plasticity in the responses of fitness related traits to different factors results critical to understand the full potential of species dynamics.

Nowadays, research using provenance tests is becoming popular worldwide because of the highly valuable information contained therein – e.g. among population genetic variation, degree of locally adapted populations, etc.– and the numerous applications that can be given to the data collected in common environments –e.g. predict the performance of a population at a planting site under future circumstances, etc.– (Savolainen *et al.* 2007; Alberto *et al.* 2013). Accordingly to this renewed interest in provenance data, great efforts have been devoted to improve modelling techniques. The earlier techniques were highly specific, and their findings

were restricted either to specific sites or to populations (e.g. Rehfeldt 1991; Matyas 1996; Morgenstern 1996; Alía *et al.* 2001; Rehfeldt *et al.* 2003). On the contrary, the most recent methods try to be "universal" (Neill, Hamann & Wang 2008; Wang, O'Neill & Aitken 2010; Leites *et al.* 2012a) and then to be able to extrapolate their results to almost any population within the species' distribution range. However, as a consequence, these methods can overlook specific population responses that can be key to understand population's dynamics. Then, the analysis of provenance data using different but complementary modelling techniques can help to find a compromise between too specific and too general results.

In Mediterranean ecosystems, pine trees are keystone species –i.e. they define much of the structure of the community to which they belong by providing core ecological structures and functions, and by stabilizing local environmental conditions in ways that other species can assemble into it (Ellison *et al.* 2005)–. Moreover, pine species provide a set of primary goods and ecosystems services –such as soil protection, water retention, wood production, and social entertainment– that made themselves key elements for society. Among pine species, *Pinus sylvestris* displays the largest distribution across the Eurasian region, reaching its southern limit in the Iberian Peninsula. Here, this species had glacial refugia and it is expected that local adaptation has contributed to high levels of phenotypic variation among and within populations.

In this study, we assessed the effect of climate and geographical variables drawing the intraspecific phenotypic variation of tree height in *Pinus sylvestris,* accounting for both local adaptation and plastic responses. To do so, we used data generated in a multisite provenance test covering the

environmental variability of the species distribution in the Iberian Peninsula. We focused in tree height as it has been previously shown as a crucial trait reflecting tree species' ecological strategies. Indeed, tree height depicts carbon gain strategies as well as provides relevant information about how tree species live, grow and reproduce (Moles & Leishman 2008; Moles *et al.* 2009).

We have chosen two complementary modelling techniques to analyze the provenance data. On the one hand, we implemented the universal response function –URF–. This function allows to overcome drawbacks from older methods, characterized by being either population- or planting site- specific, claiming to be universally applicable and be able to predict responses for any population growing in any climate. In 2010, Wang *et al.* developed a universal response function that considered at the same time the environmental effects of planting site on the population phenotypes (the reaction norm) and the among-population genetic differentiation resulting from local adaptation to climate (accounted for by transfer functions). On the other hand, we also applied population response functions, also known as reaction norms (Matyas 1994; Rehfeldt *et al.* 1999). These funcions describe the relationship between the performance of a specific population across environments, and thereof across different climate conditions. When comparing a group of population' response functions, we can readily observe among-population genetic variation at each planting site. Focusing on intraspecific variation in tree height at two different scales could aid to not overlook potential differential natural selection forces for specific populations. To our knowledge, the use of two complementary modelling

techniques to analyze data of provenance test has never been performed before.

The analysis of provenance data of *Pinus sylvestris* by means of two different modelling techniques, providing more population-specific and more generalizable results, will provide a highly reliable description of the role of intraspecific variation in the species tree height. To our knowledge, and despite the potential of this approach, the use of two complementary modelling techniques to analyze provenance data has never been performed before.

Material and methods

Plant material

The plant material was produced from seeds collected in natural populations of *Pinus sylvestris* along the species range in the Iberian Peninsula (Table S1a). Seeds were collected in seed lots from at least 25 mother trees with a 50-meter separation distance among each other to avoid interbreeding (González-Martínez *et al.* 2006). Afterwards, plants originating from the seed lots were established in comparative common garden tests.

Multisite common gardens of *Pinus sylvestris* were established between November 1990 and November 1991 using two-year plantlets (Table S1b). The experimental design consisted on a randomized block design, with four blocks and a sixteen-tree square plot for each population planted at 2.5×2.5 m spacing. In this study, we focused on measurements of total height (cm) at 15 years old in 16 Spanish populations (Table S1a,b).

Climate and geographical data

Fifty-eight climatic and geographical variables were used to characterize the planting sites and populations' site of origin of *Pinus sylvestris*. Specifically, we included mean, maximum and minimum average monthly (i) temperature (t_i, tmc_i and tmf_i, respectively), mean temperature of the warmest month (MWMT), mean of the maximum temperatures of the warmest month (MaxWMT), mean temperature of the coldest month (MCMT), mean of the minimum temperatures of the coldest month (MinCMT), mean annual temperature (MT), continentality (TD, which is the difference between MWMT and MCMT; this index reflects the degree by which climate is influenced by either maritime or continental air masses), annual and seasonal precipitation (p, Winter-Spring-Autumn-Summer-p), drought length period (A) and intensity (K), growing degree-days (dd5), length frost period in months (fp), altitude, latitude and longitude. Following Rehfeldt *et al.* (1999), we also included two moisture indices representative of the amount of precipitation available for plant growth: annual heat–moisture index (AHM, see Eq. 1) and summer heat–moisture index (SHM, see Eq. 2). All these values were estimated from Gonzalo-Jiménez's (2008) climatic model –climate normal period of 1961-1999– with a 1-km² spatial resolution. We assumed that the period employed would represent the climate inhabited by the parent generation of trees planted in the common garden.

$$AHM = \frac{TM + 10}{(p/1000)} \qquad\qquad \text{[Eq. 1]}$$

$$SHM = \frac{MWMT}{\left(Summer_p\,/1000\right)}\,^1 \qquad\qquad [\text{Eq. 2}]$$

Modelling procedures

We used two modeling approaches: the Universal response function (Wang *et al.* (2010)) and population responses functions described in Matyas (1994) and Rehfeldt *et al.* (1999).

1) The universal response function, URF

Variable selection

To select the most suitable explanatory variables to be included in the URF, we computed Pearson and Spearman correlation coefficients −ρ− (to identify linear and non-linear co-variation) between tree height and climatic and geographical variables. We retained those explanatory variables associated to planting sites when $\rho \geq |0.5|$, and those from population site of origin if $\rho \geq |0.08|$ (here, correlations were less robust). Afterwards, we assessed colinearity among the selected variables. Those pairs of variables with $\rho \leq |0.6|$ would be susceptible to enter at the same time in the same URF.

Model structure

Our model structure was based on a combination of methods from Wang *et al.* (2010) and Leites *et al.* (2012a). First, we used the mathematical equation

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[1] Summer$_p$ refers to Summer-p, previously defined. Notation has been changed just in Eq. 2 to avoid confusion.

developed by Wang *et al.* (2010), and second, we implemented mixed modelling techniques to include hierarchical data more appropriately as in Leites *et al.* (2012a,b). Then, the hierarchy of the data –blocks nested into populations, and those nested within planting sites– was accounted for in the random part of the model, resolving any expectable within population correlation effects. The fixed part of the model followed the URF structure proposed by Wang *et al.* (2010) and it is represented in [Eq. 3]:

$$y_{ij} = b_0 + b_1 X_{1i} + b_2 X_{1i}^2 + b_3 X_{2j}^2 + b_4 X_{2j}^2 + b_5 X_{1i} X_{2j} + e_{k(j(i)))} \quad [\text{Eq. 3}]$$

where y_{ij} is the observation of the population j at the planting site *i*; *b*'s are the intercept and regression coefficients; X_{1i} is one climate or geographic variables from the planting site *i*, and X_{2j} is one climate or geographic variables from the population *j*. $X_{1i} X_{2j}$ is the interaction between X_{1i} and X_{2j}. For consistency with previous studies (e.g. Rehfeldt *et al.* 1999; Rehfeldt, Wykoff & Ying 2001; Hamann & Wang 2006; Leites *et al.* 2012a), we have made use of quadratic functions as well.

Modelling process

The modelling process followed four steps. First, as we are dealing with highly collinear variables, we fitted a battery of URF containing just one variable to characterize the planting site and one variable to characterize population's site of origin, within the linear mixed-effect model framework (*lme4* R package (Bates *et al.* 2015), *lmer* function). This was done to overcome colinearity issues following Leites *et al.* (2012) advices. In this step, the main objective was to select the best combination of explanatory

variables. Second, from the battery of models fitted, a potential set of good models were selected based in AIC criteria (Akaike 1992; Burnham & Anderson 2002). Third, the models selected models in step 2 were ranked regarding their relative importance by computing Akaike weights, w_i, [Eq. 3] (Canham & Uriarte 2006).

$$w_i = \frac{exp\left(-\frac{1}{2}\Delta_i\right)}{\sum_{i=1}^{m} exp\left(-\frac{1}{2}\Delta_i\right)} \qquad \text{[Eq. 3]}$$

where w_i are the Akaike weights, ranging from 0 to 1; high values indicate a higher probability of being a good model predictor, i.e. a good explanatory variable. Δ_i is calculated as the difference between the minimum AIC value from the set of selected models in step 2 and the AIC value of the evaluated ($\Delta_i = AIC_{min}-AIC_i$). Fourth and final step, the statistical significance ($p < 0.10$) of the explanatory variables was assessed following the methodology proposed by Bolker *et al.* (2009) and Zuur *et al.* (2009) (see Supplementary material, Appendix 1) of alternate model comparisons. Goodness-of-fit of the best model was assessed by plots of predicted vs. observed values.

Assessment of local adaptation

We tested for local adaptation at the population level following Wang *et al.* (2010). For that, we calculated the first-order partial derivate of the best URF model with respect to the population variable and settled it to zero –in agreement to mathematical properties, this solution might indicate the existence of a maximum or minimum; and a negative value in the second

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partial derivate indicates the existence of a maximum–. Afterwards, the remaining parameter in the equation (i.e. the climate variable from the planting site) is substituted by the corresponding value from the population's site of origin. Specifically, in our case study, we assessed local adaptation for the 16 Spanish populations covering the whole variation in population origins. Then, the equation was resolved as explained above to find out which values of the population's site of origin variable would provide the highest height. If this value coincides with that from the population's site of origin, then we can conclude that the population is locally adapted.

2) Population response functions

<u>Variable selection</u>

Exploratory analyses among planting site climate and geographic variables and tree height were developed in order to assess linear and non-linear relationships and to pre-select the more suitable variables (R package *car* (Fox & Weisberg 2011) and the function *scatterplotMatrix*). Pearson and Spearman correlation coefficients –ρ– were used to check mathematically the convenience of the selected variables. Moreover, along this process, and due to the reduced number of planting sites –six–, we tried to maximize the climatic gradient by avoiding the selection, for instance, of climatic variables that were similar in different sites.

<u>Modelling process</u>

We developed population response functions within Wang *et al.* (2006) framework, but taking advantage of mixed-effect models to account for the hierarchical data-structure and to control sources of variation from unknown

variables. Specifically, the random structure of the model included blocks nested into populations and these latter, in turn, nested within planting sites.

Univariate models were constructed using tree height as response variable and one explanatory variable, from those selected previously, at a time. We compared and ranked models based in AIC criteria –as for URFs above, Akaike (1992)–. Afterwards, in order to allow straightforward comparisons among population responses, we selected a unique type of univariate model, i.e. the same planting site-climate variable combination, to represent the whole set of populations tested. For that, we counted how many times a univariate model resulted in a relative higher substantial support (i.e. we counted models having their ΔAIC within 1-2 units of the minimum). The same process was repeated to fit bivariate models –i.e. models including two explanatory variables–. Finally, we tried to improve the most parsimonious model (either uni or bi-variate) by including either quadratic and/or interaction terms.

Along the whole process of modelling, AIC criteria were used to compare and detect models' improvement. All models were fitted using the *lmer* function from the *lme4* R package (Bates *et al.* 2015). Moreover, throughout this process, and due to the reduced number of planting sites – six–, we tried to maximize the climatic gradient by avoiding the selection, for instance, of climatic variables that were similar in different sites.

Results

Universal response functions, URF

Based on Pearson and Spearman correlation coefficients $-\rho-$, we selected a total of 32 climatic variables from planting sites and 8 from population site of origin. Among the former, we found high collinearity; from a total of 496 pairs of comparisons $-(32 \times 31)/2-$ we only found 69 combinations with a correlation coefficient $\rho \leq |0.6|$, thus just 14 % of comparisons presented acceptable low covariation for being susceptible to be included together in the same model. Contrarily, colinearity among population's site of origin variables was almost negligible.

A total of 256 models were fitted (Table S2); from this total, a set of 32 models were selected and Akaike weights were computed (Table S3). The best-supported mixed-effect model according to Akaike weights included spring precipitation of the planting site (Spring-$p_{\text{Pl.site}}$) and drought length period of population's site of origin (A_{Pop}) as predictors (Table S3). The statistical significance and the functional form $-$linear, quadratic, etc.$-$ of the two explanatory variables were tested by alternative modeling comparison (Table 1).

The best-supported mixed-effect model (Table 2), included the additive and interaction effects of the spring precipitation of the planting site (Spring-$p_{\text{Pl.site}}$) and drought length period of population's site of origin (A_{Pop}), and also the quadratic term of the latter (left side of Table 2). This model produced unbiased estimates of height with a R^2 of observed vs predicted values of 0.84, and accomplished well the requirements of normality (Figure S1).

Table 1 Model comparison (see Table S2). The Akaike Information Criterion (AIC) was used to assess the structure of the final model. The best-supported mixed-effect model is highlighted in bold. (see Table S3). Akaike Information Criterion (AIC) was used to assess the best structure of the best model. The final model is highlighted in bold.

Model	Fixed part	Random part	AIC
1	Full	-	51926
2	Full	(1|Pl.site)	51330
3	Full	(1|Pl.site/Pop)	50862
4	Full	(1|Pl.site/Pop/Block)	49581
5	Null	(1|Pl.site/Pop/Block)	49623
6	Spring-p $_{Pl.site}$	(1|Pl.site/Pop/Block)	49610
7	A_{Pop}	(1|Pl.site/Pop/Block)	49619
8	$A_{Pop} + A_{Pop}^2$	(1|Pl.site/Pop/Block)	49593
9	Spring-p$_{Pl.site}$ + A_{Pop}	(1|Pl.site/Pop/Block)	49606
10	Spring-p$_{Pl.site}$ × A_{Pop}	(1|Pl.site/Pop/Block)	49605
11	**Spring-p$_{Pl.site}$ × A_{Pop} + A_{Pop}^2**	**(1|Pl.site/Pop/Block)**	**49580**
12	Spring-p$_{Pl.site}$ × A_{Pop} + Spring-p$_{Pl.site}^2$+ A_{Pop}^2	(1|Pl.site/Pop/Block)	49581

Pl. site = Planting site; **Pop** = Population; **Spring-p**$_{Pl.site}$: Spring precipitation of the planting site; **A**$_{Pop}$: drought length period of populations' site of origin.
The full model in the Fixed part –Full– has the following structure:
Height ~ Spring-p$_{Pl.site}$ × A_{Pop} + Spring-p$_{Pl.site}^2$+ A_{Pop}^2

Table 2 Estimated parameters from the best supported mixed-effect model for tree height (cm) –Model 11– (see Table 1). Mean, standard error–SE- and t-values are given for the fixed part of the model. In the random part, the standard deviation of each variable component is given.

Fixed part	Parameter	Mean (cm)	SE (cm)	t-value	Random part	σ (cm)
Intercept	b_0	354.00	19.90	17.79	Block/Pop/Pl.site	53.07
Spring-p$_{Pl.site}$	b_1	124.60	19.58	6.36	Pop/Pl.site	19.73
A_{Pop}	b_2	3.96	4.57	0.89	Pl.site	45.76
A_{Pop}^2	b_3	-30.32	5.36	-5.65	ε	63.63
Spring-p$_{Pl.site}$ × A_{Pop}	b_4	-6.23	3.70	-1.68		

Pl. site = Planting site; **Pop** = Population; **Spring-p**$_{Pl.site}$: Spring precipitation of the planting site; **A**$_{Pop}$: drought length period of populations' site of origin.

The 3D-plot shown in Figure 1 represents the fixed part of the best supported mixed-effect model (Figure 1). The linear, ascendant and monotonous trend described along the Spring-$p_{Pl.site}$ axis (Figure 1), together with the lack of Spring-$p_{Pl.site}$ quadratic term (Table 2), suggest that the full potential to show higher heights has not been reached yet in the Iberian Peninsula. However, there is an optimum length drought period in which populations reached their maximum tree height (Figure 1). Moreover, along the A_{Pop}-axis, we can readily observe among-population genetic variation at each planting site. Finally, the interaction term Spring-$p_{Pl.site}$ × A_{Pop} was statistically significant suggesting population-specific responses across planting sites.
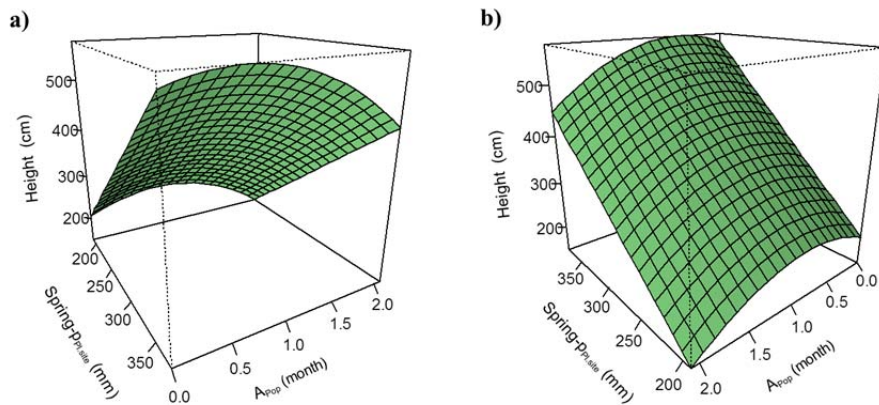
Figure 1 Tree height predictions (cm) for *Pinus sylvestris* based on a "best" model that contained spring precipitation of planting site (Spring-$p_{Pl.site}$) and length drought period from population origin (A_{Pop}). a) and b) display different perspectives of the same tree height predictions.

The random part of the best mixed-effect model captured a significant part of variation which is not explained by solely the explanatory variables in the fixed part of the model (right side on Table 2). In the best supported mixed-effect model, the σ of the random effect associated to populations nested within planting sites presented the lowest value, suggesting a low unexplained variation. This also indicates that genetic variation was well accounted for. However, the random variation associated to planting sites was higher.

We also assessed whether the set of populations assayed in the provenance trial installed in Spain were locally adapted. In general, we found that populations were not locally adapted (Figure 2). Except two populations –La Cenia (North-east Iberian Peninsula) and La Granja (Center Iberian Peninsula)–, most populations are apparently living under suboptimal climates, either under colder (Figure 2, populations situated below black circles) or warmer (population situated above the black circles) conditions.
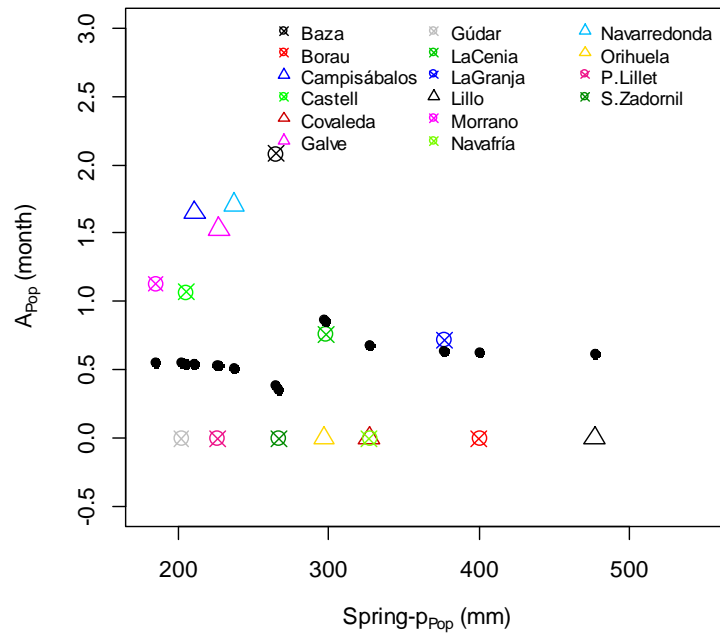
Figure 2 Graphical output to depict whether a population is living in its optimal environment. Black circle indicates the hypothetical optimal tree height and open symbols the observed values. Y-axis represents the length drought period from populations' site of origin (A_{Pop}). X-axis represents Spring precipitation values from population's site of origin (Spring-p_{Pop}).

Population response functions

Based on exploratory analyses, we selected a total of 12 planting site climate variables. Specifically, we selected: average monthly minimum temperatures of December, January, April and June (tmfDec, tmfJan, tmfApr, tmfJun); average monthly temperature of April and June (tApr, tJun), annual mean temperature (MT), annual precipitation (p), spring precipitation (Spring-p), drought length period in months (A), days with degrees temperature above 5°C (dd5), and annual heat moisture index (AHM). Moreover, tmfJun, tJun

and AHM presented non-linear relationships with total tree height. In these cases, we also tested them as quadratic terms in population response models.

We fitted a total of 192 univariate –linear and quadratic– models, i.e. 16 alternative models × 16 *P. sylvestris* populations (Table S4). The best univariate model represented well eleven populations out of the sixteen, with the population responses being driven by spring precipitation (Spring-p). Bivariate models increased, in general, significantly the power to explain population responses –by means of AIC comparisons–, except in four out the sixteen populations. Furthermore, the best bivariate model represented well twelve out of the sixteen populations, and was composed by Spring precipitation (Spring-p) and drought length (A) (Table S5). In the last step of the modelling process, aiming to find out the best mathematical form for the selected variables, we found a tie between three out the four alternative model structures, with each model structure giving support to five populations each (Table S6). Population response functions along climatic gradients under these three model structures are provided in Figure 3.

Linear response functions for each population
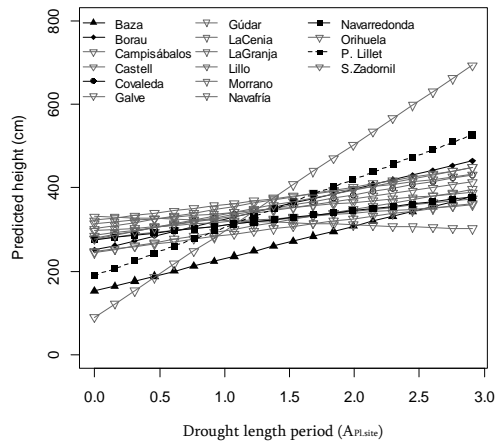
a)

Linear response functions for each population
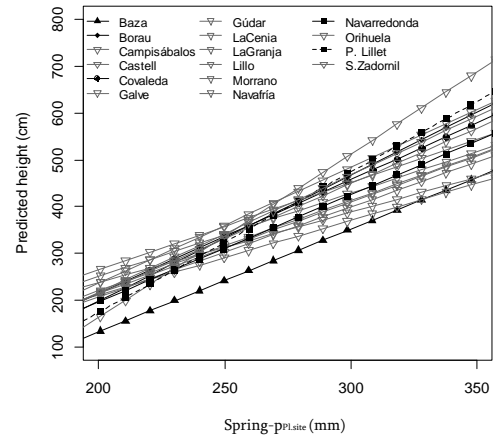
b)

Quadratic response functions for each population

c)

Quadratic response functions for each population

d)

Quadratic response functions for each population

e)

Quadratic response functions for each population

f)
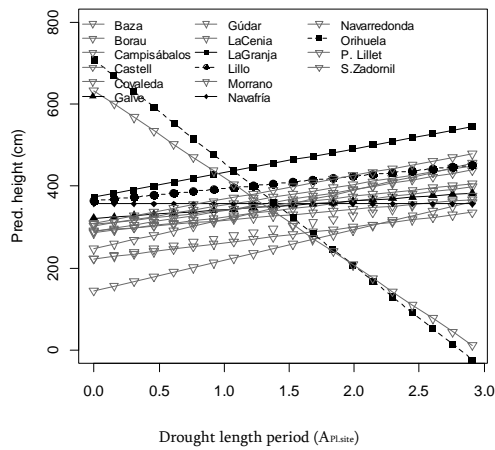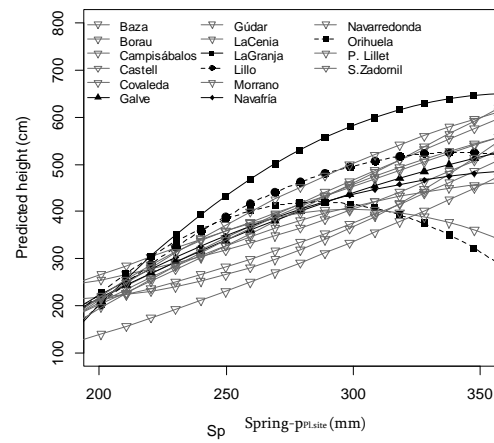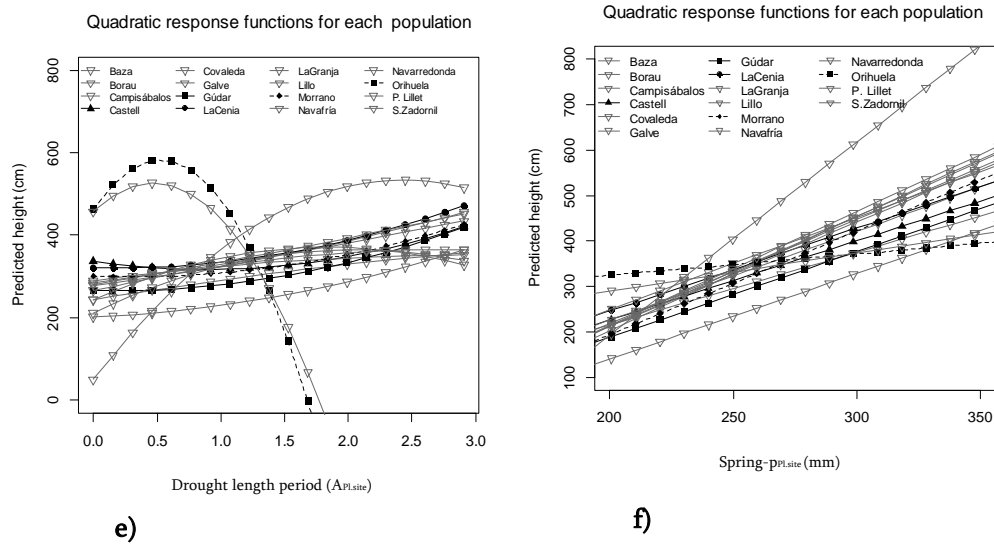
Figure 3 Population response functions for tree height (cm) of *Pinus sylvestris* populations. Figures a) c) and e) depict responses along the drought length period of the planting site ($A_{Pl.site}$ in months). Figures b), d) and f) depict responses along the Spring precipitation (mm) gradient of the planting site (Spring-$p_{Pl.site}$).

These predictions are based in the following model structures –random effects are not included–: a) and b) display responses based on Height ~ Spring-$p_{Pl.site}$ + $A_{Pl.site}$ model; c) and d) display responses based on Height ~ Spring-$p^2_{Pl.site}$ + $A_{Pl.site}$ model; and e) and f) display responses based on Height ~ Spring-p + $A^2_{Pl.site}$ model. Population predictions that presented the best fit are highlighted in black, otherwise are in gray.

Discussion

Advantages of the complementary use of two modelling techniques

The complementary use of the two modelling techniques (URF and population response functions) explained a large amount of the intraspecific phenotypic variation in tree height of *P. sylvestris* in the Iberian Peninsula – as a result of plastic and adaptive responses– to climate. On the one hand,

according to the URF, two climate variables (spring precipitation of the planting site and drought length period of population's site of origin) described fairly well the intraspecific phenotypic variation in tree height, providing at a single glance a general view of the *Pinus sylvestris* height performance. On the other hand, the fitted population response functions allow to discern differences among the studied populations of *Pinus sylvestris*. In this sense, our results show that certain environmental conditions have permitted to specific populations to reach their hypothetical maximum height growth (e.g. Galve, LaGranja, Orihuela and Lillo populations, Figure 3b), as well as that moderate drought lengths seem to be beneficial for the species height in all populations but for Orihuela.

Modelling procedures

Our modelling procedures aimed to avoid any uncertainty in our predictions. To do so, we incorporated mixed-modeling techniques to fit both the URF and the population response functions. Besides, for the URF, we have carefully followed a robust methodology in our model selection. Finally, we tried to fit the best population response functions for each population

Incorporating mixed modelling techniques brings great advantages with respect to common modelling procedures (see Leites *et al.* (2012)). Thus, potential problems regarding to correlated data –e.g. data collected within blocks– can be solved with an appropriate structure in the random part of the model (e.g. Wang *et al.* 2006; Wang, O'Neill & Aitken 2010; O'Neill & Nigh 2011). Moreover, mixed-modelling techniques gather the major sources of unexplained phenotypic variation. Finally, the interpretation of random effects is straightforward.

In the URF, we followed a robust methodology regarding mixed-effect model selection. In this sense, the large amount of models fitted, 256, allowed to test a considerate universe of plausible possibilities –including those a priori less expected–. Also, incorporating Akaike weights to choose the best variable to describe phenotypic variation in tree height was essential. In fact, the final model increased the probability of being selected up to. a 56% when the Spring-p$_{Pl.site}$ was selected among the rest of the climate variables (see Table S3). To our knowledge, this is the first time that Akaike weights have been used to rank variable importance when modelling data of provenance tests (see for example, Wang, O'Neill & Aitken 2010; Chakraborty *et al.* 2015).

Finally, the methodology followed to fit population response functions was flexible enough to select the more appropriated function for each population along the studied climate gradients. Here, we avoided to fit curvilinear forms when it was not necessary, in contrast to Leites *et al.* (2012a) procedures, as this could led to misinterpreations. For example, linear responses can indicate that populations cannot reach the hypothetical optimal height under the existing growing conditions (Figure 1, a-b). In contrast, curvilinear responses can indicate that the maximum height is susceptible to be reached in those climate conditions (Figure 1, b-f).

Intraspecific phenotypic variation: the biological interpretation

Understanding the underlying factors driving the final appearance of populations –population's phenotype– provides remarkable information to manage natural forests and forest plantations for sustainability, conservation and productivity purposes in a climate change context. Based upon the two

approaches, we found that the height growth of *Pinus sylvestris* is arrested by water availability. In general, previous studies have selected temperature related variables –mean annual temperature, or mean or minimum temperature of the coldest month– (e.g. Rehfeldt, Wykoff & Ying 2001b; Neill *et al.* 2008; Wang *et al.* 2010; Leites *et al.* 2012a). Notwithstanding, a previous study, using the same data, species and ca. age, showed that the allocation of resources was mainly driven by water availability instead of by low temperatures (Vizcaíno-Palomar *et al.* under review). Strikingly, most of the populations benefit from moderate periods of drought, except that of Orihuela.

Finally, a large part of *P. sylvestris'* populations inhabit suboptimal climate potentially precluding them to reach their full potential height growth. This result is in agreement with previous studies focused also on *Pinus sylvestris* (Rehfeldt *et al.* 2003) or in other conifer species as *Pinus banksiana* Lamb (Thomson & Parker 2008) *or Pinus contorta Dougl. ex Loud.* (Rehfeldt *et al.* 1999, 2001b) The existence of tree populations under suboptimal climate conditions have been associated to the called "adaptational lag", and can be due to the gene flow or to a recent changes in local climate in an evolutionary timescale (Aitken *et al.* 2008).

Conclusions

By means of analyzing provenance data of *Pinus sylvestris* in the Iberian Peninsula, we demonstrate that the use of two complementary modelling techniques is necessary to obtain a broad picture of the intraspecific phenotypic variation in fitness traits. For our study case, the use of population response functions provided insights of *Pinus sylvestris'*

populations specific issues, whereas the use of the universal response function allowed obtaining a generalizable description of the intraspecific phenotypic variation in height. Our results show that the height growth of *Pinus sylvestris* is mainly driven by water availability, and highlight that a large part of *P. sylvestris'* populations inhabit suboptimal climate, precluding them to reach their full potential height growth.

Acknowledgments

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Supplementary material

Table S1 Summary information of: **a)** *Pinus sylvestris* populations and **b)** Planting sites location and characteristics.

a)

Population	County	Country	Latitude (dec degree)	Altitude (m)
Puebla de Lillo	León	Spain	43.058	1,645
San Zadornil	Burgos	Spain	42.851	927
Morrano	Huesca	Spain	42.210	633
Borau	Huesca	Spain	42.697	1,510
Pobla de Lillet	Barcelona	Spain	42.228	1,123
Covaleda	Soria	Spain	41.943	1,637
Navarredonda	Ávila	Spain	40.353	1,522
Orihuela	Teruel	Spain	40.511	1,725
Gúdar	Teruel	Spain	40.410	1,665
Baza	Granada	Spain	37.374	2,084
Galve de Sorbe	Guadalajara	Spain	41.219	1,374
Campisábalos	Guadalajara	Spain	41.249	1,443
Valsaín	Segovia	Spain	40.821	1,688
Navafría	Segovia	Spain	41.004	1,585
Castell de Cabrés	Castellón	Spain	40.647	1,145
La Cenia	Tarragona	Spain	40.746	1,196

b)

Planting site	County	Country	Spring-p (mm)	A (month)
Aragüés	Huesca	Spain	377	0
Baza	Granada	Spain	191	2.91
Curueño	León	Spain	204	1.44
Gúdar	Teruel	Spain	198	0
Manzanal	León	Spain	214	1.37
Navafría	Segovia	Spain	294	1.10

Table S2 Comparisons of alternative models of tree height (cm) according to Eq. 1. In the table, rows are the variables characterizing population's site of origin, and columns do the same for planting sites. The models with the lower AIC values are highlighted in bold.

	tmfJan	tmfFeb	tmfMar	tmfApr	tmfSet	tmfOct	tmfNov
p	49559.63	49560.72	49560.71	49560.68	49559.38	49560.05	49560.59
Summer-p	49551.74	49551.38	49550.99	49551.4	49550.93	49550.89	49550.8
Autumn-p	49554.15	49555.08	49555.01	49555.01	49554.04	49554.45	49554.9
A	**49532.21**	**49532.59**	**49532.21**	**49531.96**	**49531.83**	**49531.69**	**49531.99**
K	49544.33	49545.56	49545.14	49544.43	49545.36	49544.33	49545.01
OSC	49557.2	49557.39	49557.21	49557.17	49557.2	49556.97	49557.1
TD	49557.09	49557.3	49557.11	49557.05	49557.11	49556.87	49557
SHM	49546.44	49547.21	49546.92	49546.58	49546.46	49546.21	49546.73

(continued)

	tmfDec	tmcMar	tmcApr	tmcMay	tmcOct	tFeb	tMar
p	49558.35	49559.41	49560.07	49558.67	49560.1	49560.11	49559.29
Summer-p	49551.16	49551.82	49551.58	49550.63	49551.94	49551.59	49550.86
Autumn-p	49553.06	49553.99	49554.5	49553.19	49554.42	49554.5	49553.77
A	**49531.46**	**49531.13**	**49531.4**	**49529.85**	**49531.76**	**49531.95**	**49530.81**
K	49543.63	49543.3	49543.66	49542.69	49544.19	49544.17	49543.09
OSC	49556.55	49556.47	49556.62	49555.77	49557.24	49557.24	49556.15
TD	49556.43	49556.32	49556.48	49555.64	49557.14	49557.14	49556.02
SHM	49545.56	49545.58	49545.95	49544.68	49546.54	49546.43	49545.26

(continued)

	tApr	tMay	tOct	tMov	p	pe	Winter-p
p	49560.58	49559.93	49560.85	49560.74	49556.66	49559.02	49557.89
Summer-p	49551.67	49551.3	49552.14	49552.16	49547.51	49549.84	49548.82
Autumn-p	49554.94	49554.34	49555.19	49554.99	49551.56	49553.63	49553.03
A	**49531.83**	**49530.8**	**49532.45**	**49532.49**	**49528.38**	**49530.87**	**49529.91**
K	49544.15	49543.92	49544.94	49544.59	49542.96	49544.63	49545.09
OSC	49557.02	49556.76	49557.87	49557.59	49555.07	49556.5	49557.31
TD	49556.9	49556.65	49557.78	49557.5	49554.96	49556.4	49557.2
SHM	49546.43	49545.82	49547.12	49547.12	49542.98	49545.56	49544.34

	Spring-p	Summer-p	Autumn-p	A	K	TM	MinCMT
p	49551.91	49559.4	49558.02	49561.92	49555.19	49560.08	49559.4
Summer-p	49542.89	49550.01	49548.82	49553.48	49550.74	49551.91	49551.46
Autumn-p	49546.79	49553.93	49552.83	49556.35	49550.22	49554.55	49553.88
A	**49523.42**	**49531.12**	**49529.82**	**49534.38**	**49530.72**	**49532.03**	**49531.9**
K	49537.66	49544.8	49544.27	49547.26	49543.61	49544.53	49543.91
OSC	49550.06	49556.57	49556.09	49559.16	49555.87	49557.63	49556.8
TD	49549.92	49556.48	49555.99	49559.07	49555.78	49557.53	49556.69
SHM	49537.76	49545.86	49544.56	49548.99	49545.05	49546.59	49546.14

(continued)

	fp	dd5	AHM	SHM
p	49559.6	49559.15	49553.34	49557.06
Summer-p	49550.39	49550.99	49546.99	49551.09
Autumn-p	49553.99	49553.67	49548.73	49551.97
A	**49531.19**	**49531.15**	**49527.14**	**49531.45**
K	49543.76	49543.44	49541.42	49544.56
OSC	49556.4	49556.63	49554.08	49556.64
TD	49556.29	49556.52	49553.97	49556.55
SHM	49545.69	49545.53	49541.36	49545.83

t#, tmc# and *tmf#* mean minimum, maximum and mean average monthly (#) temperatures (°C); *p* means annual precipitation (mm); *pe* accumulated precipitation from the driest summer month (mm); *Winter-Summer-Autumn* and *Spring-p* mean seasonal precipitation (mm); *A* means length drought period (month); *K* means intensity drought period based upon the climodiagram areas; *TM* means mean annual temperature (°C); *MinCMT* means mean of the minimum temperatures from the coldest month (°C); *fp* means frost period (month); *dd5* means degree days-period over 5°C; *AHM* means annual heat moisture index; and *SHM* means summer heat moisture index.

Table S3 A set of 32 models were pre-selected according to AIC values (see Table S1). Akaike weights, w_i, were computed to select the best models. The best three models are highlighted in bold and the one with the highest support according to Akaike weights is shaded in light grey.

Model	Planting Site	Population	AIC	ΔAIC	w_i
1	tmfJan	A	49532.21	8.79	0.007
2	tmfFeb	A	49532.59	9.17	0.006
3	TmfMar	A	49532.21	8.79	0.007
4	tmfApr	A	49531.96	8.53	0.008
5	tmfSet	A	49531.83	8.41	0.008
6	tmfOct	A	49531.69	8.27	0.009
7	tmfNov	A	49531.99	8.56	0.008
8	tmfDec	A	49531.46	8.04	0.010
9	tmcMar	A	49531.13	7.71	0.012
10	tmcApr	A	49531.40	7.98	0.010
11	tmcMay	A	49529.85	6.42	0.022
12	tmcOct	A	49531.76	8.33	0.009
13	tFeb	A	49531.95	8.53	0.008
14	tMar	A	49530.81	7.39	0.014
15	tApr	A	49531.83	8.40	0.008
16	tMay	A	49530.80	7.38	0.014
17	tOct	A	49532.45	9.03	0.006
18	tNov	A	49532.49	9.07	0.006
19	**p**	**A**	**49528.38**	**4.96**	**0.047**
20	pe	A	49530.87	7.44	0.014
21	Winter-p	A	49529.91	6.49	0.022
22	**Spring-p**	**A**	**49523.42**	**0.00**	**0.558**
23	Summer-p	A	49531.12	7.70	0.012
24	Autumn-p	A	49529.82	6.39	0.023
25	A	A	49534.38	10.96	0.002
26	K	A	49530.72	7.30	0.015
27	TM	A	49532.03	8.61	0.008
28	*MCMT*	A	49531.90	8.48	0.008
29	fp	A	49531.19	7.77	0.011
30	dd5	A	49531.15	7.72	0.012
31	**AHM**	**A**	**49527.14**	**3.72**	**0.087**
32	SHM	A	49531.45	8.02	0.010

Check previous table (Table S2) for the climate variable names

Table S4 AIC values for univariate (maximum quadratic) response functions for each population. In the first column, rows contain climate variables from planting sites used to fit the functions and the first row of the columns contains populations' names. For each population, the model with the lowest AIC value is highlighted in bold. The last column of the table includes a counter of how many times a model presents the lowest AIC and it is shaded in light grey. "Null" refers to a model without any explanatory variables, and "poly" indicates that the model included the quadratic term of the corresponding variable.

Variables	Baza	Borau	Campisábalos	Castell	Covaleda	Galve	Gúdar	La Cenia
Null	2965.65	3117.65	3473.36	3742.35	3441.32	3414.81	3394.03	3472.63
tmfJan	2955.18	3101.4	3471.71	3739.23	3439.73	3408.36	3394	3471.69
tmfApr	**2941.32**	**3084.07**	3469.67	3733.33	3436.61	3405.37	3389.97	3467.33
tmfJun	2966.36	3117.43	3474.62	3744.31	3442.64	3416.81	3395.32	3474.14
poly(tmfJun,2)	2947.89	3094.38	3457.52	3729.58	3419.15	3396.13	3377.57	3459.94
tmfDec	2957.05	3104.05	3470.32	3738.4	3438.45	3406.62	3393.37	3470.95
tApr	2945.05	3091.05	3468.48	3733.16	3436.11	3405.29	3389.51	3466.22
tJun	2960.74	3109.84	3475.16	3742.75	3442.9	3414.41	3395.72	3474.23
poly(tJun,2)	2962.73	3111.84	3462.56	3734.77	3429.04	3403.73	3385.17	3464.7
p	2953.63	3099.79	3462.03	3720.91	3417.09	3388.79	3376.3	3461.56
Spring p	2944.13	3085.77	**3451.64**	**3706.68**	**3401.65**	**3372.38**	3366.68	**3451.49**
A	2960.88	3108.9	3474.68	3741.66	3441.81	3411.78	3395.34	3474.08
TM	2954.79	3100.73	3472.55	3738.55	3439.72	3408.46	3393.49	3471.7
dd5	2951.11	3095.71	3470.39	3736.28	3437.8	3405.75	3392.05	3469.67
AHM	2959.7	3107.11	3464.88	3730.24	3426.09	3394.43	3385.11	3466.67
poly(AHM, 2)	2944.59	3091.42	3460.87	3708.7	3411	3385.81	**3364.85**	3455

(continued)

Variables	La Granja	Lillo	Morrano	Navafría	Navarredonda	Orihuela	P. Lillet	S. Zadornil	counter
Null	3209.7	3213.2	2821.19	2979.37	2681.99	2470.64	2434.7	3362.62	0
tmfJan	3172.79	3210.87	2820.98	2953.11	2679.36	2437.28	2410.46	3360.61	0
tmfApr	3171.21	3210.21	2817.37	2948.29	2677.72	**2428.76**	2403.47	3357.27	4
tmfJun	3208.32	3215.08	2822.88	2974.47	2683.99	2471.3	2435.66	3364.11	0
poly(tmfJun,2)	3169.93	**3188.53**	2807.74	2952.49	2656.31	2434.4	2411.83	3341.99	3
tmfDec	3177.03	3208.99	2820.3	2956.34	2678.02	2442.72	2414.13	3359.27	0
tApr	3174.34	3209.25	2817.67	2953.08	2677.57	2434.89	2410.01	3356.16	0
tJun	3199.63	3214.35	2822.66	2962.86	2682.2	2464.05	2429.05	3364.11	0
poly(tJun,2)	3200.06	3194.83	2815.45	2964.82	2664.23	2465.39	2431.04	3349.45	0
p	3195.04	3196.76	2794.49	2958.96	2662.86	2457.31	2417.34	3345.36	0
Spring p	3178.46	3189.07	2781.49	2949.21	**2654.42**	2439.22	**2401.62**	**3331.09**	**11**
A	3197.61	3213.31	2821.23	2962.28	2681.11	2455.24	2415.1	3363.48	0
TM	3183.13	3210.77	2820.38	2946.29	2678.84	2445.85	2414.57	3360.82	0
dd5	**3168.61**	3209.13	2819.23	**2942.73**	2677.67	2432.41	2408.21	3358.53	2
AHM	3194.53	3195.81	2809.11	2958.06	2665.44	2449	2409.74	3350.86	0
poly(AHM, 2)	3193.26	3196.17	**2761.01**	2956.29	2662.41	2442.91	2408.88	3341.29	2

Check previous table (Table S2) for the climate variable names

Table S5 AIC values of bivariate response functions for each population. In the first column, rows contain the planting site climatic variables used to fit the functions and the first row of the columns contains population's names. For each population, the model with the lowest AIC value is highlighted in bold. The last column includes a counter of how many times a model presents the lowest AIC and it is shaded in light grey.

Variable	Baza	Borau	Campisábalos	Castell	Covaleda	Galve	Gúdar	La Cenia	La Granja
Spring p	2944.13	3085.77	3451.64	3706.68	3401.65	3372.38	3366.68	3451.49	3178.46
Spring p + tmfJan	2943.65	3086.04	3452.85	3707.44	3398.04	3373.93	3362.21	3451.91	3172.05
Spring p + tmfApr	2942.79	3083.54	3453.39	3708.37	3402.1	3374.32	3368.03	3453.49	3172.27
Spring p + tmfJun	2945.69	3087.7	3451.36	3708.44	3399.52	3374.11	3365.93	3452.04	3180.18
Spring p + tmfDec	2943.24	3085.64	3453.11	3706.75	3398.26	3374.06	3361.24	3451.69	3173.39
Spring p + tApr	2943.43	3084.2	3453.63	3707.96	3403.19	3374.32	3368.52	3453.18	3170.27
Spring p + tJun	2945.93	3087.77	3451.69	3708.56	3399.93	3374.16	3366.33	3452.4	3179.97
Spring p + p	2942.97	3084.52	3447.07	3705.42	3400.92	3372.45	3366.91	3446.92	3169.87
Spring p + A	**2936.87**	**3079.88**	**3446.85**	3704.75	**3389.88**	**3371.63**	**3356.8**	3445.66	3179.07
Spring p + TM	2945.31	3087.57	3452.12	3708.28	3398.99	3374.13	3364.83	3452.16	3177.63
Spring p + dd5	2945.94	3087.77	3453.09	3708.51	3400.19	3374.29	3365.51	3452.95	**3169.47**
Spring p + AHM	2937.72	3081.18	3449.93	**3698.97**	3398.08	3373.78	3359.79	**3443.65**	3180.03

(continued)

Variable	Lillo	Morrano	Navafría	Navarredonda	Orihuela	P. Lillet	S. Zadornil	counter
Spring p	3189.07	2781.49	2949.21	2654.42	2439.22	2401.62	3331.09	4
Spring p + tmflJan	3190.5	2775.48	2949.42	2655.35	2432.21	2401.77	3330.9	1
Spring p + tmflApr	3190.12	2782.58	2947.9	2655.66	2429.61	2400.77	3332.8	3
Spring p + tmflJun	3190.68	2779.29	2946.07	2654.64	2440.04	2403.09	3330.69	1
Spring p + tmflDec	3190.85	2773.49	2949.86	2655.66	2433.71	2402.08	3331.11	1
Spring p + tApr	3190.92	2783.18	2947.54	2656.19	2428.23	2400.59	3333.08	3
Spring p + tJun	3190.66	2779.88	2945.78	2654.8	2439.7	2402.98	3331.07	1
Spring p + p	3190.06	2778.85	2950.86	2655.01	2429.48	2401.24	3326.6	7
Spring p + A	**3188.35**	**2767.66**	2951.06	**2653.12**	**2428.26**	**2401.01**	**3322.36**	12
Spring p + TM	3190.71	2778.19	2943.66	2655.06	2435.89	2401.94	3330.96	3
Spring p + dd5	3190.82	2779.17	**2943.26**	2655.58	**2428.09**	**2400.57**	3332.03	4
Spring p + AHM	3190.88	2771.46	2950.29	2655.44	2439.1	2403.25	3327.17	4

Check previous tables (Tables S2) for the climate variable names

Table S6 AIC values of bivariate quadratic response functions for each population based in the best model selected in Table S5 with best combination of explanatory variables. In the first column, rows contain the population's names, and the first row of the columns reflects the climatic variables from the planting sites and the mathematical form employed to fit the functions. For each population, the model with the lowest AIC value was highlighted in bold. The last row includes a counter of how many times a model presented the lowest AIC.

Model	Spring p + A	poly (Spring p, 2) + A	Spring p + poly (A, 2)	Spring p × A
Baza	**2936.87**	2938.30	2938.17	2938.22
Borau	**3079.88**	3081.76	3081.68	3081.71
Campisábalos	3446.85	3445.07	3448.72	**3444.46**
Castell	3704.75	3705.03	**3699.27**	3705.35
Covaleda	**3389.88**	3391.61	3391.75	3391.57
Galve	3371.63	**3371.30**	3373.11	3371.32
Gúdar	3356.80	3357.33	**3354.78**	3357.44
La Cenia	3445.66	3447.66	**3445.16**	3447.61
La Granja	3179.07	**3162.80**	3163.38	3163.09
Lillo	3188.35	**3179.10**	3183.64	3179.96
Morrano	2767.66	2766.45	**2763.52**	2765.95
Navafría	2951.06	**2948.72**	2949.57	2949.25
Navarredonda	**2653.12**	2653.73	2654.42	2653.72
Orihuela	2428.26	**2427.32**	**2427.32**	**2427.32**
P. Lillet	**2401.01**	2402.35	2402.35	2402.35
S. Zadornil	3322.36	3322.23	3324.32	**3321.89**
counter	5	5	5	3
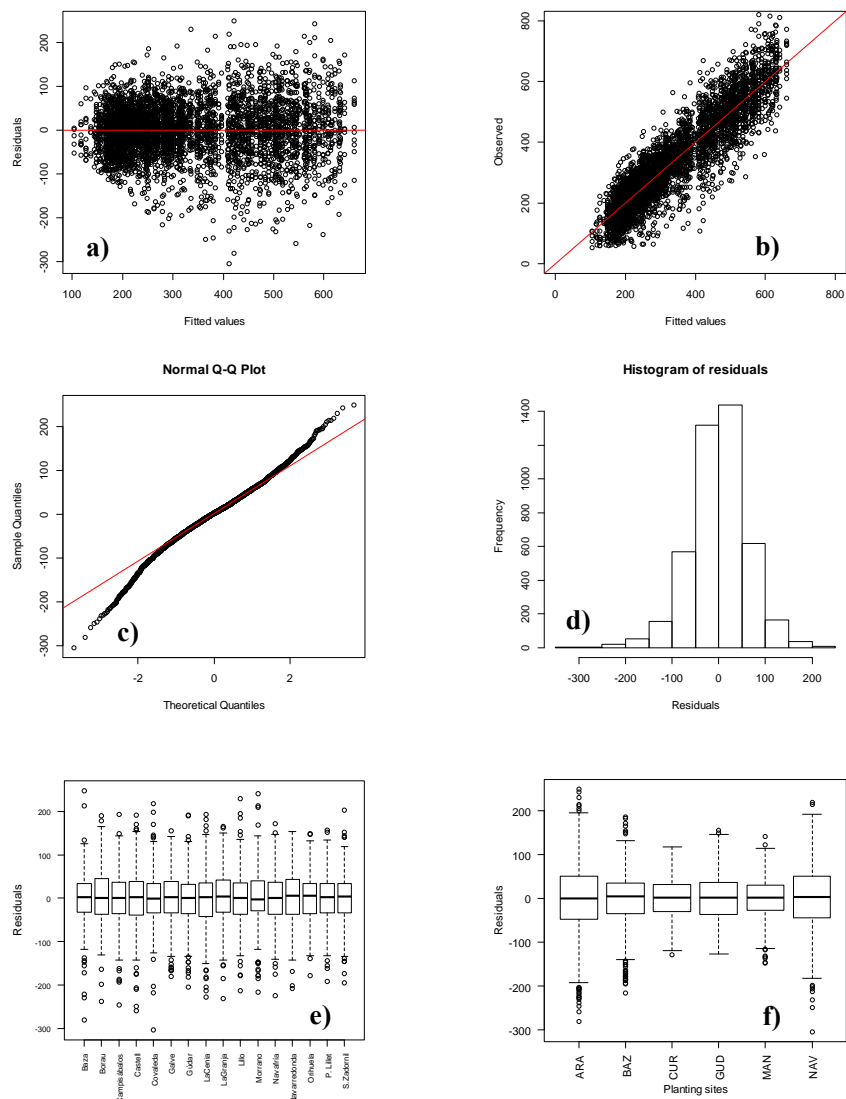
Figure S1 Graphical summary of model fit of the URF for *Pinus sylvestris* (Model 11 in Table 1): a) plot of fitted tree height (cm) versus residuals values; b) plot of observed tree height (cm) versus fitted values; c) and d) evaluation plot of residuals' quality; e) and f) boxplots of the within-group residuals for population (e) and planting site (f).

Appendix 1

To build the most parsimonious and appropriate mixed-effect model according to Zuur *et al.* (2009) and Bolker *et al.* (2009), we followed the next two steps: (i) different structures in the random part of the model are judged based in AIC criteria (Akaike 1992; Burnham & Anderson 2002), while the fixed part is held with the most saturated model structure; (ii) afterwards, the process is reversed. The structure of the fixed part is adjusted while the random part is held with the structure selected in the previous step. The process to find the best suitable combination of explanatory variables is done by adding in one variable at a time and evaluating the improvements by model comparisons based in AIC differences. The lower the AIC values are, the better the model. The random effects were tested using Maximum Likelihood of the parameter (ML) and fixed effects using restricted maximum-likelihood (REML).

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Chapter 5

Adaptation and plasticity in aboveground allometry of four contrasting pine species along environmental gradients

Resumen

Pregunta: En las especies forestales, la relación alométrica entre la altura total y el diámetro a la altura del pecho puede ser concebida como el resultado evolutivo del balance entre los recursos por encima y por debajo del suelo. La alometría se relaciona con la productividad y la resiliencia de las especies en distintos entornos. Además, refleja el compromiso de la especie entre la eficacia-seguridad y su capacidad competitiva. Dada su importancia, es necesario un mayor entendimiento acerca de cómo este rasgo varía dentro y entre especies. Este conocimiento podrá ser utilizado para determinar el potencial de una especie y/o población para adaptarse a lo largo de gradientes ambientales.

Localización: Península Ibérica

Método: Se evaluó la variación de la alometría altura-diámetro en cuatro especies de pino comunes en Europa. Para ello, se cuantificó mediante el empleo de variables geográficas del sitio de origen de las poblaciones (indicando adaptación local) y variables del clima del sitio de crecimiento (indicando aclimatación).

Resultados: Nuestros resultados mostraron que una gran parte de la variación total de la alometría altura-diámetro es explicada por las variables geográficas: altitud y latitud; y las variables climáticas: temperatura y precipitación, así como también por la variabilidad existente entre las poblaciones dentro de la especies. Por un lado, el clima del sitio de crecimiento separó las especies xerófilas (*Pinus halepensis* y *P. pinaster*) de los dos especies de ambientes más templados (*P. sylvestris* y *P. nigra*). Por otro lado, las especies de *Pinus* difirieron en los niveles de variación genética

intraespecífica en su respuesta a la temperatura y la precipitación. *P. sylvestris*, presentó, en general, el mayor nivel de variación, mientras que *P. halepensis* fue la única especie que no presentó variación genética intraespecífica.

Conclusiones: Nuestros resultados sugieren que las cuatro especies han sido sometidas a diferentes procesos históricos y limitaciones climáticas que podrían haber configurado su actual alometría altura-diámetro, lo cual condiciona la existencia de diferentes estrategias de vida. Dado que la resistencia y resiliencia de nuestros bosques a lo largo de gradientes de temperatura y aridez depende tanto de la adaptación como de los niveles de plasticidad fenotípica de sus poblaciones, nuestros resultados señalan la idoneidad de tenerlos en cuenta en la toma de decisiones para el desarrollo de la planificación de la estrategia forestal.

Palabras clave: Análisis bayesianos, gradientes ambientales, rasgos funcionales, modelos estadísticos, Península Ibérica, variación intraespecífica, ensayos de procedencias.

Abstract

Question: Plant species aboveground allometry can be viewed as a functional trait that reflects the evolutionary trade-off between above- and belowground resources. In forest trees, allometry is related to productivity and resilience in different environments, and it is tightly connected with tree species compromise between efficiency-safety and competitive ability. A better understanding on how this trait varies within and across species is needed, and it can be used to determine the potential of a species/population to cope along environmental gradients.

Location: Iberian Peninsula.

Methods: We assessed, in a hierarchical framework, species variation in height-diameter allometry in four common European *Pinus* species and quantified this variation as a function of the geographic variables of the population's site of origin (indicating local adaptation) and the climate of the growing site (indicating acclimation).

Results: Our results showed that a large and significant part of the total variation of the height-diameter allometry is explained by both geographic (altitude and latitude) and climatic variables (temperature and precipitation), and by inter-population variability within the species. Climate of the growing site separated xeric species (*Pinus halepensis* and *P. pinaster*) from the two, more mesic, species (*P. sylvestris* and *P. nigra*). Moreover, *Pinus* species differed in the levels of intraspecific genetic variation in their response to temperature and precipitation. *P. sylvestris*, a boreal species, displayed, generally, the largest level of variation, while *P. halepensis* was the only species without intraspecific genetic variation.

Conclusions: Our findings suggest that the four species have been subjected to different historical and climatic constraints that might have driven their aboveground allometry and promoted different life strategies. As full potential of forest resilience and resistance along temperature and aridity gradients depend on the populations' adaptation and levels of phenotypic plasticity, our results to account the ecological and historical background of the particular species and population to take decisions about forest strategy planning.

Keywords: Bayesian analysis; environmental gradients; functional trait; statistical modeling; Iberian Peninsula; intra-species variability; provenance tests.

Introduction

Aboveground allometry is considered a functional trait that links the changes in total height to those in stem diameter and reflects the evolutionary outcome in plant species dynamics for above and belowground resources (Hallé, Oldeman & Tomlinson 1978). Both height and stem diameter are tightly associated with species foraging and resource allocation strategy (Tilman 1988): while tree height reflects a strategy for securing carbon profit via light capture (Moles *et al.* 2009), stem diameter is closely related to mechanical support and water-absorbing capacity (McMahon 1973; Niklas 1993; Bullock 2000). Due to inevitable tradeoffs in plant allocation strategies along resource gradients –sensu Tilman (1988)– we can expect a finite set of allometric outcomes. Consequently, aboveground allometry emerges as a comprehensive and integrative trait in which allocation patterns could be driven, and or be constrained, by adaptive or neutral genetic processes at different scales (species, population or individual), by plastic responses to the environments, or by both.

Tree height-diameter allometry has profound effects on species fitness and, consequently, on ecosystem structure. It correlates with bioclimatic variables (e.g. Aiba & Kohyama 1996; López-Serrano *et al.* 2005), and can change along biotic and abiotic gradients such as those for temperature, aridity and competition (e.g. Lines *et al.* 2012; Banin *et al.* 2012). To what extent, those changes are the result of the genetic adaptation of the species (i.e. over several generations), or of plastic responses (i.e. phenotypic adjustments in response to local conditions and resource availability), or whether this plasticity has an adaptive component, are still matters under investigation. Height and diameter exhibit moderate

heritability, separately (Costa & Durel 1996; Hannrup, Wilhelmsson & Danell 1998) or combined in allometric relationships (Bailey *et al.* 2004), hence these traits can be subjected to adaptive evolutionary change.

Intraspecific variation in allometry has usually been neglected and most studies have focused either on the species level or on the broad geographical scales that disregarded population variability (e.g. López-Serrano *et al.* 2005; Chave *et al.* 2005; Dietze, Wolosin & Clark 2008; Lines *et al.* 2012; but see Pretzsch & Dieler, 2012). However, different studies have highlighted the importance of intraspecific variation to better understand, among others, community dynamics and ecosystem functioning (e.g. Lecerf & Chauvet 2008; Violle *et al.* 2012; Carmona *et al.* 2014). Therefore, a better understanding of both interconnected levels of variability (species and populations) in tree height-diameter allometry is necessary to forecast the full potential of tree species to adapt or evolve under climate change conditions (e.g. Benito-Garzón *et al.* 2011; Valladares *et al.* 2014).

Multi-locality and multi-population common garden tests constitute a valuable source of information in the study of intraspecific genetic variation (e.g. Matyas 1996; Alberto *et al.* 2013). In the last two decades, these experiments have been rediscovered to address population adaptation within the current global warming framework (e.g. Rehfeldt *et al.* 1999; Wang, O'Neill & Aitken 2010; O'Neill & Nigh 2011). In this study, we used total height and stem diameter measured in common garden tests to assess allometry relationships for the four most important European pine species: *Pinus sylvestris* L., *P. nigra* Arnold*, P. pinaster* Aiton and *P. halepensis* Miller. These species display contrasting demographic backgrounds and genetic compositions segregating in a predictable pattern along temperature

and water availability gradients across Europe (Richardson 1998; Tapias *et al.* 2004; Soto *et al.* 2010). We implemented a flexible log-linear model taking into consideration each species' population origin and associated geographic characteristics (to account for intraspecific genetic diversity), and the climatic characteristics of the growing site (to account for acclimatization potential). We tested three hypotheses: (i) Height-diameter allometry patterns in pines are constrained by both the species and the intraspecific variation related to population origin; (ii) Intraspecific variation in height-diameter allometry reflects adaptive patterns along climatic and geographic gradients; (iii) Phenotypic plasticity modulates species and populations response to different environments. Testing these hypotheses allowed us to quantify and assess the underlying abiotic drivers, at both the species and the intra-specific levels, in allometry variation and to identify adaptive responses if existent. Understand phenotypic integration of tree species responses to abiotic conditions could then assist the forecasts of forest species, and populations, performance in the context of global warming.

Material and methods

Plant material and common garden provenance tests

Aboveground allometry was measured in multi-site, within Spain, common garden provenance tests for four pines species: *Pinus sylvestris*, *P. nigra*, *P. pinaster* and *P. halepensis*. Populations from the distribution range of the species, mostly from the Iberian Peninsula (Spain and Portugal), were sampled by collecting seed lots from at least 25 mother trees with a 50-meter separation distance. Plants originating from the seed lots were collected in

different populations (22 for *P. sylvestris*, 23 for *P. nigra*, 52 for *P. pinaster* and 56 for *P. halepensis*) and established in comparative common garden tests for each species (Figure 1 and see Table S1 in Supporting Information).
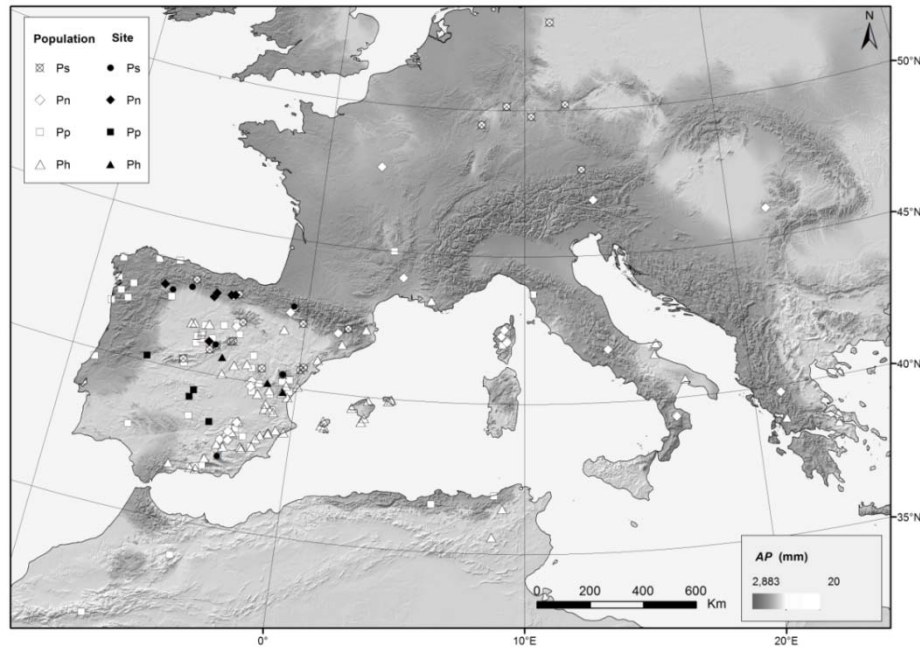
Figure 1 Common garden sites (Site, filled symbols) and population's sites of origin (Population, unfilled symbols) are represented in the map. Each pine species is represented in a different symbol Ps: *P.* sylvestris and it is represented by a circle; Pn: *P. nigra,* it is represented by a star; Pp: *P. pinaster* by a square, and Ph: *P. halepensis* by a triangle.

Measurements were collected at 11 ± 1 years of age, depending on the common garden tests, for two variables: *height* (total height in cm, measured with a pole) and *dbh* (diameter at breast height in mm, measured with a caliper). A common age was chosen to minimize species differences along their ontogenies (López-Serrano *et al.* 2005), and the effects of intraspecific competition. Thus, in *P. pinaster*, the fastest growing species in

the study, inter and intra population competition was not significant for any of the two variables, height and dbh, at age 32 in the same common garden test (Alía, Moro & Denis 2001a), suggesting that intraspecific competition could be neglected in our analyses.

In total, we used data from 4,853 *P. sylvestris* trees from 22 populations planted in 6 sites; 3,644 *P. nigra* trees from 23 populations in 8 sites; 9,976 *P. pinaster* trees from 54 populations in 4 sites; and 1,928 *P. halepensis* trees from 56 populations in 3 sites.

Climatic and geographical data

Each growing site was characterized by a set of 47 climatic variables: minimum, average and maximum mean monthly temperature, minimum and maximum temperature of the coldest and warmest months and total and seasonal precipitation. These variables were estimated by using Gonzalo-Jiménez's (2008) climatic model for the Iberian Peninsula –correspondent to the climate normal period of 1961-1999–, with a 1-km^2 spatial resolution.

We carried out extensive exploratory data analyses to select the subset of climatic variables at the growing sites most relevant to plant allometry for the four species (i.e. highly correlated to *height* and *dbh*) (see Table S3). The selected variables were MMT (mean minimum temperature of coldest month, °C), and AP (annual precipitation, mm). Both MMT and AP affect physiological and growth processes of plant species in the Mediterranean region (Thompson 2005). Altitude (*ALT*, m a.s.l.), longitude (*LON*, centesimal degrees), and latitude (*LAT*, centesimal degrees) were taken for each population origin sampled. To allow comparison among species, climatic and geographic variables were standardized.

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We summarized the growing sites and the climatic characteristics of the population's sites of origin by performing a principal component analysis (see Figure S1). Although the number of growing sites is low (3 to 8), they cover the average climate of each species with contrasting climates allowing for species comparisons (Ruiz-Benito *et al.* 2013).

Statistical models

We selected a common allometric model for the four species to facilitate parameter comparisons among them. We estimated tree height as a function of diameter by using three classic allometric functions (Linear, Power, and Gompertz), and two link functions (normal and log-normal) and implemented generalized linear models (GLMs). The best allometric model fitting the data was selected using the Deviance Information Criteria, DIC (Spiegelhalter *et al.* 2002). A power function with a log-normal link function was the best model for two out of the four species, and the second best model for the other two species (see Table S2).

Based on this allometric model, we constructed a hierarchical model (Clark 2005, 2007). These models are more appropriate to connect and represent the biological hierarchy of the data, e.g. populations within species. The process to construct the final model followed three steps. We consider several variations of the basic model (i.e. constant parameter values) and selected the final model based on both biological relevance and the DIC criterion, this final model estimated tree height allometry as a combination of climate at the growing site (*s*) and geographic characteristics at the origin site of the population (*p*).

Considering an individual i, from population p growing in growing site s, its height-diameter allometry was modelled as:

Likelihood: $height_i \sim \log Normal\ (H_i,\ \sigma^2)$

and process model: $H_i = \ln\left(a_{p(i),s(i)}\right) + c_{p(i)} \times dbh_i$ [Eq. 1]

where, $\ln\left(a_{p(i),s(i)}\right)$ the scaling coefficient was estimated as:

$\ln\left(a_{p(i),s(i)}\right) = \alpha_{1p} + \alpha_{2p} \times MMT_s + \alpha_{3p} \times AP_s$ [Eq. 2]

Because all explanatory variables were standardized, parameter α_{1p} was the allometric curve's intercept at average climate conditions of across all growing sites. Differences among α_{1p} represent intraspecific genetic differences among populations. The parameters α_{2p} and α_{3p} represent, respectively, the specific height-diameter allometric responsiveness of each population p to temperature (MMT) and rainfall (AP) at growing site s, i.e. the reaction norms. The existence of significant intraspecific differences in α_{2p} and α_{3p} indicates genetic differences in plasticity among populations that can be either adaptive or non-adaptive. We formally tested significant intraspecific differences in α_{*p} for each species by computing all pairwise combinations of population differences. Two populations were significantly different if zero was not included in the confidence interval around their difference. Additionally, we quantified the level of inter-population genetic variation as the percentage of the total number of significant pairwise comparisons relative to the total number of pairwise comparisons within species. Range of variability among populations within species was computed as the standard deviation of α_{*p}.

The scaling exponent, $c_{p_{(i)}}$, was estimated as a function of the population's origin latitude and altitude:

$$c_{p_{(i)}} = \beta_1 + \beta_2 \times LAT_p + \beta_3 \times ALT_p \qquad \text{[Eq. 3]}$$

where β_1, β_2 and β_3 represent, respectively, the intercept and species response to latitude (*LAT*) and altitude (*ALT*). These two variables are surrogates for the environmental conditions under which each population evolved, and would indicate a clinal adaptive pattern to the original climate of the population.

Model parameters estimation

Parameters were estimated following a Bayesian approach highly suited for hierarchical analyses (Gelman & Hill 2007). Each of the population level parameters, α^*_p, was estimated from a species-level prior normal distribution, with hyperparameters μ^* and $\sigma_{\alpha^*}^2$, $\alpha^*_p \sim N(\mu^*, \sigma_{\alpha^*}^2)$, estimated from non-informative prior distributions $\mu^* \sim N(0, 1000)$ and $\sigma_{\alpha^*} \sim Uniform(0, 100)$. These species-level parameters μ^* and $\sigma_{\alpha^*}^2$ would correspond *sensu stricto* to the average among the studied populations. However, we refer to these parameters as a species proxy response.

Parameters β^* were also estimated from non-informative prior distributions, $\beta^* \sim N(0, 1000)$. Variance associated with the individual random effects was estimated as $1/\sigma^2 \sim Gamma(0.01, 0.01)$. As standard deviation of residual errors around a fitted power function might increase with diameter, we tested whether the residuals were a linear function of diameter, as recommended in Lines et al. (2012). However, the approach did

not improve the fit in any of the studied cases, so we considered unnecessary to account for diameter size in the estimation of the variance.

Models were run in OpenBUGs (version 3.2.2 rev 1063) (Thomas *et al.* 2006). Three chains were run for ~50,000 iterations and parameters convergence was reached after ~25,000 iterations. After the burn-in period, chains were thinned (every 100) to reduce autocorrelation, then posterior parameter values (mean and 95% credible intervals) were calculated. Plots of predicted vs. observed values were also used to evaluate model fit (unbiased models having a slope of one and R^2 values indicating goodness-of-fit). A slope parameter was considered to be statistically significant when the 95% credible interval (CI) did not include zero. Population level parameters were considered significantly different when their 95% CI did not overlap (or the 95% CI around their difference did not include zero).

Adaptive patterns in height-diameter allometry along climatic and geographic gradients

To detect neutral *vs* adaptive patterns of variation in the parameters, we used two different approaches. First, we tested the influence of previously defined gene pools for each species on α_{p} parameters. Gene pools are proxies of genetic relationships among populations, indicating common demographic or evolutionary factors. In *P. sylvestris*, *P. pinaster* and *P. halepensis*, populations were associated to gene pools previously defined using molecular markers in Robledo-Arnuncio *et al.* (2005) and in Jaramillo-Correa *et al.* (2015)*.* In *P. nigra*, populations were grouped by sub-species. One-way ANOVA was used to detect these patterns and post-hoc comparisons among gene pools with a HSD Tukey's test were employed. When homogeneity and

normality assumptions were not reached, non-parametric Kruskal-Wallis test and post-hoc comparisons with a Nemenyi test, corrected for ties if necessary, were used. Second, to identify climatic or geographical clines due to local adaptation in phenotypic plasticity, we estimated Pearson correlation coefficients (ρ) of all α_P with climate and altitude of the site of origin.

Phenotypic plasticity in height-diameter allometry along climatic gradients

The allometric model allows us to estimate the aboveground allometry in pines along simulated climatic gradients (MMT and AP*)* covering ample part of the entire distribution range of the species. Phenotypic plasticity in the allometric relationship was assessed at species level by estimating the height at a fixed *dbh* (100 mm) along two climatic gradients. We used parameters' means, variances and covariances of the final models for each species. We defined two climatic gradients for these simulations: i) mean minimum temperatures of the coldest month (MMT: -6, +6 °C), and ii) annual precipitation (AP: 240-1800 mm). To run the simulations along each climatic gradient, we set the rest of variables (annual precipitation, mean minimum temperature of coldest month, latitude and altitude) to the mean values associated with each species' distributional range: 894 mm, -3.42 °C, 43° 37' 48" and 1,123 m for *P. sylvestris*; 712 mm, -0.24 °C, 41° 31' 48" and 1,028 m for *P. nigra*; 788 mm, +0.77 °C, 40° 09' 36"and 852 m for *P. pinaster* and 453 mm, +0.6 °C, 39° 19' 36" and 539 m for *P. halepensis*. We also checked alternative fixed *dbh* (average *dbh* in each species and 150 mm), but the patterns remained similar.

Phenotypic plasticity is presented graphically, as the models output estimates along the gradients, and quantified numerically by a Phenotypic Plasticity index (PI_v) following Valladares et al. (2006)

$$PI_v = \frac{(H_{max} - H_{min})}{H_{max}} \qquad\qquad [Eq. 4]$$

where Hmax and Hmin are maximum and minimum values of estimated heights along each climatic gradient. This index ranges between zero and one, meaning no plasticity or high plasticity, respectively.

Results

Height allometric patterns across species and intraspecific variation

The four final models produced unbiased estimates of *height* with high R^2 of observed *vs* predicted values (0.90 in *P. sylvestris*, 0.91 in *P. nigra*, 0.85 in *P. pinaster* and 0.89 in *P. halepensis*).

Pinus pinaster had the lowest intercept value, measured by parameter μ_1, and it did not overlap with the other three species. *P. nigra* and *P. sylvestris* had intermediate but overlapping values, while *P. halepensis* had the highest value and did not overlap with any of the other pine species (Table 1). The intraspecific variability, standard deviation of α_{1p}, also varied among species. *P. pinaster* displayed the greatest value (0.054 cm), followed by *P. nigra* (0.039 cm), *P. sylvestris* (0.028 cm) *and P. halepensis* (0.024 cm) (Table 1). Moreover, there was significant intraspecific variation in α_{1p} in all species, measured by the number of significant pairwise comparisons within

species relative to the total number: *P. pinaster* was the species with the highest percentage of significant differences (50.24 %); followed by *P. nigra* (40.32 %), *P. sylvestris* (31.17 %) and *P. halepensis* (17.21 %).

Temperature (MMT) also affected tree height allometry, being this parameter, μ_2, statistically significant and positive in three out of the four species and significant but negative in *P. sylvestris* (Table 1). *P. halepensis* showed the strongest response to temperature, approximately twice to four times larger, when compared to the rest of species. Moreover, we found evidence of population differentiation in response to temperature (MMT), i.e. differences in phenotypic plasticity, in three out of the four species (*P. halepensis* was the exception), as significant intraspecific variation was found in α_{2p}. Specifically, the level of inter-population genetic variation varied with the species. Thus, *P. sylvestris* displayed the greatest level of genetic differences in phenotypic plasticity in response to MMT (38.10 %), in comparison to the other two species: *P. nigra* (9.88 %) and *P. pinaster* (9.57 %). Finally, the four species showed some degree of intraspecific variability, *P. sylvestris* having the greatest with a standard deviation of 0.032 cm, followed by *P. pinaster* with 0.026 cm, *P. nigra* with 0.011 cm and *P. halepensis* with 0.007 cm.

Table 1 Parameter estimates from the selected best model. It shows the overall species' response, i.e. μ^* and β^* posterior mean estimates and standard deviation (in parenthesis) and the intraspecific variability (sd). The range of parameter values among populations, i.e. posterior mean estimates of α^*_p, are shown in square brackets. **Bold** numbers indicate fixed-effect coefficients that were statistically significant (95% CI does not include zero). Letters indicate comparisons based upon CI when coefficients were statistically significant; different letters indicate differences among species for each of the parameters when statistically significant.

			P. sylvestris	P. nigra	P. pinaster	P. halepensis
$ln(a_{p,s})$	Intercept:	μ_1	**4.143** (0.013)b	**4.121** (0.014)b	**3.056** (0.016)c	**4.344** (0.021)a
	α_{1p}		[4.107, 4.229]	[4.047, 4.198]	[2.907, 3.170]	[4.281, 4.396]
	sd(α_{1p})		0.028	0.039	0.054	0.024
	MMT:	μ_2	**-0.030** (0.009)d	**0.037** (0.005)c	**0.086** (0.007)b	**0.158** (0.007)a
	α_{2p}		[-0.120, 0.026]	[0.014, 0.063]	[-0.002, 0.169]	[0.143, 0.174]
	sd(α_{2p})		0.032	0.011	0.026	0.007
	AP:	μ_3	**0.080** (0.006)a	**-0.018** (0.004)b	**-0.023** (0.006)bc	**-0.049** (0.007)c
	α_{3p}		[0.043, 0.128]	[-0.032, 0.000]	[-0.059 ,0.032]	[-0.053 ,-0.043]
	sd(α_{3p})		0.019	0.007	0.018	0.002
c_p	Intercept:	β_0	0.426 (0.003)b	0.412 (0.003)bc	0.700 (0.003)a	0.397 (0.005)c
	LAT:	β_1	**0.016** (0.004)a	**0.010** (0.003)a	0.005 (0.002)	0.003 (0.001)
	ALT:	β_2	-0.006 (0.004)	-0.004 (0.003)	-0.007 (0.002)	-0.002 (0.001)

Annual precipitation (AP) also influenced tree height allometry. Values for parameter μ_3 were statistically significant and negative in three out the four species, but positive in *P. sylvestris*. The estimated values for *P. sylvestris* and *P. halepensis* did not overlap, but the pairs composed by *P. nigra* and *P. pinaster* and *P. pinaster* and *P. halepensis* did. The strongest response in absolute terms to rainfall was found in *P. sylvestris* (Table 1). Similarly, we found genetic differences in phenotypic plasticity to rainfall (AP) in three out of four species, the exception again being *P. halepensis*. The level of inter-population genetic variation was the greatest in *P.*

sylvestris (29.87%), followed by *P. pinaster* (3.60%) and *P. nigra* (2.77%). Furthermore, the four pine species presented some degree of intraspecific variability. *P. sylvestris* and *P. pinaster* presented similar degrees, with standard deviations of 0.0191 cm and 0.0184 cm, respectively; *P. nigra* had a standard deviation of 0.0075 cm and *P. halepensis* of 0.0023 cm (Table 1). In all the cases, intraspecific variability in response to AP was smaller than the previously reported in response to MMT.

Adaptive patterns in height-diameter allometry along climatic and geographic gradients

Overall, we found that tree height allometry variation was climatically and/or geographically adaptive, as evidenced by the following three findings.

First, we found a significant geographical association between the scaling exponent parameter (c_P) and the latitude of origin for two out the four pine species (*P. sylvestris* and *P. nigra*), but not for the other two, more xeric, species (*P. pinaster* and *P. halepensis*) (Table 1).

Second, gene pools significantly influenced the allometric curve's intercept values in *P. pinaster* and *P. nigra* ($p < 0.001$ and $p < 0.05$, respectively), but not in the two others (Table 2a). In addition, posterior mean values of α_{1p} were significantly correlated ($p < 0.05$) to different climatic variables of population's site of origin (Table 2b). Parameters α_{1p} were correlated for *P. pinaster* with spring precipitation ($\rho = 0.28$) and mean temperature of the warmest month ($\rho = -0.28$); for *P. sylvestris* with altitude ($\rho = 0.56$) and annual precipitation ($\rho = 0.54$) and negatively with minimum, maximum and mean monthly temperature from April to June (ranging from $\rho = -0.42$ to -0.63); and for *P. nigra* with mean minimum monthly

temperature (ranging from $\rho = 0.45$ to 0.60). *P. halepensis*, however, did not present any significant correlation.

Table 2 a) Summary of one-way ANOVAs to test gene pools effects on α^*_p. When a non-parametric test was used it is shown by the symbol ≈. **b)** Post-hoc comparisons among gene pools adjusted by Tukey's HSD for *P. nigra* and *P. pinaster*. Different letters indicate differences among gene pools.

a)

Species	Parameter	F / K	P-value
P. sylvestris	α_{1p}	0.60	n.s.
	α_{2p}	0.57	n.s.
	α_{3p}	0.57	n.s.
P. nigra	α_{1p}	6.95	**
	α_{2p}	7.20≈	n.s.
	α_{3p}	2.53≈	n.s.
P. pinaster	α_{1p}	12.43	***
	α_{2p}	14.23≈	n.s.
	α_{3p}	3.84≈	n.s.
P. halepensis	α_{1p}	1.07	n.s.
	α_{2p}	0.44	n.s.
	α_{3p}	0.08	n.s.

b)

	Gene pools	α_{1p}	std	
P. nigra	spp. *laricio*	4.15	0.04	a
	spp. *salzmannii*	4.12	0.01	ab
	spp. *dalmatica*	4.08	*	ab
	spp. *nigra*	4.06	0.02	b
P. pinaster	Morocco	3.11	0.00	a
	Atlantic Iberian	3.10	0.02	a
	Eastern Spain	3.06	0.03	ab
	Southern Spain	3.06	0.05	abc
	Corsica	3.05	0.00	abc
	Central Spain	3.02	0.03	bc
	Italy	2.95	*	cd
	Eastern North Africa	2.91	0.00	d

Third, posterior mean values of α_{2p} and α_{3p} were significantly correlated to climatic variables of population's sites of origin ($p < 0.05$). Chiefly, *P. pinaster*, *P. sylvestris* and *P. nigra* showed correlations to some either climatic or geographic variables with α_{2p}, but none in *P. halepensis* (Table 3), whereas only *P. sylvestris* presented significant and positive correlations α_{3p} with mean annual temperature (Table 3).

Table 3 Heat map for Pearson's correlation coefficients, ρ, between α'_p and climate variables from the populations' sites of origin. Dark grey indicates high positive correlation coefficients, light grey indicates high negative, and white color indicates low. Bold numbers means significant correlations at $p < 0.05$.

	P. sylvestris			P. nigra			P. pinaster			P. halepensis		
	$\alpha_{1\pi}$	$\alpha_{2\pi}$	$\alpha_{3\pi}$	$\alpha_{1\pi}$	$\alpha_{2\pi}$	$\alpha_{3\pi}$	$\alpha_{1\pi}$	$\alpha_{2\pi}$	$\alpha_{3\pi}$	$\alpha_{1\pi}$	$\alpha_{2\pi}$	$\alpha_{3\pi}$
Latitude	**-0.45**	0.14	0.19	-0.05	-0.24	-0.09	**-0.48**	0.03	-0.07	0.20	0.17	-0.04
Longitude	**-0.45**	-0.02	0.11	-0.29	0.22	0.37	0.00	0.09	0.08	0.03	-0.04	0.00
Altitude	**0.56**	-0.17	-0.29	0.06	-0.23	-0.31	0.16	**-0.33**	0.01	0.01	0.02	0.07
MMTJan	-0.03	0.29	0.23	**0.61**	**0.42**	0.16	-0.07	**0.27**	0.01	0.03	-0.05	-0.17
MMTFeb	0.00	0.31	0.20	**0.54**	0.34	0.13	-0.05	0.25	0.02	0.02	-0.06	-0.17
MMTMar	-0.21	0.36	0.32	**0.45**	0.31	0.17	-0.06	0.25	0.00	0.02	-0.06	-0.15
MMTApr	**-0.48**	0.37	0.38	0.34	0.22	0.14	-0.07	0.20	-0.01	0.06	-0.01	-0.16
MMTMay	**-0.53**	0.40	0.41	0.32	0.18	0.14	-0.13	0.18	0.00	0.06	-0.06	-0.18
MMTJun	**-0.52**	**0.44**	**0.44**	0.33	0.21	0.12	-0.16	0.13	-0.03	0.06	-0.07	-0.22
MMTJul	**-0.48**	**0.53**	**0.47**	**0.45**	0.23	0.10	-0.20	0.05	-0.04	0.06	-0.11	-0.22
MMTAug	**-0.43**	**0.56**	**0.46**	**0.48**	0.25	0.11	-0.22	0.04	-0.07	0.09	-0.06	-0.20
MMTSep	-0.38	**0.50**	0.39	**0.51**	0.35	0.17	-0.19	0.16	-0.04	0.06	-0.04	-0.16
MMTOct	-0.30	**0.46**	0.40	**0.55**	0.36	0.19	-0.16	0.21	-0.04	0.07	-0.01	-0.14
MMTNov	-0.32	0.42	0.37	**0.57**	0.35	0.18	-0.09	0.24	0.00	0.09	-0.01	-0.15
MMTDec	-0.17	0.30	0.26	**0.59**	0.38	0.18	-0.06	**0.27**	0.01	0.06	-0.04	-0.18
MWTJan	-0.02	**0.44**	0.33	0.33	0.29	0.07	0.10	0.16	-0.02	-0.07	-0.13	-0.14
MWTFeb	-0.17	**0.46**	0.40	0.17	0.21	0.07	0.01	0.14	-0.04	-0.15	-0.16	-0.13
MWTMar	**-0.44**	**0.53**	**0.51**	-0.05	0.10	0.08	-0.06	0.06	0.01	-0.25	-0.20	-0.08
MWTApr	**-0.59**	**0.43**	**0.48**	-0.17	-0.01	0.05	-0.13	0.04	-0.02	-0.16	-0.10	-0.05
MWTMay	**-0.63**	**0.47**	**0.46**	-0.23	-0.13	-0.05	-0.20	-0.09	0.02	-0.18	-0.12	-0.10
MWTJun	**-0.57**	**0.54**	**0.52**	-0.21	-0.15	-0.09	-0.15	-0.14	0.01	-0.25	-0.11	-0.04
MWTJul	-0.23	**0.48**	0.37	-0.15	-0.15	-0.13	-0.15	-0.21	0.03	-0.25	-0.10	0.02
MWTAug	-0.20	**0.52**	0.39	-0.11	-0.17	-0.14	-0.14	-0.15	0.01	-0.25	-0.08	0.02
MWTSep	-0.37	**0.53**	**0.43**	-0.05	-0.07	-0.06	-0.14	-0.06	0.01	-0.24	-0.16	-0.06
MWTOct	-0.33	**0.55**	**0.50**	0.04	0.04	0.01	-0.15	0.11	0.00	-0.17	-0.08	-0.09
MWTNov	-0.16	**0.50**	0.42	0.28	0.16	0.05	-0.05	0.20	0.00	-0.06	-0.05	-0.11
MWTDec	-0.09	**0.47**	0.40	0.39	0.30	0.11	0.06	0.23	-0.03	-0.01	-0.13	-0.17
MTJan	-0.02	0.41	0.31	**0.50**	0.38	0.13	0.01	0.23	-0.01	-0.02	-0.09	-0.17
MTFeb	-0.10	**0.43**	0.34	0.37	0.29	0.10	-0.03	0.21	-0.01	-0.06	-0.12	-0.17
MTMar	-0.37	**0.49**	**0.46**	0.18	0.21	0.13	-0.07	0.18	0.00	-0.11	-0.13	-0.15
MTApr	**-0.56**	0.42	**0.45**	0.06	0.10	0.09	-0.12	0.14	-0.02	-0.03	-0.05	-0.13
MTMay	**-0.61**	**0.45**	**0.45**	0.00	0.00	0.03	-0.20	0.06	0.01	-0.05	-0.10	-0.17
MTJun	**-0.57**	**0.51**	**0.50**	0.01	0.00	-0.01	-0.19	-0.02	0.00	-0.10	-0.11	-0.18
MTJul	-0.42	**0.59**	**0.50**	0.07	-0.01	-0.06	-0.21	-0.13	0.00	-0.15	-0.16	-0.16
MTAug	-0.35	**0.61**	**0.48**	0.13	-0.02	-0.06	-0.22	-0.08	-0.03	-0.11	-0.11	-0.15
MTSep	-0.41	**0.58**	**0.46**	0.22	0.12	0.05	-0.21	0.06	-0.02	-0.07	-0.11	-0.16
MTOct	-0.34	**0.56**	**0.50**	0.33	0.22	0.11	-0.18	0.18	-0.02	-0.02	-0.04	-0.13
MTNov	-0.26	**0.53**	**0.44**	**0.47**	0.28	0.12	-0.08	0.24	-0.01	0.03	-0.03	-0.14
MTDic	-0.14	**0.44**	0.37	**0.52**	0.36	0.15	0.00	0.26	-0.01	0.03	-0.08	-0.18
AP	**0.54**	-0.23	-0.20	-0.13	-0.17	-0.07	0.23	**0.40**	0.06	0.19	0.21	0.20
WintP	**0.45**	-0.33	-0.22	0.27	-0.20	-0.22	0.24	**0.40**	0.05	0.09	0.26	0.17
SpringP	**0.58**	-0.36	-0.31	-0.12	-0.17	-0.06	**0.28**	**0.35**	0.06	0.09	0.18	0.25
SumP	-0.17	0.00	0.11	**-0.52**	-0.04	0.17	0.18	0.07	0.03	0.12	0.01	0.08
AutP	**0.52**	-0.27	-0.14	0.16	0.04	-0.08	0.21	**0.34**	0.05	0.24	0.08	0.02
MAT	-0.39	**0.54**	**0.47**	0.25	0.17	0.07	-0.14	0.13	-0.01	-0.04	-0.11	-0.17
WT	**-0.43**	**0.57**	**0.47**	0.08	-0.02	-0.06	**-0.28**	-0.03	-0.01	-0.09	-0.15	-0.17
MWT	-0.21	**0.45**	0.35	-0.15	-0.16	-0.13	-0.14	-0.19	0.04	-0.22	-0.13	0.00
MT	0.00	0.40	0.30	**0.48**	0.37	0.12	-0.03	**0.32**	-0.02	-0.01	-0.12	-0.17
MMT	-0.01	0.30	0.24	**0.59**	0.41	0.15	-0.07	**0.27**	0.01	0.03	-0.07	-0.18

MMT# is the mean minimum temperature of the month #; MWT# is the mean maximum temperature of the month #; MT# is the mean temperature of the month #; WintP is total winter precipitation; SpringP is total spring precipitation; SumP is the total summer precipitation; AutP is total autumn precipitation; AP is the annual precipitation; MAT is the mean annual temperature, WT is the mean temperature of the warmest month, MWT is the mean maximum temperature of the warmest month, MT is the mean annual temperature, MMT is the mean minimum temperature of the coldest month.

Phenotypic plasticity in height-diameter allometry along climatic gradients

Based on the predictions along the gradients of mean minimum temperature of coldest month (MMT) or annual precipitation (AP) at a fixed *dbh* (100 mm), the four species showed some degree of plasticity and phenotypic variation (Figure 2 and 3). For the MMT gradient, *P. pinaster*, *P. halepensis* and *P. nigra* tended to be taller for a given diameter under warmer conditions, whereas *P. sylvestris* showed the opposite trend (Figure 2). We observed a greater degree of plasticity in *P. pinaster* and *P. halepensis* compared to *P. sylvestris* and *P. nigra* (also indicated by the index of plasticity employed). Finally, *P. pinaster* presented the highest intraspecific variation among the four.
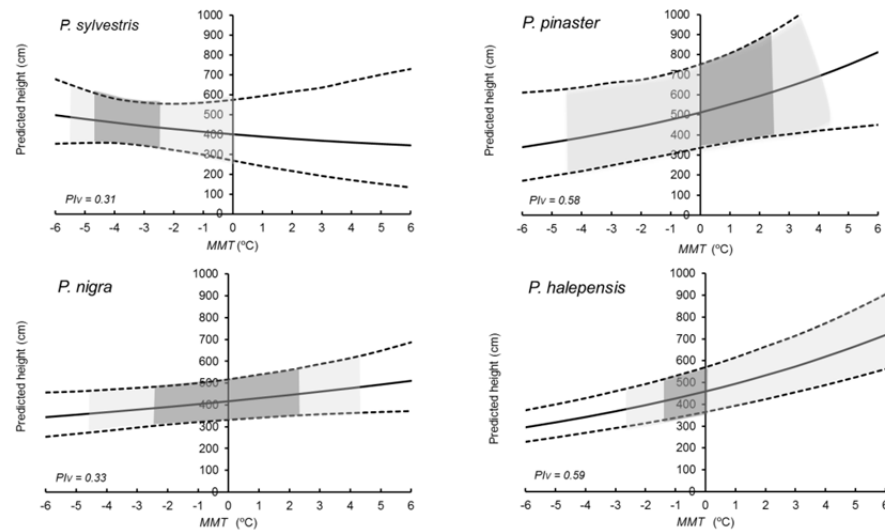
Figure 2 Predicted height (cm) at a given dbh = 100 mm along a gradient of mean minimum temperatures of the coldest month (MMT) for the four pine species studied. Black lines represent mean response and dotted lines represent 95% credible interval. The light grey area represents the MMT covering the distributional range of the species, and the dark grey area represents the range of MMT for the growing sites. At the bottom left corner, phenotypic plasticity index PIv, is shown.

Pinus nigra and *P. pinaster* had negligible plasticity in height along the annual precipitation gradient, as also revealed by the plasticity index (Figure 3). However, the other two species presented some degree of plasticity in height along this gradient. *P. sylvestris* tended to be taller under wetter conditions, while *P. halepensis* tended to be shorter. This last species presented the greatest plasticity in height, i.e., the steepest slope (Figure 3) and the largest value in the plasticity indices, PI$_v$. *Pinus pinaster* showed the highest intraspecific variation among the four.
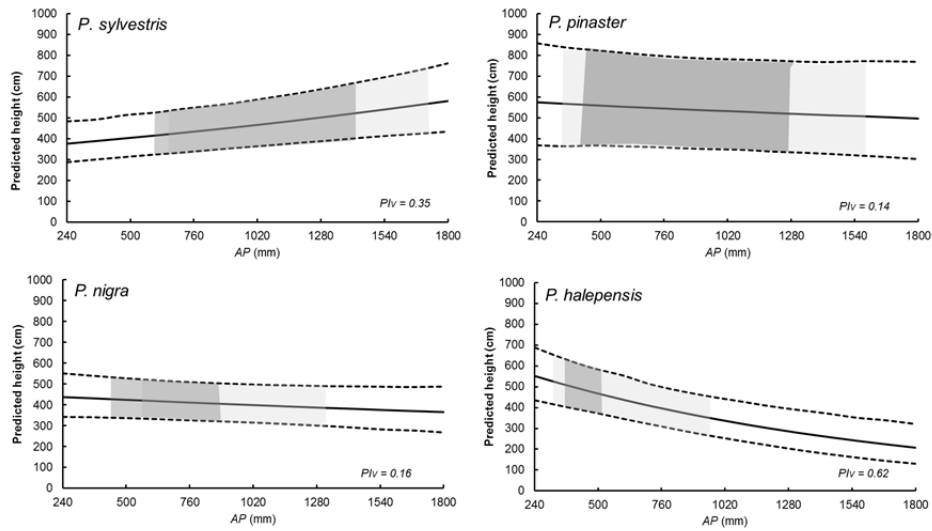
Figure 3 Predicted height (cm) at a given dbh = 100 mm along a gradient of mean annual precipitation (AP) for the four pine species studied. Black lines represent mean response and dotted lines represent 95% credible intervals. The light grey area represents the AP covering the distributional range of the species, and the dark grey area represents the range of AP for the growing sites. At the bottom right corner, phenotypic plasticity index, PIv, is shown.

Discussion

We assessed and quantified the relationship between abiotic drivers and the height-diameter allometric relationship of four widely planted pine species. We evaluated this variation at two scales, species and population. Our hierarchical approach, accounting for population variation within each species, allowed us to better understand the trade-offs in height-diameter variation in pines, and to better assess the contributions of the species' standing genetic variation, their demographic genetic background and their phenotypic plasticity, adaptive or non-adaptive, in response to

environmental variability. We show that intraspecific variation is essential in determining the adaptation and plastic responses to environmental conditions and that some species responses to climate are adaptive either to present climate or to past conditions (approached by analyses based on standing gene pools, which reflect the demographic history of the species).

Allometric patterns across species and intraspecific variation

The species-level parameters related to allometry (μ_r and β_r), for most comparisons did not overlap among species, reflecting the existence of evolutionary species-specific allocation strategies to cope with the current environment. *P. sylvestris* showed the opposite pattern in how climatic variable affected aboveground allometry than the other three species, performing better at colder temperatures and wetter conditions. This is a temperate-boreal species, which tends to be shorter (for a given diameter) in warmer or arid conditions. This suggests a distinct allocation strategy based on a greater responsiveness to aridity. Thus, a seeming separation is found between more xeric pines (*P. halepensis* and *P. pinaster*) and the temperate-boreal species (*P. sylvestris*). *Pinus nigra*, despite its association with mountainous areas, had an intermediate behavior showing more conservative responses i.e., less responsive to a given range of conditions.

Resource allocation patterns between height and diameter have been previously related to local climate (e.g. Wang *et al.* 2006; Lines *et al.* 2012). In European pine species, resource allocation patterns were more responsive to changes in the mean minimum temperature of the coldest month, MMT, than to changes in annual precipitation, AP. This might seem a somehow surprising result, given the fact that forests are strongly constrained in these

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ecosystems by water availability (Andreu *et al.* 2007; Martínez-Vilalta *et al.* 2008). However, European pine species in the southern range of their distributions are well adapted to water shortage and display drought avoidance mechanisms (Zuccarini *et al.* 2014). For instance, they might exhibit specific wood anatomy characteristics such us thick cell walls, thick pit membrane, narrow lumens or different root hydraulic resistance (Tyree & Zimmermann 2002; Zuccarini *et al.* 2014). Thus, it is likely that they may have developed less adaptive mechanisms to cope with cold stress, but have more plastic responses to short-term fluctuations in temperature.

There was some variability among populations in their responsiveness to both temperature and precipitation (see Figure S2, S3 and S4). *P. sylvestris* was the species with the greatest degree of inter-population genetic variation, but was closely followed by *P. pinaster*, whilst *P. halepensis* presented none. Both *P. sylvestris* and *P. pinaster* presented widespread and connected distributions in the past, in contrast with their current situation, in which populations are mainly isolated, or fragmented, as a result of changes related to glacial events in the Quaternary (Cheddadi *et al.* 2006). Populations might have developed under distinct natural pressures, probably the result of recent adaptation via locally adapted phenotypic variation, in agreement with previous phenotypic and molecular studies (Alía *et al.* 2001b; Robledo-Arnuncio *et al.* 2005). By contrast, *P. halepensis*' null degree of genetic variation agrees with the fact that the western populations of these species are genetically uniform, due to a relatively recent long-range colonization from its ancestral range in the eastern Mediterranean Basin (Grivet *et al.* 2009).

Adaptive patterns in height-diameter allometry along climatic and geographic gradients

Overall, tree height allometry variation was adaptive to climate and geography, in some cases expressing the past of a species (*P. nigra* and *P. pinaster)*, as gene pools reflecting the demographic history were associated with different allometric relationships.

In agreement with previous studies along latitudinal gradients the more mountainous species, *P. sylvestris* and *P. nigra*, presented a clear latitudinal variation in their parameters (Chuine & Cour 1999; Körner 2003; Alberto *et al.* 2013). The lack of latitudinal clines in *P. halepensis* and *P. pinaster* could be related to either insufficient span in our data or to a real lack of latitudinal variability. Interestingly, this is the first time that adaptive patterns have been shown for a composite trait such as tree height allometry. Our results confirm that this trait and its confined variation are under natural selection control and consequentially play an important role in both the adaptation and acclimation potential of tree species to future conditions.

Phenotypic plasticity in height-diameter allometry along climatic gradients

Phenotypic plasticity along the temperature and rainfall gradients, measured as the degree of variation of the predicted height at a given *dbh* of a species, *sensu stricto* of an average population, varies among species. Along the temperature (MMT) gradient, the four species presented the ability to respond to a varying environment in energy-stimulus. Under warmer conditions, the more xeric pine species, but also *P. nigra*, are expected to increase their heights, as previously shown (e.g. Lopatin 2007). Warmer conditions during the cold season might allow a higher photosynthetic

capacity, resulting in a higher rate of carbon assimilation (Way & Oren 2010). However, *P. sylvestris* is expected to display a shorter height under warmer conditions. A similar result was reported by Reich & Oleksyn (2008) in a large study spanning latitudes from 46° to 68° N. They observed that *P. sylvestris* responses to climate differed between northern and southern populations: while in southern populations height decreased as temperature increased, the opposite was observed in northern populations. It was suggested that, at least for this temperate-boreal species, warmer temperatures might rather enhance heat stress and heat-induced moisture stress than alleviate cold stress.

A varying environment in terms of rainfall resulted in species-dependent responses. Under drier conditions, *P. sylvestris* tended to be shorter, displaying height variation along the rainfall gradient and hence plasticity. This agrees with a well-documented pattern in many parts of the world, e.g. Méndez-Alonzo *et al.* (2008). This plasticity has often been attributed to the changing hydraulic structure of vessels in drought areas. In contrast, *P. halepensis* showed a height increase under arid conditions, while *P. pinaster* and *P. nigra* displayed a minor degree of plasticity in terms of rainfall. These three species have efficient water-use strategies. Previous studies (Tyree & Zimmermann 2002; García Esteban *et al.* 2009) have shown that both *P. nigra* and *P. halepensis*' specific wood anatomy characteristics (e.g. thick cell walls, thick pit membrane, narrow lumens) make them well adapted to droughts. Still, the degree of intraspecific variation could also be playing a key role: for instance, *P. pinaster* showed large intraspecific variation that might compensate lack of plasticity.

In conclusion, these four species of pines are a heterogeneous group with a recognized ability to adapt to extremely variable environments. Our findings enhance the eco-evolutionary knowledge we already have about them, but still reveal that height-diameter allometry patterns have developed under different natural selection pressures, despite sharing a sizeable part of their distribution area in the studied region. This might have resulted in species, like *P. halepensis*, where phenotypic plasticity is more important than genetic variation; while for others, e.g. *P. pinaster*, genetic variation and local adaptation might be more relevant. Altogether, past and present environmental circumstances have outlined the likely possible outcomes of integrated phenotypes. In fact, the full potential of forest resilience and resistance along new temperature and aridity gradients, i.e. climate-change driven, would depend on the populations' adaptation and levels of phenotypic plasticity.

Acknowledgments

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Considering an individual i, from population p growing in growing site s, its height-diameter allometry was modelled as:

Likelihood: $height_i \sim \log \text{Normal}\,(H_i,\, \sigma^2)$

and process model: $H_i = \ln\left(a_{p(i),s(i)}\right) + c_{p(i)} \times dbh_i$ [Eq. 1]

where, $\ln\left(a_{p(i),s(i)}\right)$ the scaling coefficient was estimated as:

$\ln\left(a_{p(i),s(i)}\right) = \alpha_{1p} + \alpha_{2p} \times \text{MMT}_s + \alpha_{3p} \times \text{AP}_s$ [Eq. 2]

Because all explanatory variables were standardized, parameter α_{1p} was the allometric curve's intercept at average climate conditions of across all growing sites. Differences among α_{1p} represent intraspecific genetic differences among populations. The parameters α_{2p} and α_{3p} represent, respectively, the specific height-diameter allometric responsiveness of each population p to temperature (MMT) and rainfall (AP)at growing site s, i.e. the reaction norms. The existence of significant intraspecific differences in α_{2p} and α_{3p} indicates genetic differences in plasticity among populations that can be either adaptive or non-adaptive. We formally tested significant intraspecific differences in α_{*p} for each species by computing all pairwise combinations of population differences. Two populations were significantly different if zero was not included in the confidence interval around their difference. Additionally, we quantified the level of inter-population genetic variation as the percentage of the total number of significant pairwise comparisons relative to the total number of pairwise comparisons within species. Range of variability among populations within species was computed as the standard deviation of α_{*p}.

Table S2 Summary of the different allometric and linking functions tested for each species. DIC criterion and ΔDIC ($DIC_i - DIC_{min}$) provides objective measures of the strength of the empirical support for the competing models. The model with the lowest DIC is highlighted in bold letters for each species.

Species	Allometric function	Linking function	DIC	Δ DIC
P. sylvestris	Linear	Normal	52430	2630
	Power	Normal	53270	3470
	Gompertz	Normal	60590	10790
	Linear	**Log Normal**	**49800**	**0**
	Power	Log Normal	52360	2560
	Gompertz	Log Normal	58940	9140
P. nigra	Linear	Normal	27540	1180
	Power	Normal	28190	1830
	Gompertz	Log Normal	26590	230
	Linear	Log Normal	30950	4590
	Power	**Log Normal**	**26360**	**0**
	Gompertz	Normal	35460	9100
P. pinaster	Linear	Normal	119800	300
	Power	Normal	119700	200
	Gompertz	Normal	124200	4700
	Linear	Log Normal	119700	200
	Power	**Log Normal**	**119500**	**0**
	Gompertz	Log Normal	130700	11200
P. halepensis	Lineal	Normal	15350	230
	Power	Normal	15430	310
	Gompertz	Normal	19050	3930
	Linear	Log Normal	15240	120
	Power	Log Normal	15230	110
	Gompertz	**Log Normal**	**15120**	**0**

Table S3 Heat map for Pearson's correlation coefficients, ρ, between *height* and *dbh* together with the geographic and climatic variables from the growing sites for the four species (dark grey indicates high positive correlation coefficients, light grey indicates high negative, and white color indicates low). The selected variables are shown in bold letters.

	P. sylvestris		*P. nigra*		*P. pinaster*		*P. halepensis*	
	dbh	height	dbh	height	dbh	height	dbh	height
Longitude	0.58	0.57	-0.65	-0.62	-0.28	-0.35	0.39	0.46
Latitude	0.12	0.19	0.15	0.07	0.21	0.25	-0.40	-0.48
Altitude	0.25	0.18	-0.35	-0.32	-0.37	-0.47	-0.69	-0.73
MMTJan	-0.61	-0.62	0.45	0.45	0.32	0.40	0.73	0.80
MMTFeb	-0.63	-0.66	0.48	0.48	0.24	0.31	0.73	0.80
MMTMar	-0.67	-0.69	0.53	0.53	0.19	0.25	0.73	0.80
MMTApr	-0.70	-0.71	0.40	0.41	0.33	0.42	0.73	0.80
MMTMay	-0.39	-0.42	0.46	0.48	0.21	0.28	0.58	0.66
MMTJun	-0.04	-0.06	0.48	0.50	0.10	0.15	0.48	0.56
MMTJul	-0.05	-0.11	0.56	0.58	-0.12	-0.12	0.69	0.76
MMTAug	-0.13	-0.20	0.53	0.56	-0.06	-0.05	0.57	0.65
MMTSep	-0.54	-0.60	0.57	0.58	-0.03	-0.01	0.66	0.74
MMTOct	-0.68	-0.70	0.56	0.55	0.10	0.14	0.72	0.79
MMTNov	-0.68	-0.70	0.53	0.53	0.12	0.17	0.72	0.79
MMTDec	-0.61	-0.62	0.40	0.39	0.31	0.39	0.73	0.80
MWTJan	-0.31	-0.33	0.32	0.37	0.40	0.51	0.55	0.63
MWTFeb	-0.50	-0.51	0.33	0.37	0.36	0.46	0.61	0.69
MWTMar	-0.64	-0.64	0.33	0.37	0.29	0.38	0.22	0.29
MWTApr	-0.68	-0.68	0.17	0.22	0.35	0.45	-0.51	-0.58
MWTMay	-0.62	-0.63	0.01	0.07	0.32	0.40	-0.67	-0.75
MWTJun	-0.49	-0.52	0.12	0.18	0.16	0.22	-0.72	-0.79
MWTJul	-0.49	-0.54	0.07	0.14	0.05	0.08	-0.69	-0.76
MWTAug	-0.39	-0.44	-0.05	0.02	0.06	0.10	-0.73	-0.79
MWTSep	-0.45	-0.49	-0.08	-0.01	0.13	0.18	-0.73	-0.80
MWTOct	-0.46	-0.48	0.11	0.17	0.27	0.35	-0.25	-0.23
MWTNov	-0.27	-0.28	0.27	0.32	0.37	0.47	0.59	0.67
MWTDec	-0.26	-0.29	0.22	0.26	0.47	0.59	0.67	0.75
MTJan	-0.47	-0.49	0.42	0.44	0.41	0.52	0.69	0.77
MTFeb	-0.61	-0.63	0.42	0.44	0.34	0.44	0.72	0.79
MTMar	-0.69	-0.69	0.47	0.49	0.26	0.34	0.73	0.80
MTApr	-0.69	-0.69	0.26	0.31	0.35	0.44	0.67	0.71
MTMay	-0.60	-0.62	0.21	0.27	0.30	0.39	-0.56	-0.57
MTJun	-0.35	-0.38	0.31	0.36	0.15	0.20	-0.72	-0.78
MTJul	-0.34	-0.39	0.33	0.38	-0.01	0.01	-0.68	-0.76
MTAug	-0.30	-0.36	0.22	0.28	0.00	0.02	-0.71	-0.77
MTSep	-0.50	-0.55	0.23	0.29	0.07	0.11	-0.60	-0.62
MTOct	-0.57	-0.59	0.39	0.43	0.21	0.28	0.66	0.74
MTNov	-0.51	-0.53	0.45	0.47	0.28	0.36	0.69	0.77
MTDic	-0.43	-0.45	0.32	0.34	0.45	0.56	0.72	0.79
AP	0.65	0.70	0.42	0.37	0.28	0.34	0.67	0.71
WintP	0.53	0.59	0.52	0.47	0.29	0.36	0.27	0.25
SpringP	0.77	0.79	0.24	0.19	0.35	0.43	0.58	0.60
SumP	0.62	0.66	-0.46	-0.49	0.02	0.00	-0.05	-0.01
AutP	0.59	0.65	0.44	0.39	0.31	0.38	0.73	0.78
MAT	-0.57	-0.60	0.47	0.50	0.13	0.19	0.68	0.76
WT	-0.24	-0.30	0.36	0.41	-0.10	-0.11	-0.71	-0.79
MWT	-0.42	-0.47	0.13	0.19	-0.06	-0.05	-0.70	-0.77
MT	-0.47	-0.49	0.49	0.50	0.37	0.47	0.70	0.77
MMT	-0.61	-0.62	0.50	0.50	0.32	0.40	0.73	0.80

MMT# is the mean minimum temperature of the month #; MWT# is the mean maximum temperature of the month #; MT# is the mean temperature of the month #; WintP is total winter precipitation; SpringP is total spring precipitation; SumP is the total summer precipitation; AutP is total autumn precipitation; AP is the annual precipitation; MAT is the mean annual temperature, WT is the mean temperature of the warmest month, MWT is the mean maximum temperature of the warmest month, MT is the mean temperature of the coldest month, and MMT is the mean minimum temperature of the coldest month.
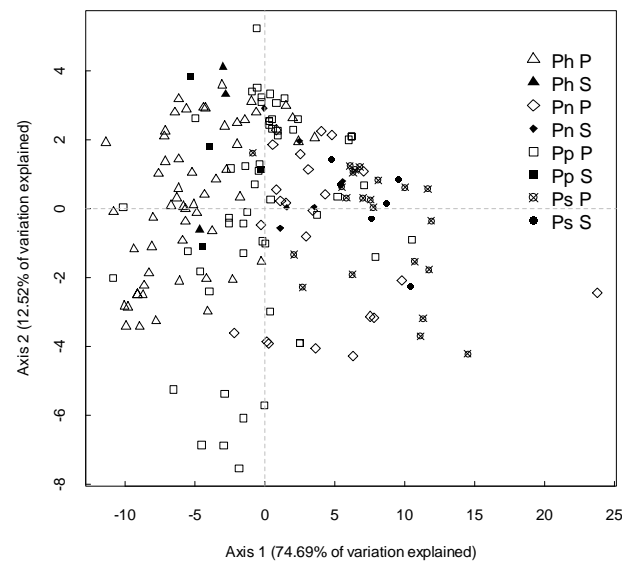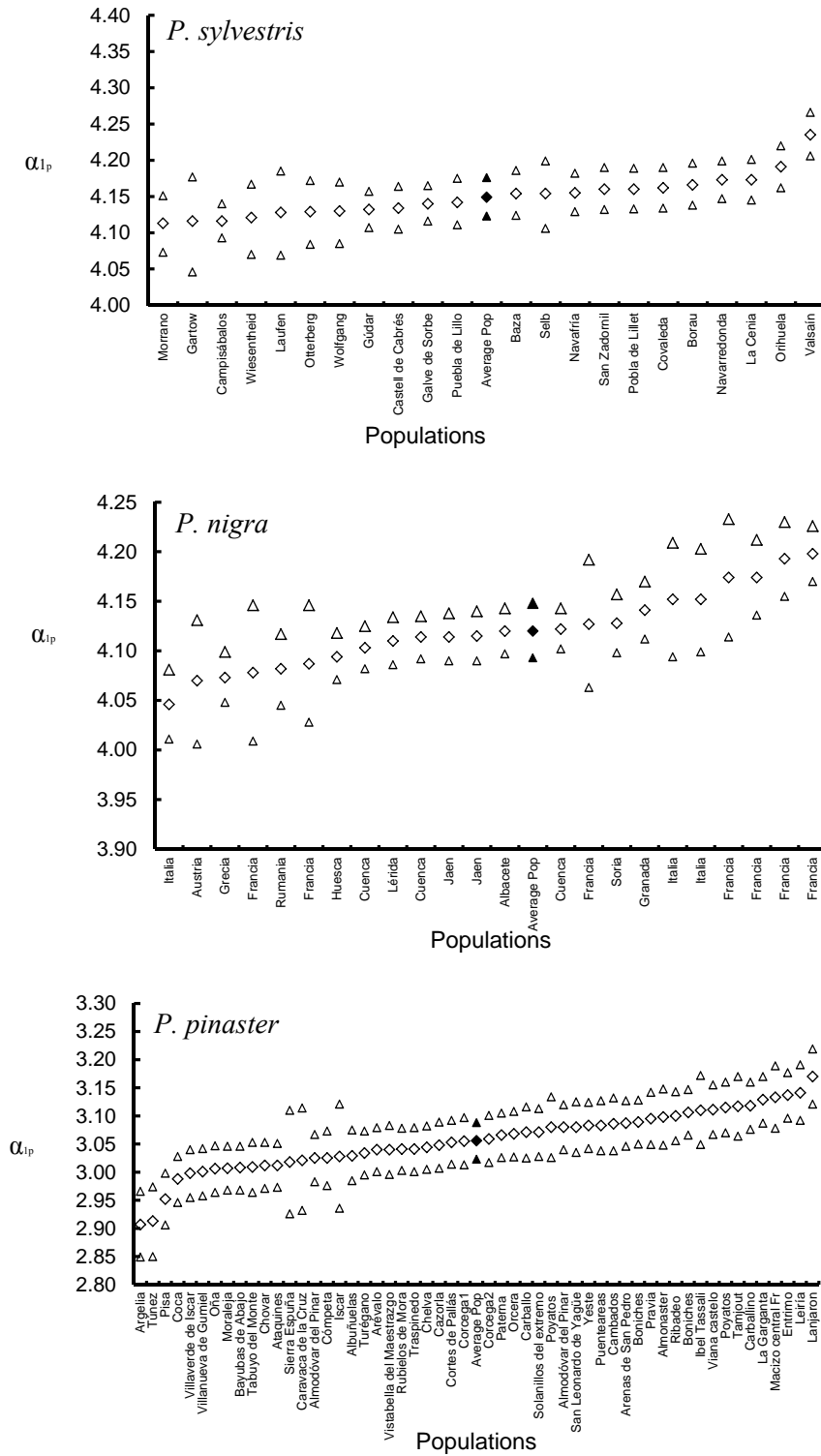

Figure S1 Principal Component Analysis (PCA) of pine populations (P) and growing sites (S) based on their climatic variables (see Material and Methods) of the following four pine species: *Pinus halepensis* (Ph), *P. nigra* (Pn), *P. pinaster* (Pp) and *P. sylvestris* (Ps).
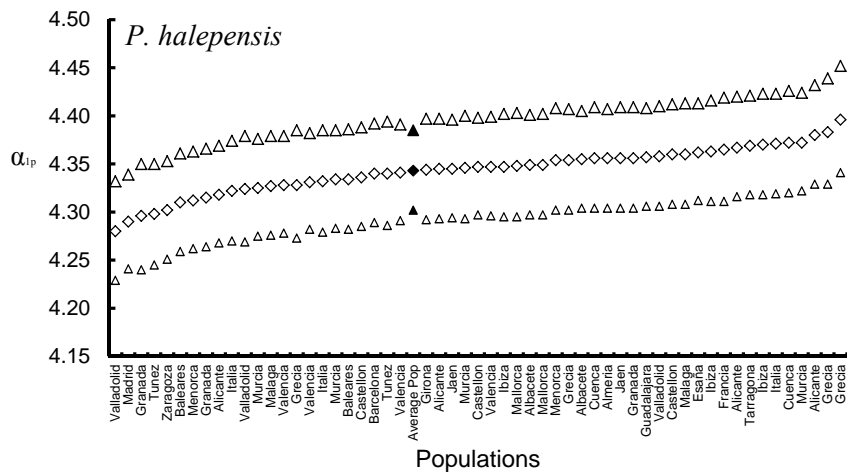
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P. sylvestris

α_{1p}

Populations: Morrano, Gartow, Campisábalos, Wiesentheid, Laufen, Otterberg, Wolfgang, Gúdar, Castell de Cabrés, Galve de Sorbe, Puebla de Lillo, Average Pop, Baza, Selb, Navafría, San Zadornil, Pobla de Lillet, Covaleda, Borau, Navarredonda, La Cenia, Orihuela, Valsaín

P. nigra

α_{1p}

Populations: Italia, Austria, Grecia, Francia, Rumania, Francia, Huesca, Cuenca, Lérida, Cuenca, Jaen, Jaen, Albacete, Average Pop, Cuenca, Francia, Soria, Granada, Italia, Italia, Francia, Francia, Francia, Francia

P. pinaster

α_{1p}

Populations: Argelia, Túnez, Pisa, Coca, Villaverde de Iscar, Villanueva de Gumiel, Oña, Moraleja, Bayubas de Abajo, Tabuyo del Monte, Chovar, Ataquines, Sierra Espuña, Caravaca de la Cruz, Almodóvar del Pinar, Cómpeta, Iscar, Albuñuelas, Turégano, Arévalo, Vistabella del Maestrazgo, Rubielos de Mora, Traspinedo, Chelva, Cazorla, Cortes de Pallás, Corcega1, Average Pop, Corcega2, Paterna, Orcera, Carballo, Solanillos del extremo, Poyatos, Almodóvar del Pinar, San Leonardo de Yagüe, Yeste, Puenteareas, Cambados, Arenas de San Pedro, Boniches, Pravia, Almonaster, Ribadeo, Boniches, Ibel Tassaili, Viana castelo, Poyatos, Tamjout, Carballino, La Garganta, Macizo central Fr, Entrimo, Leiria, Lanjaron

Populations

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Figure S2 The figure shows α_{1p} values (represented by diamonds) for the four pine species studied, and their 95% credible intervals (represented by triangles). X-axis indicates the population name. The hyperparameter, μ_1, is represented with a black diamond.
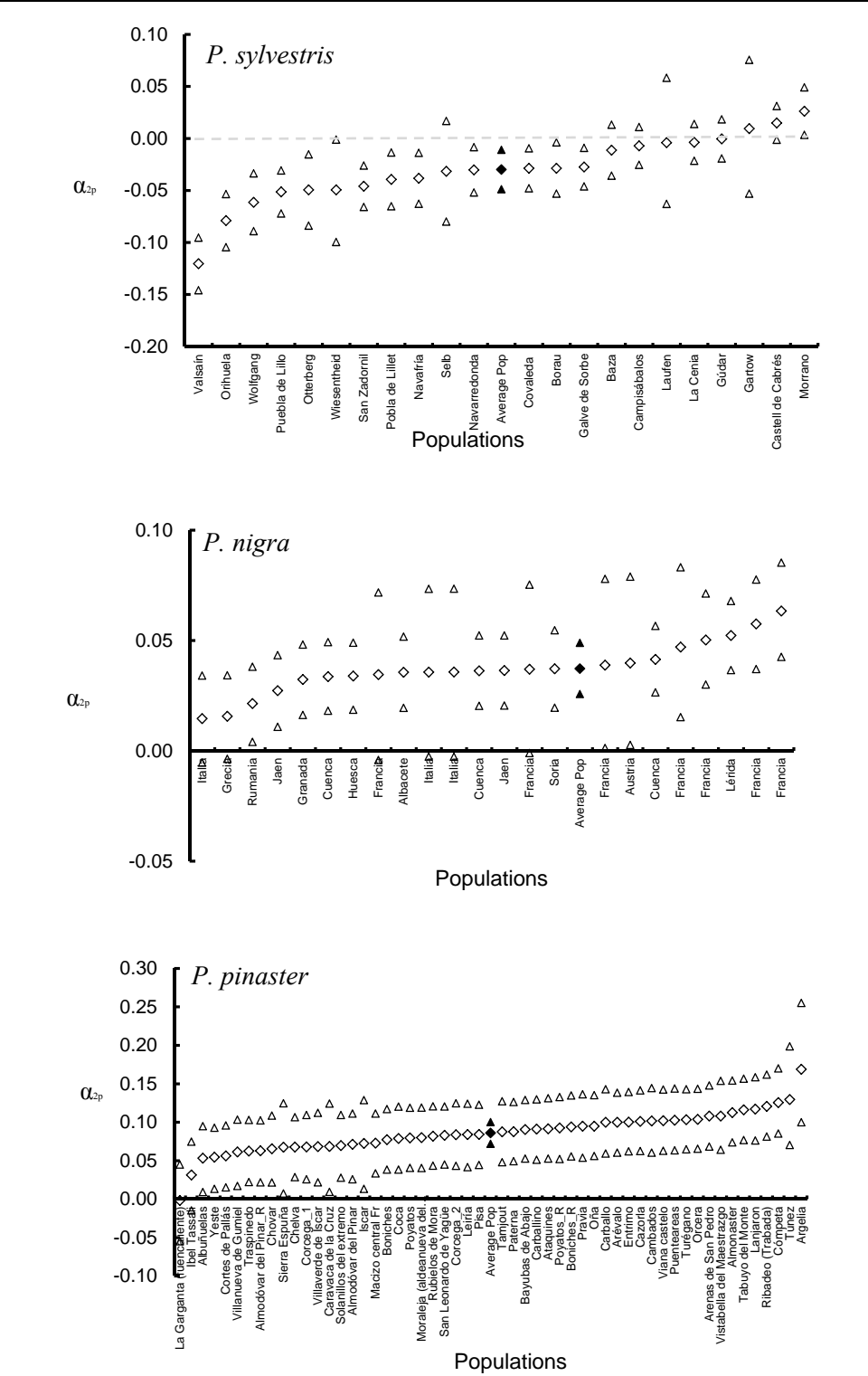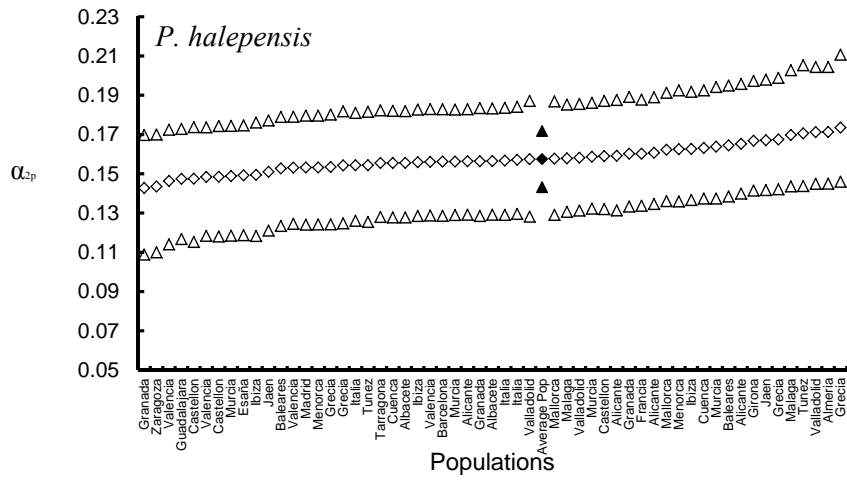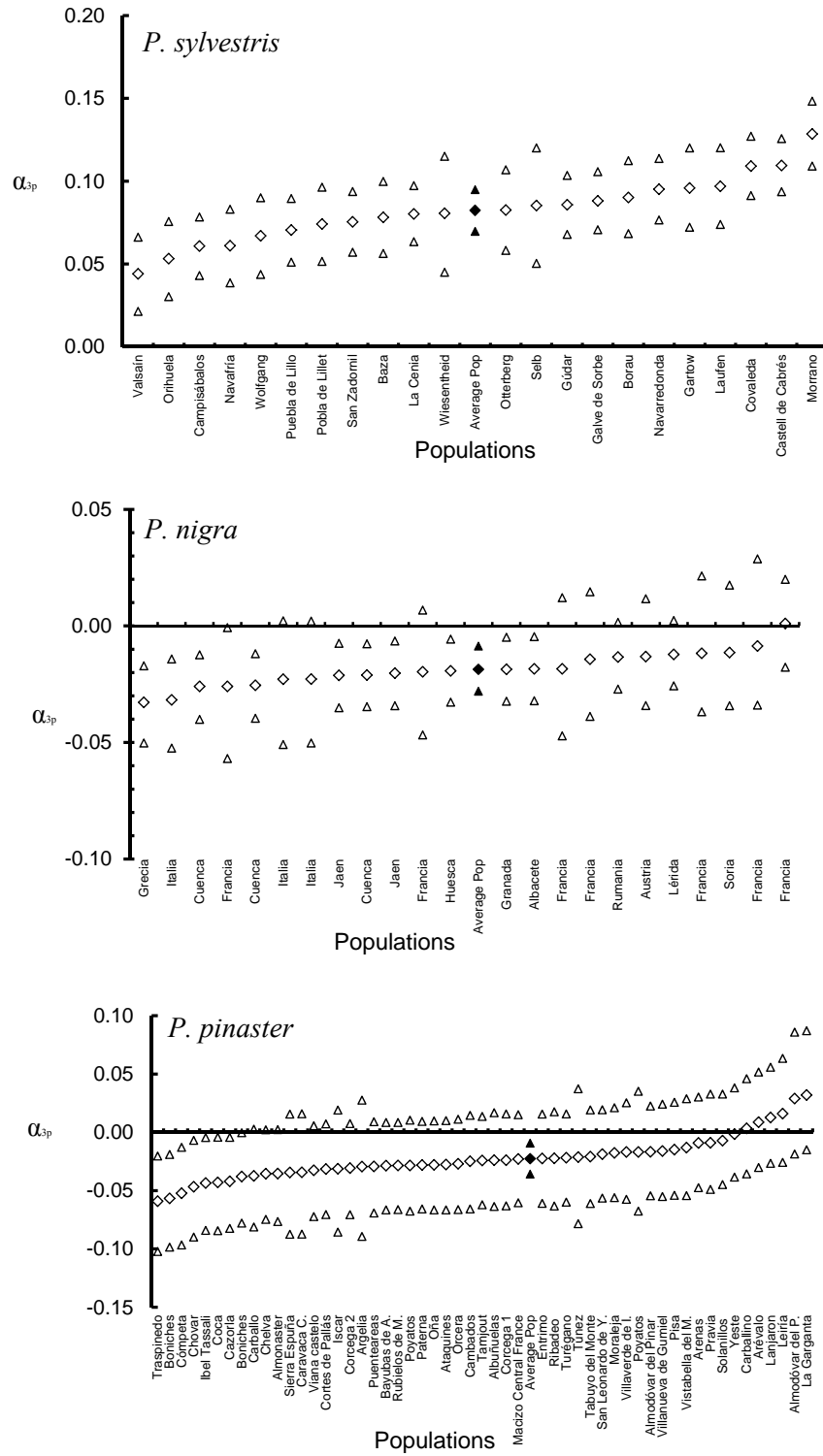
Figure S3 The figure shows α_{2p} values (represented by diamonds) for the four pine species studied, and their 95% credible intervals (represented by triangles). X-axis indicates the population name. The hyperparameter, μ_2, is represented with a black diamond.
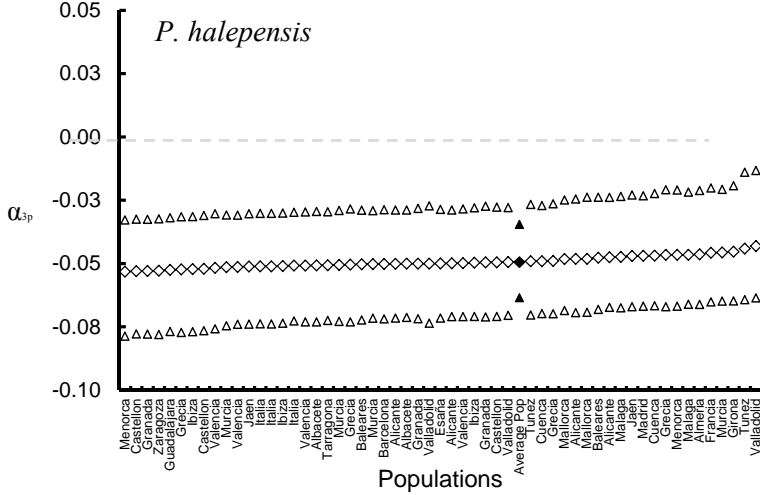
P. sylvestris

P. nigra

P. pinaster

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Figure S4 The figure shows α_{3p} values (represented by diamonds) for the four pine species studied, and their 95% credible intervals (represented by triangles). X-axis indicates the population name. The hyperparameter, μ_3, is represented with a black diamond.

Chapter 6

Climate and population origin shape pine tree height-diameter allometry: implications for forest management

This chapter is based upon the following manuscript:

Vizcaíno-Palomar, N., Ibáñez, I., Benito-Garzón, M., González-Martínez, S.C., Zavala, M.A., Alía. R. Climate and population origin shape pine tree height-diameter allometry: implications for forest management (Under review in New Forests)

Resumen

En las especies de árboles forestales, la relación alométrica entre la altura total y el diámetro a la altura del pecho refleja la respuesta evolutiva de los patrones de asignación de recursos entre la parte aérea y la subterránea. Como resultado, estos patrones difieren entre y dentro de las especies debido a procesos de adaptación local y plasticidad fenotípica. Estas variaciones en la relación alométrica altura-diámetro determinan la productividad, la resistencia y la resiliencia de los árboles a las variaciones en el clima, y por tanto, nos pueden indicar del éxito del material vegetal empleado en proyectos de restauración. En este estudio evaluamos el efecto del cambio climático y el origen de la población en la alometría altura-diámetro para cuatro especies de pinos testados a lo largo de un amplio gradiente climático en el sur de Europa occidental. Basándonos en modelos alométricos específicos, evaluamos los cambios en la alometría bajo las condiciones climáticas actuales y futuras (año 2050, RCP 4.5) en una muestra representativa de poblaciones en cada especie. Nuestros resultados mostraron que *Pinus halepensis* y *P. pinaster* fueron las especies más plásticas, mientras que *P. sylvestris* y *P. nigra* manifestaron cambios de menor entidad o insignificantes. Además, nuestros resultados indicaron que tanto el sitio de ensayo como el origen de la población explican una fracción significativa de la variación en la alometría altura-diámetro. Nuestros modelos pronostican que la alometría –altura-diámetro– cambiará bajo condiciones ambientales futuras, y que las nuevas condiciones ambientales se traducirán, en general, en un aumento de la variación fenotípica dentro de las especies. Para algunas de las especies, esto podría permitir la selección de fenotipos mejor adaptados a las nuevas condiciones climáticas. Estos cambios previsibles en la alometría

altura-diámetro del árbol (entre especies y dentro de la misma especie) podrían conllevar efectos eco-evolutivos sobre la dinámica del bosque. Por lo tanto, los planes forestales de restauración y reforestación deberían considerar estos posibles cambios ya que podrían interferir con los objetivos perseguidos –ambientales y/o productivos–.

Palabras clave: Cambio climático, plantaciones forestales, variación intraespecífica, resiliencia, variación fenotípica, plasticidad, restauración.

Abstract

Tree height-diameter allometry, the link between tree height and trunk diameter, reflects the evolutionary response of a particular species' allocation patterns to above and belowground resources. As a result, it differs among and within species due to both local adaptation and plasticity. These variations in tree height-diameter allometry determine tree productivity, resistance and resilience to climate variation, and hence, the success of plant material used in restoration projects. In this study, we test the effect of climate change and population origin on tree height-diameter allometry in four pine species planted along a wide climatic range in South-western Europe. Tree height-diameter allometry changes were assessed for a representative sample of populations of each species under present and future climatic conditions (year 2050, RCP 4.5), and based upon species specific height-diameter allometric models. We found that *Pinus halepensis* and *P. pinaster* were the most plastic species, while *P. sylvestris* and *P. nigra* showed negligible variation. We also found that both testing site and population origin explained a significant fraction of variation in tree height-diameter allometry. Our models forecasted that pine tree height-diameter allometry will change under future environmental conditions and that new environmental conditions will result, in general, in an increase of phenotypic variation within species. For some of the species, this might allow the selection of phenotypes better suited to novel climatic conditions. These foreseeable changes in tree height-diameter allometry (among and within-species) could entail eco-evolutionary effects on forest dynamics. Therefore, restoration and reforestation plans should consider these effects as they may interfere with production and/or environmental goals.

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Keywords: Climate change, forest plantations, intraspecific variation, resilience, phenotypic variation, plasticity, restoration.

Introduction

Understanding how species and populations will perform under future climatic conditions has become essential for sustainable forest management. Forests provide a variety of societal benefits, from wood products to non-timber goods and services, e.g. primary production, soil protection, regulation of local and regional climate, watershed management, carbon sequestration (Costanza *et al.* 1997; Millennium Ecosystem Assessment 2005; Bullock *et al.* 2011). Under impending climate change (IPCC 2007, 2014), restoration efforts are oriented to ensure forest resilience and ecosystem stability (Clewell, Aronson & Winterhalder 2004; FAO 2010), but also to curb the effects of rapid climate change. Seeking for easily measurable traits but of crucial relevance to understand the performance of keystone tree species is of utmost importance, as it might aid with silvicultural management practices.

Tree height-diameter allometry is an integrated trait composed by two easily and commonly measurable traits, tree height and trunk diameter. The growth of one of these traits confines the growth of the other: tree height reflects a strategy for securing carbon profit via light capture (Moles *et al.* 2009), and stem diameter is closely related to mechanical support and water-absorbing capacity (McMahon 1973; Niklas 1993; Bullock 2000). Linked together, tree height and trunk diameter represent species' growth allocation strategies in response to above and belowground resources (Hallé, Oldeman & Tomlinson 1978), which in turn are modified by biotic –e.g. competition– and abiotic –e.g. light and precipitation– components (López-Serrano *et al.* 2005; Lines *et al.* 2012), and can be associated with tree resistance and resilience. Investment in above and belowground biomass is

tightly connected to the climatic conditions experienced by a tree species, but also to past eco-genetic background, which in turn may have resulted in remarkable intraspecific variation due to local adaptation processes (Vizcaíno-Palomar *et al. under review*).

   Although uncertainty at short temporal and spatial scales remains (Kao & Ganguly 2011), climatic projections point at an increase in temperature and climatic variability, with extreme events becoming more frequent (IPCC 2014). According to that, we expect that changes in environmental conditions and resource availability would lead to shifts in plant allocation strategies and hence in tree height-diameter allometry. For example, under increasingly stressful conditions (i.e. higher evapotranspiration and lower precipitation), trees would mainly allocate more resources to belowground growth (larger diameters) than to height growth (shorter trees) (e.g. Lines *et al.* 2012). These changes could then impact life-history traits, with important consequences on population and community dynamics. We could expect, for example, a delay or advance in trees reaching sexual maturity (Munguía-Rosas *et al.* 2011). Also, biomechanical features of tree architecture could be altered, and trees could be forced to either grow closer to their critical buckling height or to develop lesser density wood (Niklas 1994; Ryan & Yoder 1997; Lines *et al.* 2012). These effects are likely to have an impact on forestry productivity, thus efficient management practices will need to be revisited. For example, we could find a mismatch between actual net primary productivity estimations and stand productivity indexes (Kearsley *et al.* 2013), or alterations in the projected quality and volume of timber production, or even modifications in the desirable rotation length of forest plantations.

In the Mediterranean region, pine species occupy extensive natural areas where they tend to dominate under harsh conditions or in early successional stages. The persistence of these populations is critical for maintenance of key ecosystem services in this region. Altogether, these features have promoted extensive afforestation programs using different pine species (Montero 1997, FAO 2006). Pine species are a heterogeneous group that exhibit contrasting demographic responses along environmental gradients (Gómez-Aparicio *et al.* 2011; Ruiz-Benito *et al.* 2013). Moreover, pines display high levels of variation among populations in phenotypic traits with a likely adaptive value, such as height and diameter (Guyon & Kremer 1982; Danjon 1994; Alía, Moro & Denis 1997; Chambel, Climent & Alía 2007; Aranda *et al.* 2009).

This study assesses the effects of climate change and population of origin on tree height-diameter allometry among four important pine species present along a wide climatic range in the Iberian Peninsula (South-western Europe): *Pinus halepensis* Miller, *P. pinaster* Aiton, *P. nigra* Arnold, and *P. sylvestris* L. We build our analyses upon the modelling approach we developed taking advantage from a series of multi-site provenance tests installed in the Iberian Peninsula (Vizcaíno-Palomar *et al.*, under review). Basically, our models describe the tree height-diameter allometry relationships as a function of climate at the growing site (to account for among testing site variation) and geographic variables of the population origin (to account for among population variation). In this study, we further develop our analyses to assess the role of plasticity and population genetic effects on the responses of particular phenotypes to future climatic conditions. Specifically, we assess (i) the species-specific changes of

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phenotypic variation in tree height-diameter allometry under future climates, (ii) the inter- and intra- specific phenotypic plasticity in tree height-diameter allometry in response to future climates; and finally, we discuss (iii) the implications of plastic allometric changes for species' management, ecology and assessment of potential risks. To achieve these objectives, we simulate and project tree height-diameter allometry of a representative sample of populations under present and future climatic conditions expected by 2050 accordingly to the IPCC AR5 in RCP 4.5 (2013).

Materials and methods

Tree species

We focused on four widespread pine species in Europe commonly used in restoration and afforestation practices: *Pinus halepensis, P. pinaster, P. nigra* and *P. sylvestris*. *Pinus halepensis* is distributed all around coastal regions of the Mediterranean basin, exceptionally inland in Spain, Tunisia and Italy. *Pinus pinaster* occurs naturally from southwestern Europe to north-western Africa, showing several adaptations to local conditions. *Pinus nigra* is often associated with Mediterranean mountain areas. Finally, *P. sylvestris* displays the largest tree distribution across the Eurasian region, and reaches its southern limit in southwestern Europe where it is predominantly confined to mountainous areas.

Plant material and testing sites

For this study, we selected a subset of populations for each species (7 populations for *P. halepensis* and *P. sylvestris;* and 6 for *P. pinaster* and *P. nigra*) that cover their ecological range in the Iberian Peninsula (Figure 1, Table S1). The selection of populations was based on a principal component analysis (PCA in R Core Team, 2012) of all the populations (155 for all the species) included in a series of multi-site provenance tests (Figure S1) (Vizcaíno-Palomar *et al.*, under review). PCA was based on altitude, seasonal and annual precipitation, mean annual temperature, mean and maximum temperature from the warmest month, and mean and minimum temperature from the coldest month. These populations were planted at three sites – testing sites– for the case of *P. halepensis*; four sites for *P. pinaster*; and six sites for *P. nigra* and *P. sylvestris* cases. Sites were distributed along South-western Europe (Figure 1, and Table S2).
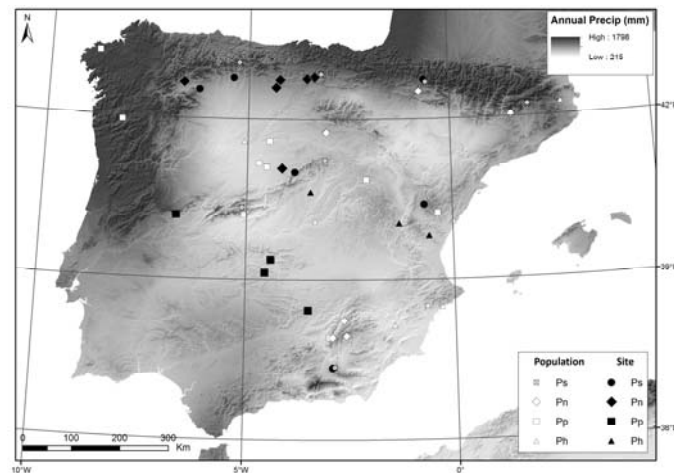

Figure 1 Populations (open symbols) and sites –testing sites– (filled symbols) selected for the study. The figure shows information about annual precipitation and topographic relief.

Phenotypic data estimates

For this study, we generated phenotypic data estimates of population tree height at a fixed dbh (100 mm; ~11 years-old trees) for each representative population under present climatic conditions of the testing sites and under those conditions expected for 2050 by the RCP 4.5 (see '*Climate data*' section). These estimates of height are interchangeable with the concept of aboveground allometry data as they represent heights but all at the same fixed size of diameter.

These phenotypic data estimates were based upon parameters estimated in Vizcaíno-Palomar *et al.,* (under review). The model was used to describe tree height-diameter allometric relationships for the four pine species. These previous analyses used the same multi-site common garden network, but a larger number of populations (56 populations in 3 sites for *P. halepensis*; 54 populations in 4 sites for *P. pinaster*; 23 populations in 8 sites for *P. nigra*; and 22 populations in 6 sites for *P. sylvestris*) (Figure S1). The best tree height-diameter allometric model followed a power function with a log-normal link function, as follows:

$$height_i = a_{p(i),s(i)} \times dbh_i^{c_{p(i)}} \qquad \text{[Eq. 1]}$$

where $height_i$ is the total height of an individual tree i (cm) and dbh_i is the diameter measured at breast height of the same tree (mm). The scaling coefficient a represents intra-specific genetic differences among populations in their responsiveness to the testing sites s, characterized by two environmental variables (MMT and AP) –this is a genetic plastic component–; and population differences, p –this is a pure genetic component–. The scaling exponent c is defined by two geographical

variables (altitude and latitude) of the population origin –pure genetic component–, p, nested within species (see Appendix 2 for a detailed description of the model). Estimates of a and c parameters from Vizcaíno-Palomar *et al.* (under review) were used to get phenotypic data estimates for the selected sample of populations (see '*Plant material*' section), at a fixed dbh of 100 mm, for present climatic conditions and those expected for 2050 by the RCP 4.5 (see '*Climate data*' section).

Following the procedure described above, we obtained 246 phenotypic data estimates of height at 100 mm dbh, i.e. measures of aboveground allometry. In general, averages and standard deviation –both measurements in cm– of height estimates for each species and climatic conditions were the following: *P. halepensis* (present: 482.94 ± 50.88; future: 591.49 ± 42.77), *P. pinaster* (present: 545.59 ± 48.17, future: 673.70 ± 100.74), *P. nigra* (present: 398.32 ± 18.24, future: 429.99 ± 24.69), and *P. sylvestris* (present: 436.65 ± 43.27, future: 390.60 ± 24.73).

Climate data

The values for present climatic conditions (MMT and AP) were extracted from Gonzalo-Jiménez (2008) climatic model with a 1-km^2 spatial resolution. Future climate conditions (year 2050) at the same resolution (Table S2) were averaged from 10 global circulation models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC5, MIROC-ESM-CHEM, MIROC-ESM, MRI-CGCM3, NorESM1-M) available in WorldClim (Hijmans *et al.* 2005). We used the representative concentration pathway (RCP) 4.5. RCPs are greenhouse gas concentration –GHG- (not emissions) trajectories adopted by the IPCC in the last Assessment Report (AR5) (IPCC 2013). RCP

4.5 is an intermediate scenario that forecasts an average increase of temperatures of 1.4°C ranging between 0.9ºC and 2.01ºC by 2050.

Data analysis

Linear mixed-effect model to analyse phenotypic data estimates among species

Based on the phenotypic data estimates of tree height at a dbh of 100 mm, we used linear mixed-effect models to test for the effect of species, *Sp*, climate, *C*, testing sites, *s*, and populations, *p*, on tree heights estimates (i.e. aboveground allometry). Species and climate, as well as the species by climate interaction, $Sp \times C$, were entered as fixed factors. Testing sites, *s,* and populations nested within testing sites, *p(s),* were considered random effects to: (i) get unbiased estimates for the fixed variables controlling the variability associated with testing sites and populations, and (ii) to partition the phenotypic variation in different components of variance, specifically that associated to testing sites (u_s), and to populations nested to testing sites ($u_{p(s)}$) (Gelman & Hill 2007). We built the best-supported mixed model structure following Zuur *et al.* (2009) and Bolker *et al.* (2009); and comparisons of alternative models by means of AIC criteria (Akaike 1992), an indicator of the parsimony and the likelihood of the model. We obtained estimates of the parameters by minimizing the likelihood of the residuals from the fixed-effect portions of the model (Zuur *et al.* 2009). We used normal error distribution with an identity link. The *lme* function of the *nlme* package (Pinheiro *et al.* 2013) in R version 3.0.3 (R Core Team 2012) was employed. Posthoc pairwise comparisons, adjusted by Tukey HSD, were calculated with the *lsmeans* function of *lsmeans* package (Lenth 2014).

Based on the best supported mixed-effect model, we analysed the species-specific phenotypic variation (1) and phenotypic plasticity (2).

(1) *Species-specific phenotypic variation allometry in response to climate change*

We calculated the standard deviation of the fitted values of each population within species referred to each climatic condition (present *vs* future). The ratio between the two values (*sd$_{pre}$* and *sd$_{fut}$*, respectively) was used to assess the change in phenotypic variation with climate for each species. A ratio greater than one ($\phi_{PVar} = \dfrac{sd_{fut}}{sd_{pre}} > 1$) indicates a greater range of potential phenotypes of the species under the future climate, and $\phi_{PVar} < 1$ would indicate a reduction in future phenotypic variation. We can assume that these ratios estimates of change in phenotypic variation represent well each of the four pine species studied, as we have selected a representative sample of populations covering the distribution range of each species.

(2) *Species-specific phenotypic plasticity in allometry in response to climate change*

This was calculated as the difference between future and present tree fitted values divided by present fitted values. This is a dimensionless and provides comparable measurements among species. We statistically assessed whether this value was different from zero running the calculation with a sample size of 1,000 using total tree height and SD estimates values.

Linear fixed-effect model to analyse phenotypic data estimates at the species level

At the species level, we aimed to test three different aspects of plasticity: (i) at the spatial scale, i.e. plastic responses of populations among testing sites, s effect; (ii) at the temporal scale, i.e. plastic responses of populations between present and future conditions, C effect; and (iii) genetic differences in plasticity at either the spatial or temporal scales, $s \times p$ and $C \times p$, respectively. To do so, we analysed each species separately. We based on the phenotypic data estimates of tree height at a dbh of 100mm (see Phenotypic data estimates), and used linear fixed-effect models to test the effects of climate, C, testing sites, s, populations, p, and the interaction terms of $C \times p$, $s \times p$ on tree height estimates.

Results

Linear mixed-effect model to analyse phenotypic data estimates among species

We constructed twelve competing mixed-effect models to find out the best-supported structure for the height data estimates (Table 1). The best mixed-effect model included the two fixed factors, species (Sp) and climate (C) and its interaction term $(Sp \times C)$, with the random part accounting for variation among testing sites and populations nested within sites, $u_1 = u_s$ and $u_2 = u_{p(s)}$, respectively; and on climate effect as well, $u_3 = u_{C/s}$ and $u_4 = u_{C/p(s)}$. This model accomplished the requirements of unbiased predictions and normal distribution of residuals (Figure S2).

The fixed part of the model revealed that species, climate and its interaction term were statistically significant, pointing to specific species strategies to accommodate tree height-diameter allometry in response to climate variability (Table 2). Specifically, the model predicts height-diameter allometric changes in the near future for the two pine species (*P. halepensis* and *P. pinaster*) currently growing in more xeric climates (Figure 2; Table S3a). By 2050, these two pines are expected to exhibit significantly (*P*-value < 1×10^{-4}) taller trees at a given diameter size, whereas *P. nigra* is expected to maintain the same height (*P*-value = 0.44). *P. sylvestris* would display lower heights at a given diameter, though this difference was only marginally significant (*P*-value = 0.06).
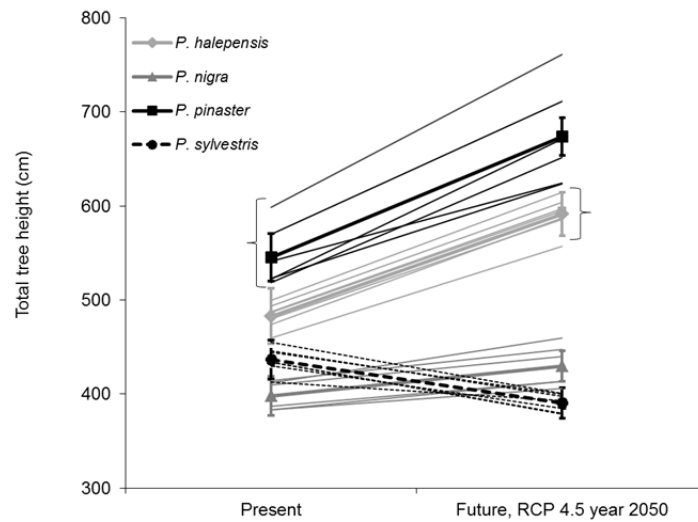

Figure 2 Tree height change at species-population levels (thick and thin lines, respectively) is shown by connecting tree height estimates at present and future climates (RCP 4.5 2050). Black wrenches are examples to indicate phenotypic variation within a species at present (*P. pinaster*) and at future climatic conditions (*P. halepensis*). Each pine species is represented by a different shade of grey. Species and populations responses are estimates from the best mixed-effect model (see Table S1). Vertical bars indicate standard error at the species level.

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The random components of the model, u_{1-4}, held significant intraspecific phenotypic variation on the tree height-diameter allometry. This variation was partitioned among testing sites, s, $\sigma_{u_1} = 49.23$ cm and variation among testing sites in their response to climate $\sigma_{u3} = 37.03$ cm. Identically, for populations within testing sites, $p(s)$, $\sigma_{u_2} = 28.26$ cm and $\sigma_{u4} = 13.21$ cm. Interestingly, the amount of intraspecific variation explained –or retained– by testing sites was greater than that accounted by populations nested into testing sites.

Table 1 Comparisons of alternate models to analyze tree height estimates (cm) at a fixed dbh (100 mm) differences among species using Akaike Information Criterion (AIC). The final model is highlighted in bold letters.

Model	Fixef	Random	df	AIC
M1	Full	-	9	2555.20
M2	Full	$1\mid s$	10	2389.94
M3	Full	$1\mid p$	10	2547.92
M4	Full	$1\mid s/p$	11	2386.00
M5	Full	$1+ C \mid s$	12	2550.49
M6	Full	$1+ C \mid p$	12	2346.09
M7	Full	$1+ C \mid s/p$	15	2230.01
M8	Null	$1+ C \mid s/p$	8	2330.14
M9	*Sp*	$1+ C \mid s/p$	11	2317.87
M10	*C*	$1+ C \mid s/p$	9	2327.65
M11	*Sp* + *C*	$1+ C \mid s/p$	12	2315.37
M12	***Sp* × *C***	***1+ C \mid s/p***	**15**	**2289.96**

Sp = Species; *p* = Population*; s* = Testing site; *C* = Climatic condition; **Full** = Full model: Species × Climate; **Null** = Null model¸ **df** = number of estimated parameters.

The final model is constructed by comparing alternative models using AIC criteria. Firstly, the random part of the model is adjusted while the fixed part, Full model, is held. Afterwards, the fixed part is adjusted using the appropriate random part previously selected. Fixed effects were tested using Maximum Likelihood of the parameter (ML), and random effects using restricted maximum-likelihood method (REML) (Zuur *et al.* 2009)(Zuur *et al.* 2009).

Table 2 ANOVA of the best linear mixed-effect model (M12, see Table 1 for further information), including fixed variables and its interaction term.

Fixed effects	df	*F*-value	*P*-value
Species	3	15.94	<0.001
Climate	1	20.84	<0.001
Species × Climate	3	21.13	<0.001

Species-specific phenotypic variation and phenotypic plasticity of aboveground allometry in response to climate change

In general, the set of phenotypic variation associated with the tree height-diameter allometry increased by 2050, under the RCP 4.5 scenarios, in all species (Figure 2). Specifically, the ratio of phenotypic variation, ϕ_{PVar}, was 1.64 in *P. pinaster*, i.e. 64% times larger in the future than under present climate conditions, 1.49 in *P. halepensis*, 1.54 in *P. nigra* and 1.15 in *P. sylvestris*.

Moreover, *Pinus halepensis* and *P. pinaster* would display the greatest response to new climates (Figure 3), i.e. they would presumably be the most plastic species among the four studied, with an average phenotypic plasticity of 0.23 ± 0.09 and 0.23 ± 0.06, respectively. They were followed by *P. nigra* with 0.08 ± 0.07 and *P. sylvestris* with a negative value of -0.11 ± 0.06, although changes for these two species would not be statistically significant (*P*-value = 0.06 and *P*-value = 0.44, respectively) (Figure 2). Interestingly, *P. sylvestris* would be expected to allocate less resources to total tree height (negative value in the phenotypic plasticity estimate), whereas the opposite was found in the other three pine species.
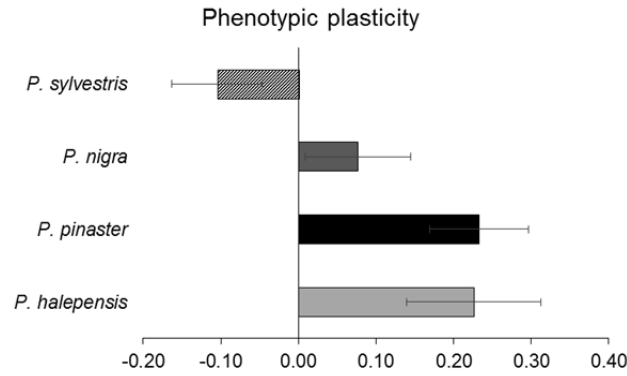
Figure 3 Phenotypic plasticity measurement estimates at species levels based on the best-mixed model estimations (see Table S1). We quantified the phenotypic plasticity as the percentage of change in tree height (the difference between future and present tree height was divided by its present total height, making measurements comparable among species and dimensionless). This value was calculated after running 1,000 simulations using the estimated mean and SD values for height. Vertical bars in black indicate standard error.

Linear fixed-effect model to analyse phenotypic data estimates at the species level

In general, we found spatial and temporal plastic responses displayed by the four species, and also genetic differences among populations, except in *P. sylvestris* in which all populations tested displayed similar heights (Table 3).

The relative contribution of each factor to the total variation in tree height data estimates varied among the four species. For instance, a great part of variation in *P. halepensis* was explained almost by solely plastic responses (ca. 85%), while ca. 40% of intraspecific phenotypic variation in *P. nigra* would be explained by among population variation (Table 3). Moreover, we did not find genetic differences for plasticity in any of the four species (i.e. $p \times C$ and $p \times s$ interactions were not statistically significant) (Table 3).

Table 3 Summary of linear fixed-effect model for each species analysed independently. Degrees of freedom, d.f., F- test value, F-value, and probability, *P-value,* are provided in the table. Also, the percentage of variation of tree height data estimates explained by each factor in the model is given (% explained).

Species		*p*	*s*	*C*	*p* × *s*	*p* × *C*	Resid.
P. halepensis	d.f.	6	2	1	12	6	14
	F-value	9.79	269.52	821.39	0.19	0.31	
	P-value	***	***	***	n.s.	n.s.	
	% explained	4.09	37.50	57.15	0.16	0.13	0.97
P. pinaster	d.f.	5	3	1	15	5	18
	F-value	5.83	23.88	82.67	0.20	0.77	
	P-value	**	***	***	n.s.	n.s.	
	% explained	14.00	34.40	39.69	1.42	1.84	8.64
P. nigra	d.f.	5	5	1	25	5	30
	F-value	42.36	18.28	188.90		2.32	
	P-value	***	***	***	.	n.s.	
	% explained	38.93	16.80	34.73	1.89	2.14	5.51
P. sylvestris	d.f.	6	5	1	6	30	35
	F-value	1.78	34.09	20.84	0.15	0.72	
	P-value	n.s.	***	***	n.s.	n.s.	
	% explained	4.73	46.00	29.93	1.98	1.91	15.45

p= Population; **s** = Testing site; **C** = Climatic condition; **Resid.** = residuals

Discussion

We assessed likely climate effects, mediated by their origin, on tree height-diameter allometry of four co-habiting pine species. In particular, we studied the allometric responses to predicted climates for 2050, averaging 10 global circulation models (RCP 4.5, IPCC 2013). Although the four species are

expected to increase phenotypic variability under future climates, only two out of the four pine species considered, *P. halepensis* and *P. pinaster,* would exhibit significant changes in tree height-diameter allometry. Moreover, none of these two species displayed genetic differences for plasticity, neither across sites nor among climatic scenarios.

Change in phenotypic variation under future climates

The four pine species are expected to increase their potential set of phenotypes, i.e. their phenotypic variation, under the expected climate conditions in 2050. These different sets of phenotypic variation would likely affect the adaptation potential of these populations under the conditions tested. As height and diameter traits exhibit moderate heritability, both separately and in combination (Costa & Durel 1996; Hannrup, Wilhelmsson & Danell 1998; Bailey *et al.* 2004), genetic changes can be expected under the new climatic conditions.

The random component of our best mixed-effect model provided complementary insights on the sources of phenotypic variation. The model emphasized that testing sites, i.e. variation among environments, contained greater phenotypic variation in tree height-diameter allometry compared to that comprised among populations within testing sites. This highlights the major role played by growing site environmental conditions (Valladares *et al.* 2002) in shaping interspecific tree height-diameter allometry patterns. However, substantial variation was still held among populations nested within testing sites. Despite their minor contribution –by the percentage of total variation explained– many studies have emphasized its relevance to assess the genetic material to be employed in study cases such as assisted

migration (e.g. Wang, O'Neill & Aitken 2010; Benito-Garzón & Fernández-Manjarres 2015; Chakraborty *et al.* 2015).

Plastic responses to future climates in tree height-diameter allometry

Plastic responses to new environments could reflect variability in the prioritization of distinct vital functions, such as light capture. In our study, we found that *P. halepensis* and *P. pinaster*, the two more xeric pines, would allocate more resources to height growth under novel climates by 2050, while the two more mountainous species would display none or negligible change in their allometry (*P. nigra* and *P. sylvestris,* respectively). These highly diverse plastic responses found among species, both in the degree of extent and direction, reflect also the species' distribution across environments in the Iberian Peninsula. In general, plastic responses have been reported for the four species in a wide variety of traits, such as diameter, height, reproduction, water use efficiency, etc. (e.g. Alía *et al.* 1997; Sánchez-Gómez *et al.* 2010b; Richter *et al.* 2012; Taeger *et al.* 2013a), but mainly at the seedlings stage, yet sapling and juveniles stage have received less attention (but see Santos-del-Blanco *et al.* 2010).

Interestingly, we found that under a drier and warmer climate (the one predicted for 2050 by the RCP 4.5), *P. halepensis* and *P. pinaster* trees would be taller at a given size. This result suggests that both species would benefit from longer growing seasons despite the expected harsher summer droughts, indicating that these species may be limited by winter temperature in their current ranges. Moreover, these results could add more evidence to support Santos-del-Blanco *et al.* (2012) and Santos-Del-Blanco *et al.* (2013)

findings. They found that *P. pinaster* and *P. halepensis* seem to be more limited by continental climates than by arid climates. Following these evidences, it could be conceivable that both species under future climates could still allocate resources to reach higher heights. However, contrary to that reasoning is found the optimal partitioning theory (Bloom, Chapin & Mooney 1985). Accordingly to this, we should expect plants shifting carbon allocation to the organs collecting the most limiting resource in respond to stressful environmental conditions, i.e. in the case of increased drought, to root biomass. Thereof, it is not clear what would be the effect in terms of fitness as a result of those predicted changes in the tree height-diameter allometry, and especially as in our study-case we have not analyzed other important traits, such as reproduction, bark size, serotiny, etc.

In contrast to the two xeric pines, *P. sylvestris* and *P. nigra* are expected to maintain a similar tree height-diameter allometry, which would not enhance risk of embolism under drier and more arid conditions. This lack of change in aboveground allocation could be partly explained by the fact that both pine species present a noteworthy root hydraulic regulation that allows them to avoid cold-induced embolism and secondary stress (Zuccarini *et al.* 2014). Also, mediterranean pines (*P. halepensis* and *P. pinaster*) are strongly polycyclic under favorable environments (Pardos *et al.* 2003; Heuret *et al.* 2006), and therefore with a more important plastic response in height growth.

Although phenotypic plasticity is understood as a strategy that favours species' survival (e.g. Benito-Garzón *et al.* 2011), lack of plasticity might be also beneficial when environmental change is not predictable, or when no phenotypic response can overcome the change (Ghalambor *et al.*

2007). Plasticity can even be potentially maladaptive, for example when facing unpredictable changes in stressful semi-arid environments (e.g. Valladares et al 2007) and heavily modified (i.e. highly plastic) phenotypes can be rendered more vulnerable to further environmental changes. We did found large amounts of plasticity in tree height-diameter allometry for different pine species, but its potential adaptive value under future climates remains unclear.

Population specific responses to future climates: the genetic component

The four species analysed present contrasting values of neutral diversity (Soto *et al.* 2010), emphasizing the importance of seed sources in afforestation programs. In fact, the three more mediterranean species (*P. halepensis, P. pinaster* and *P. nigra*) displayed those differences among populations. In contrast, *P. sylvestris* displayed a low level of genetic variation among populations. We could not detect genetic variation in plastic responses to either temporal or climate variability. Then, the ability to acclimate to new environments via adjustments of allocation to height and diameter seems to be similar within species.

Limitations

As is the case with any model predictions, our results might reflect, at least in part, biases derived from the approach adopted to fit the tree height-diameter allometry model. Our model provided a good fit for the four species studied, but there are important uncertainties in relation to potential future changes. For example, the particular climatic drivers that shape allometric

relationships can also change over time. Pine allometric relationships were highly affected by minimum winter temperatures (MMT, Vizcaíno-Palomar *et al.,* under review), but this effect may diminish in the future. More importantly allometry can change along ontogenetic stages (Poorter *et al.* 2015), along stand development stages due to density effects and competition (Stoll, Weiner & Schmid 1994), and along environmental gradients (Lines *et al.* 2012). Thus our predictions represent a limited array of scenarios.

Insights for forest management and restoration

First, our models revealed a major role of environmental conditions in shaping tree height-diameter allometry. However, we also found that genetic variability within species will also play a role shaping these responses, although the degree of impact varies among species. Specifically, we found that whenever a potential future change in allometry could be expected, the nature of this change could differ from one population to another in certain species. For instance, *P. halepensis* changes would be mainly through plastic responses, while in *P. nigra*, changes will be the result of plastic and genetic mediated responses. This result indicates the importance of accounting for source of material when selecting phenotypes in forest restoration plans to some specific sites, even when plasticity might be equal for species

Second, tree height and diameter at breast height are both among the most extensively used measurements at the nursery stage (together with diameter and length of roots), as well at the juvenile and adult stages to assess the suitability of plant material in restoration plans. Our study stresses the appropriateness of tackling both measures together to consider allocation trade-offs for selecting plant material. A single trait-based focus could lead to

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undesirable outcomes, for example, we could select taller trees to dominate over competing vegetation but then we would probably obtain, at the same time, more slender trees, less tolerant to drought. Thus, overlooking allocation trade-offs could end in large consequences at the forest restorations scale.

Third and finally, our results suggest that more xeric pines species would be at a higher risk than mountains pines of being affected by future climatic conditions due to the expected changes towards higher slenderness, which would compromise the hydraulic performance of future trees under a future scenario of longer droughts. To avoid this trend to slender trees, forest management decreasing stand density might offset the allocation resources for light capture and reduce the potential damage of drought.

Acknowledgments

has been revised by a professional scientific editor, P.C. Grant from Grant Language Services.

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Supplementary material

Table S1. Latitude and altitude of selected populations.

Species	Population	Province	Latitude	Altitude (m)
P. halepensis	Villajoyosa	Alicante	38º 29' 43.52" N	94
	Tibi	Alicante	38º 31' 7.68" N	929
	Benicasim	Castellón	40º 4' 37.4 " N	590
	Cabanellas	Girona	42º 14' 51.20" N	234
	Colmenar de Oreja	Madrid	40º 5' 24.62" N	734
	Ricote	Murcia	38º 8' 31.09" N	805
	Vega de Valdetronco	Valladolid	41º 35' 17.99" N	831
P. pinaster	Arenas de San Pedro	Ávila	40º 12' 31.22" N	642
	Vistabella del Maestrazgo	Castellón	40º 14' 46.79" N	1,306
	Solanillos del extremo	Guadalajara	40º 52' 32.38" N	1,216
	Cazorla	Jaén	37º 54' 49.70" N	986
	Carballo	La Coruña	43º 12' 8.60" N	110
	Moraleja	Segovia	41 º 6' 47.37" N	820
P. nigra	Huéscar	Granada	37º 56' 55.42" N	1,716
	Ena	Huesca	42º 31' 14.54" N	860
	Navahondona	Jaén	37º 54' 59.91" N	1,243
	Cazorla-Alcaraz	Jaén	38º 13' 59.99 "N	1,302
	Solsona	Lérida	42º 2' 33.40" N	719
	Soria	Soria	41º 45' 30.06" N	1,080
P. sylvestris	Navarredonda de Gredos	Ávila	40º 21' 16.13" N	1,550
	Pobla de Lillet	Barcelona	42º 13' 43.56" N	1,075
	San Zadornil	Burgos	42º 51' 8.74" N	975
	Baza	Granada	37º 22' 29.75"N	2,050
	Campinsábalos	Guadalajara	41º 14' 3.53" N	1,400
	Borau	Huesca	42º 41' 54.02" N	1,550
	Puebla de Lillo	León	43º 3' 33.12" N	1,550

Table S2 Climatic conditions of the planting sites for each species for Present and Future climate. MMT means mean minimum temperature of the coldest month in °C; and AP means annual precipitation in mm.

Species	Planting site	Province	Present climate		Future climate: RCP 4.5 2050	
			MMT	AP	MMT	AP
P. halepensis	Cucalón	Castellón	3.00	517	4.43	441.2
	Olmo	Madrid	0.00	475	2.22	416.6
	Ademuz	Valencia	-1.10	367	2.31	378
P. pinaster	Cabañeros	C. Real	0.00	772	0.77	461.3
	Peña Negrillas	C. Real	0.20	549	3.24	433.2
	Riofrío	C. Real	0.70	719	1.97	445
	Acebo	Cáceres	2.20	1,112	4.48,	623.6
P. nigra	Rucandio	Burgos	-0.60	692	2.45	732.3
	Trespaderne	Burgos	0.40	686	3.85	749
	Sancedo	León	0.80	871	1.64	699.7
	Herrera	Palencia	-1.50	538	1.42	601.7
	Rebolleda	Palencia	-2.10	705	0.73	695.9
	La Granja	Segovia	-0.60	460	0.34	474
P. sylvestris	Baza	Granada	-2.30	616	-2.37	676.4
	Aragüés	Huesca	-4.10	1,448	-2.6	1001.3
	Curueño	León	-2.80	835	-0.38	668.3
	Manzanal	León	-2.70	883	-1.12	771.7
	Navafría	Segovia	-4.20	954	-1.48	563.1
	Gúdar	Teruel	-4.40	677	-2.61	678.6

Table S3a Posthoc pairwise comparisons of lsmeans for height at 100 mm dbh adjusted by Tukey HSDtest for differences among present vs. future climatic conditions for the different species.

Species	P-value
P. halepensis	***
P. pinaster	***
P. nigra	0.44
P. sylvestris	0.06

P-value: * < 0.05; ** < 0.001; *** < 0.0001

Table S3b Posthoc pairwise comparisons of lsmeans for height at 100 mm dbh adjusted by Tukey HSDtest. *P values* are presented for the comparison of present *vs.* future values among species under Present and Future climatic conditions.

		P-value	
Comparison of:	To:	Present Climate	Future Climate-2050
P. halepensis	P. nigra	0.05	***
P. halepensis	P. pinaster	0.43	0.40
P. halepensis	P. sylvestris	0.72	***
P. pinaster	P. sylvestris	***	***
P. nigra	P. pinaster	***	***
P. nigra	P. sylvestris	0.70	0.88

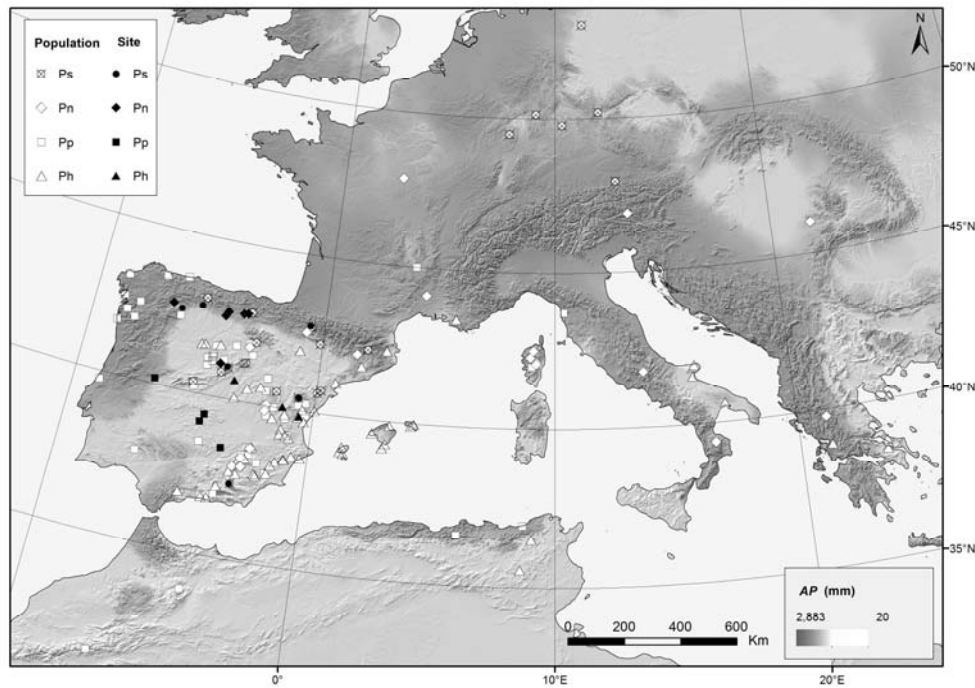P-value: * < 0.05; ** < 0.001; *** < 0.0001

Figure S1 Populations (open symbols) and testing sites (filled symbols) used to fit the tree height-diameter allometry model (Eq. 1) (Vizcaíno-Palomar et al. under revisiew). Each species is represented by a different symbol, square: Pp = *P. pinaster*, circle: Ps = *P. sylvestris*, rhomb: Pn = *P. nigra*, triangle: Ph = *P. halepensis*. The figure includes information about the annual precipitation and topographic relief.
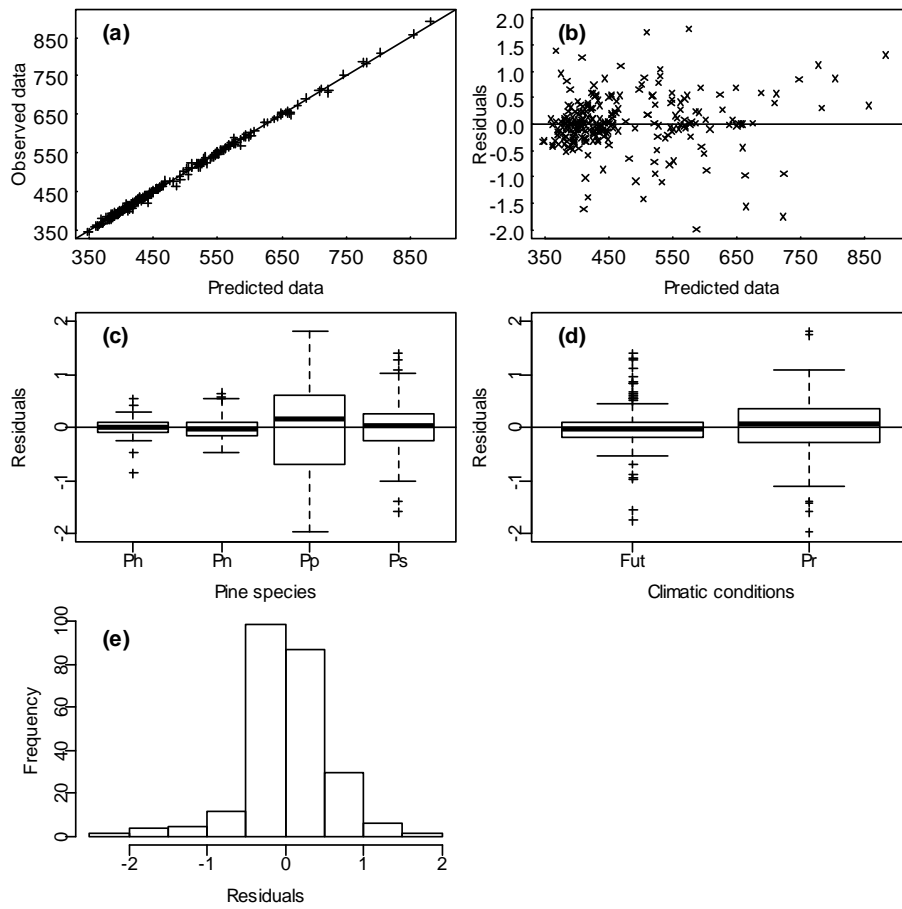
Figure S2 Exploratory plots from the best mixed-effect model for phenotypic variation: **a)** shows the predicted and observed raw data relationship; **b)** shows residuals data along the predicted data; **c)** and **d)** show boxplots of residuals distributed along pine species or climatic conditions, respectively; **e)** shows the histogram of residuals.

Appendix 1

Plant material

Seeds from different populations (i.e. from geographically and climatically heterogeneous areas) along the distribution range of the species, mostly representing the species' distribution in the Iberian Peninsula, were collected in seed lots from at least 25 mother trees with a 50-meter separation distance. Plants originating from the seed lots were established in comparative common garden tests for each species. We used a total of 4,853 *P. sylvestris* trees from 22 populations planted in 6 sites with 4 replicates and with a plot size of 16 trees; 9,976 *P. pinaster* trees from 54 populations planted in 4 sites with 4 replicates and plot size of 16 trees; 3,644 *P. nigra* trees from 23 populations planted in 8 sites with a varying number of replicates between 7 and 12 (depending on the planting site), and with a decreasing plot size with the number of replicates, specifically ranging between 4 and 8 trees; and 1,928 *P. halepensis* trees from 56 populations planted in 3 sites with 4 replicates and with a plot size of 4 trees. All the individuals used in the study were around 11 years old to avoid different responses in tree height-diameter allometry due to different ontogenetic stages (Sterck & Bongers 1998; Climent *et al.* 2011). Moreover, competition effects were neglected on the basis that in *P. pinaster*, the fastest growing species in the study, neither inter- nor intra-population competition was significant for any of the two variables at age 32 in the same common garden test (Alía *et al.* 2001a).

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Appendix 2

Tree height-diameter allometry model

Considering an individual *i*, from population *p* growing in growing site *s*, its height-diameter allometry was modelled as:

Likelihood: $height_i \sim \log \text{Normal} (H_i, \sigma^2)$

and process model: $H_i = \ln \left(a_{p(i),s(i)}\right) + c_{p(i)} \times dbh_i$ [Eq. 1]

where, $\ln \left(a_{p(i),s(i)}\right)$ the scaling coefficient was estimated as:

$\ln \left(a_{p(i),s(i)}\right) = \alpha_{1p} + \alpha_{2p} \times MMT_s + \alpha_{3p} \times AP_s$ [Eq. 2]

with MMT being the mean minimum temperature of coldest month, and *AP* the annual precipitation at the planting site. The scaling exponent, $c_{p(i)}$, was estimated as a function of the latitude, *LAT*, and altitude, *ALT*, from the population of origin:

$c_{p(i)} = \beta_1 + \beta_2 \times LAT_p + \beta_3 \times ALT_p$ [Eq. 3]

We used this model, with its associated parameters' means variances and covariances, to generate the necessary output data for our study, which aims to assess the effect of climate change on tree height-diameter allometry. Specifically, we generated total height at a fixed dbh (100 mm) of a specific population sample from each species within planting sites under present and future climatic conditions (see main text). We used OpenBUGs 1.4 to generate the output data (Thomas *et al.* 2006).

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Chapter 7

General conclusions

Conclusiones Generales

1. La respuesta de las coníferas mediterráneas al clima –tanto a escala local como regional– depende, al menos en parte, de las fuentes generadoras de variación fenotípica dentro de especie –tales como la condición sexual y/o los procesos conducentes a adaptación local y respuestas plásticas– (**Capítulos 2**, **3**, **4**, **5** y **6**).

2. Las interacciones competitivas se consideran factores determinantes de la estructura y dinámica de las poblaciones de árboles. Sin embargo, en masas jóvenes de *J. thurifera*, la condición sexual puede por sí sola explicar la variación fenotípica del crecimiento diametral dentro de una población (**Capítulo 2**).

3. Los individuos femeninos de *J. thurifera* aparentemente hacen un uso más eficiente de los recursos disponibles en comparación con los individuos masculinos y los no-reproductores, hecho que les permite crecer más rápido (**Capítulo 2**).

4. El manejo selvícola de las masas jóvenes de sabina albar, orientado a favorecer mayores crecimientos, no se vería favorecido por la ejecución de claras, ya que la competencia intraespecífica no es un factor determinante (**Capítulo 2**).

5. Las respuestas adaptativas y/o plásticas subyacen, al menos en parte, a la variación fenotípica dentro de especie para los distintos rasgos o procesos estudiados –emergencia, supervivencia, alometría altura-diámetro, altura total–(**Capítulos 3**, **4**, **5** y **6).**

6. La evidencia experimental en los pinos mediterráneos estudiados no apoya, de forma global, la existencia de adaptación local sensu Kawecki and Ebert (2004), lo que contradice la idea extendida de que la adaptación local es un proceso general en las plantas, pero sí apoya la existencia de variación genética entre poblaciones en respuesta al clima (**Capítulos 3 y 4**).

7. En ambientes mediterráneos, durante la fase de germinación y reclutamiento, los procesos estocásticos y la variación ambiental local –microambiente– parecen jugar un papel más relevante que la variación genética dentro de poblaciones. Además, la variación ambiental local favoreció a aquella población (local o no) con la estrategia vital mejor adaptada (**Capítulo 3**).

8. El diseño de planes de migración asistida –con el objetivo de mitigar los efectos negativos del cambio climático– puede verse dificultado por la estocasticidad inherente al reclutamiento en ambientes estresantes, así como por las diferentes estrategias adaptativas de cada población de pino (**Capítulo 3**).

9. Una gran parte de las poblaciones de la Península Ibérica de *P. sylvestris* habitan condiciones climáticas subóptimas que les limita alcanzar su máximo potencial de crecimiento en altura. Este hecho podría estar relacionado con la diferencia de velocidades entre adaptación y los actuales rápidos cambios en el clima (**Capítulo 4**).

10. La estrategia de asignación de recursos en altura o grosor depende de la estrategia vital de la especie; los pinos de orígenes más xéricos (*P. halepensis*, *P. pinaster*, *P. nigra*) se ven más afectados por las temperaturas mínimas; mientras que la disponibilidad de agua determina la estrategia para el pino adaptado a zonas más frías y húmedas (*P. sylvestris*). No obstante, estas estrategias vitales se modulan según los niveles de variación genética en su respuesta a la temperatura y la precipitación, excepto en *P. halepensis* (**Capítulo 5**).

11. La actual variación intraespecífica del rasgo alométrico altura-diámetro en las cuatro especies de pinos estudiadas en la Península Ibérica (*P. halepensis*, *P. pinaster*, *P. nigra* y *P. sylvestris*) es el resultado tanto de su historia evolutiva –aproximada por el estudio de las respuestas adaptativas al clima y la existencia de diferencias entre acerbos genéticos– como de las condiciones climáticas actuales (**Capítulo 5**).

12. En un escenario de cambio climático en el año 2050, las estrategias de asignación de recursos en altura en cuatro especies de pino en la

Península Ibérica se verían alteradas. Los pinos mediterráneos de orígenes xéricos (*P. halepensis* y *P. pinaster*) tenderían a ser más esbeltos, mientras que los pinos mediterráneos asociados a montañas mantendrían su alometría o disminuirían su esbeltez (*P. nigra* y *P. sylvestris*). Estos cambios han de ser tenidos en cuenta a la hora de seleccionar el material a emplear en restauración, forestación y/o reforestación (**Capítulo 6**).

General Conclusions

1. Mediterranean conifer responses to climate –both at local and regional scales– rely, at least in part, on the sources that generate phenotypic variation within species –such as gender and/or the processes leading to local adaptation and plastic responses– (**Chapters 2**, **3**, **4**, **5** and **6**).

2. Competition interactions are considered major determinants of population structure and dynamic of forest trees. However, in young *J. thurifera* stands, sexual condition alone can explain phenotypic variation in diameter growth within a population (**Chapter 2**).

3. Female *J. thurifera* individuals seem to make more efficient use of the available resources compared to male and non-reproductive individuals, a fact that enables females to grow faster (**Chapter 2**).

4. Silvicultural practices in young juniper stands aiming at favoring higher growths would not benefit from thinning as intraspecific competition seems to have little influence on the growth of remnant trees (**Chapter 2**).

5. Adaptive and/or plastic responses underlie, at least partially, phenotypic variation within species for the different traits and processes studied –emergency, survival, tree height-diameter allometry, tree height– (**Chapters 3**, **4**, **5** and **6**).

6. The experimental evidence provided by the Mediterranean pines studied do not support, globally, the existence of local adaptation sensu Kawecki and Ebert (2004), which contradicts the widespread idea that local adaptation is a general process in plants, however, we also found genetic variation within population in response to climate (**Chapters 3** and **4**).

7. In Mediterranean environments, during the germination and recruitment stages, stochastic processes and local environmental variation –i.e. the microenvironment- seem to play a more relevant

role than genetic variation within populations. In addition, the local environment favored that population (local or not) with the better-adapted life-history strategy (**Chapter 3**).

8. Designing assisted migration plans –aiming at mitigating the negative effects of climate change– can be hindered by inherent high stochasticity in recruitment in stressful environments, as well as by the different adaptive strategies of each pine population (**Chapter 3**).

9. In the Iberian Peninsula, a large part of *P. sylvestris'* populations inhabit suboptimal climate conditions potentially precluding them to reach their full potential height growth. This could be related to a mismatch between the speed of climate change and that of adaptation (**Chapter 4**).

10. The strategy to allocate resources to height or diameter growth depends on the life-history strategy of the species; pines from xeric origins (*P. halepensis*, *P. pinaster* and *P. nigra*) are more affected by low temperatures; whereas the availability of water determines the strategy for the pine adapted to colder and more humid conditions (*P. sylvestris*). However, these life-history strategies are modulated by levels of genetic variation in response to temperature and precipitation, except in *P. halepensis* (**Chapter 5**).

11. The current intraspecific variation in tree height-diameter allometric relationship in the four pine species studied in the Iberian Peninsula (*P. halepensis*, *P. pinaster*, *P. nigra* and *P. sylvestris*) is the result of both evolutionary history –approached by the study of adaptive responses to climate and the existence of population differences among gene pools– and present climate conditions (**Chapter 5**).

12. In the context of climate change in year 2050, the strategies for resource allocation in tree height-diameter in four pine species in the Iberian Peninsula would be altered. Mediterranean pines with xeric origins (*P. halepensis* and *P. pinaster*) would tend to be slenderer, while Mediterranean mountain pines would either keep similar allometry or tend to be less slender (*P. nigra* and *P. sylvestris*). These

changes should be taken into account when selecting plant material to be used in restoration, afforestation and/or reforestation (**Chapter 6**).

Curriculum Vitae

List of publications

1. **Natalia Vizcaíno-Palomar**, Benito-Garzón, M., González-Muñoz, N., Alía, R., González-Martínez, S.C. Modelling population responses to climate: a case study of *Pinus sylvestris* in the Iberian Peninsula. (In preparation).

2. **Natalia Vizcaíno-Palomar**, Ibáñez, I., González-Martínez, S.C., Zavala, M. A., Alía, R. Adaptation and plasticity in aboveground allometry of European pine species along environmental gradients. Ecology and Evolution. (In 2nd review).

3. **Natalia Vizcaíno-Palomar**, Ibáñez, I., Benito-Garzón, M., González-Martínez, S.C., Zavala, M. A., Alía, R. Climate and population origin shape pine tree height-diameter allometry: implications for forest management. New Forests. (Under review).

4. **Vizcaíno-Palomar N.**, Revuelta-Eugercios B., Zavala M.A., Alía R., & González-Martínez S.C. (2014) The role of population origin and microenvironment in seedling emergence and early survival in Mediterranean maritime pine (*Pinus pinaster* Aiton). *PloS one*, 9, e109132.

5. **Vizcaíno-Palomar N.**, Gómez-Aparicio L., Pavón-García J., Bartolomé-Esteban C., Álvarez-Jiménez J., & Zavala M.A. (2014) Main biotic drivers of tree growth in a developing Juniperus thurifera stand in central Spain. European Journal of Forest Research, 133, 1109–1119.

6. Adaptation of trees and forests to climate change: the importance of genetic variability (2014). Policy brief of BiodivERsA. www.biodiversa.org/policybriefs.

7. **Natalia Vizcaíno-Palomar**, Oliet J.A., Jacobs D.F. (2011) Biomasa y dinámica de nutrientes de encina en fase de plántula. Efectos de la disponibilidad durante el desarrollo ontogénico. In Proceedings of the 5º Congreso Forestal Español, Ref.5 CFE01-278-3A. Ed. Junta de Castilla y León. Sociedad Española de Ciencias Forestales, Spain.

8. Miguel A. Zavala, Benito-Garzón, M., **Vizcaíno-Palomar, N.**, Ruiz-Benito, P. (2011) Vulnerabilidad y resiliencia de los ecosistemas forestales al cambio climático. http://www.madrimasd.org/blogs/biodiversidad/.

Congress and meetings

1. Santiago C. González-Martínez, Alía, R., Budde, K., Eckert, A.J., Grivet, D., Heuertz, M., Jaramillo-Correa, J.P., Mayol, M., Plomion, C., Riba, M., Rodríguez-Quilón, I., Vendramin, G.G., **Vizcaíno-Palomar**, **N.** (authors in alphabetical order, except first author). Back to nature: candidate genes, population genomics and prediction of maladaptation in natural populations. ProCoGen Final Open Conference and Workshops Promoting Conifer Genomic Resources. Orléans, France. 2015. Invited Oral Presentation.

2. Marta Benito-Garzón, Fady B., **Vizcaíno-Palomar N.**, Fernández-Manjarrés J. Integration of provenance tests and National Forest Inventories to account for intraspecific variation in species distribution models. Centennial Annual meeting of the Ecological Society of America (ESA). Baltimore, USA. 2015. Invited Oral Presentation.

3. **Natalia Vizcaíno-Palomar**, Ibáñez, I., Benito-Garzón, M., Zavala, M.A., González-Martínez, S.C., Alía, R. Predicted changes in aboveground allometry in four pine species in response to climate change: Implications in restoring forests. 2nd International Congress in Restoring Forests. Indiana, USA. 2014. Oral Presentation.

4. **Natalia Vizcaíno-Palomar**, Revuelta-Eugercios, B., Zavala, M.A., Alía, R., González-Martínez, S.C. XI National Congress of the Spanish Association of terrestrial ecology (AEET). Pamplona, Spain. Local adaptation at early stages of development in maritime pine. 2013. Oral Presentation.

5. **Natalia Vizcaíno-Palomar**. 1st Remedinal Congress. Restoration and conservation the Madrilenian ecosystems. University of Rey Juan Carlos, Madrid, Spain. 2013. Participant in CIFOR-INIA session.

6. **Natalia Vizcaíno-Palomar**, Zavala, M.A., Alía, R., González-Martínez, S.C. Patterns of emergence and survival in two contrasted populations of maritime pine and environmental interactions. 2nd Scientific marathon. INIA-CIFOR, Madrid, Spain. 2012. <u>Oral Presentation</u>.

7. **Natalia Vizcaíno-Palomar**, Oliet, J.A., Douglass D.F. Biomasa y dinámica de nutrientes de encina en fase de plántula. Efectos de la disponibilidad durante el desarrollo ontogenético. II Reunión conjunta del Grupo de Trabajo de Repoblaciones Forestales de la SECF y del Grupo de Trabajo de Restauración Ecológica de la AEET. Palencia, Spain. 2011. <u>Oral Presentation</u>.

8. **Natalia Vizcaíno-Palomar**, Pavón-García, J., Gómez-Aparicio, L., Zavala, M. A. Size and gender control tree growth in early stages of stand development of a *Juniperus thurifera* forests 12th European Ecological Federation Congress. Ávila, Spain. 2011. <u>Poster</u>.

9. Beatriz Terrones, Constán-Nava S., **Vizcaíno-Palomar N.**, Climent A., Bonet A. Hábitat disponible para la especie invasora *Ailanthus altissima* (Mill.) Swingle en el P.N. del Carrascal de la Font Roja, Alicante. 2nd National Congress on invasive alien species. León, Spain. 2006. <u>Oral Presentation</u>.

Participation in research projects

1. Restauración y conservación de los ecosistemas mediterráneos: Respuesta frente al cambio global (REMEDINAL-3). Comunidad de Madrid, cofinanciada por el Fondo Social Europeo (S2013/MAE-2719). 600.300 €. Comunidad de Madrid. Dr. José M. Rey Benayas, coordinador grupo UAH. 2014 - 2018. CIFOR-INIA, Universidad Rey Juan Carlos,

Universidad de Alcalá, Universidad Complutense de Madrid, Universidad Autónoma de Madrid, Universidad Politécnica de Madrid. IP: Dr. Adrián Escudero. Universidad Rey Juan Carlos

2. Restauración Ecológica en la Comunidad de Madrid (REMEDINAL-2). Comunidad de Madrid, cofinanciada por el Fondo Social Europeo (S2009/AMB-1783). 905.000 €. Comunidad de Madrid. Dr. Miguel A. Zavala, coordinador Unidad mixta INIA-UAH. 2010 - 2014. CIFOR-INIA, Universidad Rey Juan Carlos, Universidad de Alcalá, Universidad Complutense de Madrid, Universidad Autónoma de Madrid, Universidad Politécnica de Madrid. IP: Dr. Adrián Escudero. Universidad Rey Juan Carlos.

3. Interacciones ecológicas y cambio global en el bosque mediterráneo (INTERBOS). Patrones regionales y procesos locales: modelos de la dinámica del bosque mediterráneo en respuesta al cambio global. Ministerio de Ciencia e Innovación (CGL2008-04503-C03-03/BOS). 01/01/2009 - 01/01/2012. INIA, Universidad de Cambridge, Microsoft Research, UNED. IP: Dr. Miguel Ángel de Zavala Gironés. INIA.

Other merits

Co-organizer in the workshop "Terrestrial ecosystem as complex adaptive systems: How to integrate adaptive processes in response to disturbances into Dynamic Global Vegetation Models (DGVMs)?" action COST ES0805. Madrid, España. 17th to 19th November 2010. CIFOR-INIA.

Stays in foreign centres

Université de Bourdeaux 1. BioGeCo department. Bourdeaux, France. Supervised by Dr. Sylvain Delzon. October to December 2013.

Université Paris-Sud. Department of Ecology, Systematics and Evolution. Supervised by Dr. Juan Fernández-Manjarrés. From 18th al 22 November 2013.

University of Michigan. Department of Ecology. Ann Arbor, Michigan, USA. Supervised by Dra. Inés Ibáñez. August to November 2012.

University of Cambridge. Department of Plant Sciences. Cambridge, United Kingdom. Supervised by Dr. David Coomes. September to December 2011.

Workshops and courses

1. **DAT203x Data Science and Machine Learning** Essentials. 120 hours. Microsoft |OpenEdX. 2015

2. **Introduction to Genetics and Evolution**. Dr. Mohamed Noor at Duke University, Durham, NC. 90 hours. Coursera online. 2015.

3. **Population structure and the genetic architecture** of **quantitative traits**. Dr. Antoine Kremer *et al*. 20 hours. University of Uppsala. Sweden. 2014.

4. **Statistical learning**. Dr. Trevor Hastie & Dr. Rob Tibshirani. 120 hours. Standford University online | OpenEdX. 2014.

5. **Spatial genetics**. Dra. Cristina García & Dra. Victoria Sork. 40 hours University of Granada. Spain. 2012.

6. **Likelihood Methods and Models in Ecology**. Dr. Charles Canham. 40 hours. University of Granada. Spain. 2011.

7. **Methodologies in functional and evolutionary ecology**. 40 hours. EEZA, Almería. Spain. 2011.

8. **Analysis of ecological data with R**. Dr. Marcelino de la Cruz. 12 hours. University of Alcalá. UAH, Madrid, Spain. 2011.

9. **Dendrochronology applied in ecology research and for forest management**. Dra. Emilia Gutiérrez Merino *et al*. 40 hours. Consejería de Medio Ambiente. Jaén. Spain. 2010.

10 **Analysis of ecological data with R.** Dr. Luis Cayuela. 30 hours. University of Alcalá, UAH, Madrid. Spain. 2010.

11 **Cómo se escribe y se publica un artículo de investigación**. Juan Miguel Campanario. 15 hours. University of Alcalá, UAH, Madrid. Spain. 2010.

12 **Quality control and traceability of products and processes using NIRS**. 30 hours. University of Córdoba. Córdoba, Spain. 2009.

Teaching experience

80 hours certified. Faculties of Biology and Environmental Sciences.

Language skills

Native Spanish; English B2 (EOI, June 2015)

Academic Societies:

Asociación Española de Ecología Terrestre (AEET)