TESIS DOCTORAL / DOCTORAL DISSERTATION

LUIS SANTOS DEL BLANCO

2013

Universidad de Valladolid

INIA
Instituto Nacional de Investigación
y Tecnología Agraria y Alimentaria

INSTITUTO UNIVERSITARIO DE GESTIÓN FORESTAL SOSTENIBLE Uva-INIA

ECOLOGÍA EVOLUTIVA DE LA REPRODUCCIÓN EN DOS PINOS MEDITERRÁNEOS: *PINUS PINASTER* AIT. Y *PINUS HALEPENSIS* MILL.

EVOLUTIONARY ECOLOGY OF REPRODUCTION IN TWO MEDITERRANEAN PINE SPECIES: *PINUS PINASTER* AIT. AND *PINUS HALEPENSIS* MILL.

Universidad de Valladolid

INSTITUTO UNIVERSITARIO DE GESTION FORESTAL
SOSTENIBLE Uva-INIA

DOCTORAL DISSERTATION/
TESIS DOCTORAL

EVOLUTIONARY ECOLOGY OF REPRODUCTION IN TWO MEDITERRANEAN PINE SPECIES: *PINUS PINASTER* AIT. AND *PINUS HALEPENSIS* MILL.

ECOLOGÍA EVOLUTIVA DE LA REPRODUCCIÓN EN DOS PINOS MEDITERRÁNEOS: *PINUS PINASTER* AIT. Y *PINUS HALEPENSIS* MILL.

Presentada por D. Luis Santos del Blanco para
optar al grado de
Doctor por la Universidad de Valladolid

Dirigida por:
Dr. José M. Climent Maldonado

INDEX

SUMMARY

-introduction-

The study of life-history traits is central to evolutionary biology and ecology, as life-history traits are closely linked to the environment where organisms thrive. Among them, traits related to reproduction such as the threshold size for reproduction, fecundity and the schedule of reproductive investment along life are particularly relevant. Their study helps to understand past adaptive processes as well as to infer future ones. For forest trees, this knowledge is particularly valuable and urgent, given their founding role in ecosystems all over the world.

Life-history traits can be addressed at the species level but usually, intraspecific within- and among-population variation also exists. This is the case for numerous plant species, as flowering time is commonly very variable and found to be correlated with natural selective pressures. This correlation may be due to plastic or genetic causes. A requirement for genetic responses to take place is the existence of additive genetic variation, which can be revealed by quantitative genetic studies or artificial selection experiments. Moreover, according to life-history theory, the expression of particular fitness-related traits is limited by costs or trade-offs with other traits also related to fitness.

Pinus pinaster Ait. and *P. halepensis* Mill. are two Mediterranean pine species showing numerous advantages for the study of tree reproductive traits from an evolutionary-ecological standpoint. Using these species as a model, it is possible to integrate aspects such as population differentiation, past and future local adaptation, plasticity, genetic architecture and multi-trait adaptive relationships.

-aims-

The aims pursued in the present study were, first, to document population differentiation in reproductive life-history traits. Second, to test the hypothesis that life-history traits are related to the environment by means of phenotypic plasticity and genetic change processes. Third, to estimate reproductive quantitative genetic parameters of key life-history traits. Fourth, to test the hypothesis that investment in reproduction is bound to costs or trade-offs with other adaptive traits, both at phenotypic and genetic levels.

-material and methods-

P. pinaster and *P. halepensis* are naturally distributed along extensive areas of the Mediterranean basin. *P. pinaster* has a particularly broad ecological niche, and there is wide genetic differentiation among populations, meanwhile *P. halepensis,* very tolerant to drought, shows smaller population differentiation, at least in neutral molecular markers. Data used in the present study were obtained from common garden experiments of three kinds: provenance, provenance-progeny and progeny common gardens, containing genetic material originated in natural populations or derived from breeding programs. Common garden data allowed to estimate to which extent phenotypic expression is genetically based and, when experiments were replicated under contrasting environmental conditions, to know whether this expression was also plastic. Furthermore, climatic data describing the areas where natural populations originated were obtained. Climatic data were then used to check their relationship with phenotypic traits. For *P. pinaster*, this relationship was checked after correcting for strong neutral genetic structure with nuclear microsatellite data.

Traits measured in the common gardens were related either to vegetative growth (total height, diameter at breast height, stem straightness) or with reproduction (presence/absence and quantification of reproduction, both male and female). For *P. halepensis*, reproduction was also manipulated experimentally seeking for the expression of somatic costs of reproduction. Data analysis was accomplished by linear (Gaussian data) and generalised (non-Gaussian data) mixed model analysis.

-results and discussion-

In both species, large population differentiation for reproductive life-history traits was a general finding. Moreover, mean population values –most notably regarding female function- covaried according to the environment where populations had evolved. A precocious and intense investment in reproduction was related to unfavorable environments for vegetative growth (low site index). This correlation is in agreement with the adaptive value of reproductive traits in forest trees; just as well as it has been described in other clinal traits such as timing of grow cessation. However, clinal trends were not found for the threshold size for male reproduction in *P. pinaster*. This could be related to sex allocation patterns strongly linked to tree height, as height is related to pollen dispersal conditions.

In *P. halepensis*, environmental effects (plasticity) on the expression of reproductive traits were also observed. In pararell to patterns of population genetic differentiation, a more growth-limiting environment was related to advanced ontogenetic development. In both species, it was noteworthy the absence of genotype by environment interaction for reproductive traits, contrasting with results for vegetative growth.

Plastic adaptive responses triggered in response to environmental change can reduce the need for genetic changes. On the other hand, high genetic variance and moderate to high heritabilities of reproductive life-history traits enable steady local adaptation processes. This possibility was backed by a neat response to artificial selection in *P. pinaster*.

At the somatic level, female costs of reproduction were confirmed in *P. halepensis* by means of a manipulation experiment, but not by correlational studies. In this species, investment in reproduction had a greater effect on future reproduction than on vegetative growth. At the genetic level, costs of reproduction in terms of growth showed a variable pattern depending on the species and the common garden environment. Nonetheless, several reproductive traits displayed a strong correlated response to selection for growth in *P. pinaster* i.e. evidence for the existence of a genetic trade-off between reproduction and growth. From the point of view of forest management, it is advised against the use of size as a single surrogate of fitness. Even though it is true that large trees tend to produce a greater number of offspring, this relationship is not tight and might be shadowed by trade-offs. On the contrary, under a new paradigm of adaptive forest management, the inclusion of reproduction to better define fitness is recommended.

Keywords: forest genetics, genetic variation, reproductive life-history traits, allometry of reproduction, costs of reproduction, local adaptation, fitness, evolution

RESUMEN

-introducción-

El estudio de los caracteres de historia vital es central en biología evolutiva y ecología, pues están íntimamente relacionados con el entorno vital de los organismos. Entre los caracteres de historia vital destacan los reproductivos, tales como el tamaño umbral de reproducción, la fecundidad y el reparto del esfuerzo reproductor a lo largo de la vida. Su estudio ayuda a entender procesos adaptativos pasados y a inferir los futuros. En el caso de los árboles forestales, este conocimiento es particularmente valioso y urgente dado el papel básico de los árboles forestales en ecosistemas de todo el mundo.

Los caracteres de historia vital pueden ser estudiados a nivel de especie, pero comúnmente también existe variación intraespecífica entre y dentro de poblaciones. En el caso de numerosas especies vegetales es común observar cómo el momento de floración es variable y está relacionado con presiones selectivas naturales. Esta relación puede deberse a causas plásticas o genéticas. Un requisito para que se den respuestas a nivel genético es la existencia de variación genética aditiva. Ésta puede ser revelada mediante estudios de genética cuantitativa o estudios de selección artificial. Además, según la teoría de historia vital, la expresión de caracteres relacionados con la aptitud biológica (*fitness*) está limitada por la existencia de costes o compensaciones entre funciones.

Pinus pinaster Ait. y *P. halepensis* Mill. son dos especies de pinos mediterráneos que presentan numerosas ventajas para el estudio de caracteres reproductivos en árboles desde un enfoque ecológico-evolutivo. Usando estas especies como modelo es posible integrar aspectos como la diferenciación entre poblaciones, adaptación local pasada y futura, plasticidad, arquitectura genética y relación con otros caracteres adaptativos.

-objetivos-

Los objetivos del presente estudio fueron, en primer lugar, documentar la diferenciación entre poblaciones para caracteres de historia vital reproductivos. Segundo, evaluar la hipótesis de que los caracteres de historia vital están relacionados con el ambiente mediante procesos de plasticidad fenotípica y de cambio genético. Tercero, estimar parámetros de genética cuantitativa para caracteres reproductivos de historia vital. Cuarto, comprobar la hipótesis de que la inversión en reproducción conlleva costes o compensaciones en otros caracteres adaptativos, tanto a nivel fenotípico como genético.

-material y métodos-

Las especies objeto de estudio se distribuyen de forma natural en la cuenca mediterránea, ocupando grandes extensiones. *P. pinaster* tiene un nicho ecológico particularmente amplio existiendo una notable diferenciación genética entre poblaciones, mientras que *P. halepensis* es muy tolerante a la sequía y presenta una menor diferenciación entre poblaciones, al menos en caracteres moleculares neutros. Los datos utilizados en este estudio provienen de ensayos de ambiente común (*common gardens*) de tres tipos: ensayos de procedencias, ensayos de progenies y ensayos de procedencias-progenies, que contienen material genético procedente de poblaciones naturales y de programas de mejora. Esto permite estimar hasta qué punto la expresión fenotípica de los caracteres tiene una base genética, y cuando los ensayos están replicados en ambientes contrastados, saber si esa expresión puede ser plástica. Además se recavó información sobre parámetros climáticos de las zonas de origen de las poblaciones naturales para conocer su relación con caracteres fenotípicos. En el caso de *P. pinaster*, comprobó esta relación tras controlar mediante datos de marcadores microsatélite las posibles interferencias debidas a la existencia de una fuerte estructura genética en la especie.

Los caracteres obtenidos en los ensayos de ambiente común estuvieron relacionados con el crecimiento vegetativo (altura total, diámetro normal, rectitud) y con la reproducción (presencia o ausencia y cuantificación de la reproducción masculina y femenina). Para *P. halepensis*, también se llevó a cabo un experimento de eliminación de conos femeninos para revelar la existencia de costes somáticos de reproducción. El análisis de datos se realizó aplicando modelos mixtos lineales (variables gaussianas) y generalizados (variables no gaussianas).

-resultados y discusión-

En ambas especies, la existencia de diferenciación entre poblaciones para caracteres de historia vital reproductivos fue un hallazgo generalizado. Además los valores medios por población –particularmente en lo relativo a la función femenina- covariaron con el ambiente originario de las poblaciones, de forma que una inversión temprana e intensa en reproducción se asoció con valores ambientales poco favorables para el crecimiento (baja calidad de estación). Esta correlación es coherente con la importante función adaptativa de los caracteres reproductivos en árboles forestales, tal como se ha descrito en otros caracteres con variación clinal como puede ser la fecha de cese de crecimiento. Sin embargo, en *P. pinaster* no se hayaron tendencias clinales de variación en el tamaño

umbral de reproducción masculino. Ello puede estar relacionado con patrones de asignación sexual, fuertemente dependientes de la altura del árbol, ya que ésta determina las condiciones de dispersión de polen.

La influencia del ambiente externo (plasticidad) en la expresión de caracteres reproductivos fue constatada en *P. halepensis*. De forma paralela a los patrones observados en diferenciación genética, un ambiente más limitante para el crecimiento se relacionó con una aceleración en el desarrollo ontogénico. En ambas especies es destacable la ausencia de interacción genotipo x ambiente para caracteres reproductivos, contrastando con los resultados en crecimiento vegetativo.

Las respuestas adaptativas plásticas frente a cambios ambientales pueden atenuar la necesidad de un cambio genético. Por otro lado, la existencia de valores altos de varianza genética y heredabilidades de moderadas a altas para caracteres de historia vital reproductivos facilitan los procesos continuos de adaptación local. Esta posibilidad está respaldada por la existencia de una marcada respuesta a la selección artificial en *P. pinaster*.

A nivel somático, se constató la existencia de costes de reproducción femenina en *P. halepensis* mediante un experimento de manipulación, pero no mediante estudios correlacionales. En esta especie, la inversión en reproducción tuvo un mayor efecto en la reproducción futura que en el crecimiento. A nivel genético, los costes de reproducción en términos de crecimiento presentaron patrones variables, en función de la especie y el ambiente considerados. Sin embargo, varios caracteres reproductivos mostraron una fuerte respuesta correlacionada a la selección para crecimiento en *P. pinaster*, lo que constituye evidencia de la existencia de compensaciones a nivel genético entre reproducción y crecimiento. Desde el punto de vista del manejo forestal, se desaconseja utilizar el tamaño como único indicador de aptitud biológica. Aunque es cierto que los árboles grandes tienden a producir un mayor número de descendientes, esta relación es difusa y en gran medida puede estar influida por compensaciones (*trade-offs*). Al contrario, bajo un nuevo paradigma de gestión forestal adaptativa frente al cambio global, se propone incluir los caracteres reproductivos a la hora de definir de forma más precisa la aptitud biológica.

Palabras clave: genética forestal, diversidad genética, caracteres de historia vital reproductivos, alometría de la reproducción, costes de reproducción, adaptación local, aptitud biológica, evolución

INTRODUCTION

1. INTRODUCTION

The concept of biological adaptation bears different meanings depending on the time scale considered. In forestry or agriculture, a certain genetic material is said to be well adapted, or to adapt itself well to particular environmental conditions if plants thrive well in terms of vegetative growth and survival. That is, if the combination of genotype and environment produces a phenotype that is considered suitable. In forestry, but also in everyday life, the term adaptation is thus understood as a plastic process along the plant's life.

In evolutionary terms, adaptation refers to a genetic process along generations. By adaptive processes, genetically superior individuals contribute disproportionally to the following generation, promoting genetic changes along generations (Darwin, 1859). Under the evolutionary concept of adaptation, a genetic material is considered to be adapted to a particular environment if outcompetes other genotypes. Competition here is not referred to vegetative growth, but to biological fitness i.e. number of gene copies in the next generation. The concept of biological fitness is thus central to understanding adaptation in evolutionary terms, but it is not needed, and often not used, to understand "static" adaptation.

1.1 LIFE-HISTORY TRAITS

Life-history traits define developmental patterns in terms of growth, reproduction and survival in all kind of organisms (Roff, 1992a). The most important life-history traits are size at reproduction, lifetime offspring number, distribution of reproductive effort along time and the interaction between reproduction and adult mortality (Stearns, 1976). There is abundant evidence for numerous organisms on the close relationship between environmental conditions, either natural or induced by man, and life-history reproductive traits (Grime, 1977; Roff, 1992a; Stearns, 1992). Stable environments generally favour selection on large size at maturity, iteroparity (i.e. repeated reproductive events along life), low reproductive effort and high investment in maintenance. Conversely, fluctuating environments promote the opposite constellation of traits: low sizes at maturity, semelparity (i.e. a single reproductive event before death), high reproductive effort and low investment in growth and defence (Stearns, 1976; Grime, 1977).

Reproductive life-history traits provide a fecund and comprehensive research field in genetic, evolutionary and ecology studies and help understand the interactions between constantly adapting populations and their environment. Knowledge on reproductive life-history traits is particularly scarce in forest tree species, but this knowledge is relevant and urgent as they are founding species in their ecosystems, occupying great areas on the surface of the Earth (Petit & Hampe, 2006; Neale & Kremer, 2011).

1.1.1 Life-history traits and local adaptation in plant populations

In the Plant kingdom, forest trees and annual plants illustrate well two extreme life-history strategies. Forest trees share a common pattern consisting of high juvenile mortality, delayed reproduction, large vegetative sizes and extended lifespan. And within woody species, angiosperms tend to have shorter times to reproduction than gymnosperms (Verdú, 2002). Yet, within this general pattern a wide range of variation exists, closely linked to environmental conditions (Thomas, 2011). Studies on within-species variability of life-history traits are especially valuable to illustrate local adaptation, as confounding phylogenetic factors can be minimised.

One of the first studies on genetic differentiation for life-history traits was authored by EP Lacey (1988), reporting on clinal variation for reproductive timing in *Daucus carota*. Remarkably, the studied species was artificially introduced by the 17th century in North America, where the study was performed, so that the described pattern of variation likely appeared in a short period of time. This rapid adaptive genetic change in an introduced species further stresses the relevance of life-history evolution research. Several other studies on introduced species have added to Lacey's seminal paper, also with similar results, describing a link between early reproduction and northern latitudes or harsher environments (Kollmann & Bañuelos, 2004; Alexander *et al.*, 2009; Colautti *et al.*, 2010; Lachmuth *et al.*, 2011). Interestingly, this pattern is also common in native species (Van Dijk *et al.*, 1997; Wesselingh *et al.*, 1997; Rees *et al.*, 1999; Callahan & Pigliucci, 2002; Mendez & Karlsson, 2004; Kuss *et al.*, 2008; Kagaya *et al.*, 2009; Brys *et al.*, 2011; Guo *et al.*, 2012).

Numerous studies have not only described extensive ecotypic genetic variation in reproductive life-history traits -most notably size at first reproduction- but also confirmed the existence of trade-offs between reproduction and growth. In addition to differentiation among populations, high within-population variability is a common finding in life-history studies (Wesselingh & de Jong, 1995; de Jong & Klinkhamer, 2005; Childs *et al.*, 2010). The reference list is however biased towards short-lived species, and studies on life-history traits in trees are few, commonly focused at inter-specific variation (Niklas & Enquist, 2003; Wright *et al.*, 2005) and usually confounding genetic and environmental effects (Dodd & Silvertown, 2000; Thomas, 2011).

1.1.2 Sex allocation theory

Dioecious species or monoecious species with separate male and female reproductive structures, are convenient for addressing more specific questions regarding each separate sex and their interplay (Charnov, 1982; Hardy, 2002; West, 2009). A well-developed field of theory has led to testable hypothesis and, in plants, researchers have posed relevant questions about the occurrence of dioecy (Ashman, 2006), sex ratios, i.e. relative abundance of males to females (Barrett & Yakimowski, 2010) and sex allocation, i.e. the within-individual ratio of male to total reproduction (Goldman & Willson, 1986).

Studies on wind-pollinated plants in reproductive ecology are less frequent than for animal-pollinated plants (Barrett, 2002), but have led to important hypotheses about the relative importance of male to female reproduction depending on size, i.e. size-dependent sex allocation. Fitness gain curves relate investment in a fitness function (usually investment in male or female functions) with fitness returns (Klinkhamer *et al.*, 1997; Zhang, 2006). In wind-pollinated plants, larger individuals are predicted to have relatively higher male investment as a result of the shape of both male and female fitness gain curves (Burd & Allen, 1988). As pollen can travel long distances and it is dispersed more efficiently from taller plants, male fitness gain curves are thought not to saturate (de Jong & Klinkhamer, 1994). While seeds, even though they might be also wind-dispersed, tend to remain closer to the mother plant and when produced in increasingly high numbers compete for limiting space so that fitness returns per unit resource invested in female function tend to flatten (Burd & Allen, 1988; Klinkhamer *et al.*, 1997).

There is considerable overall support for this prediction (Klinkhamer *et al.*, 1997) although conifers with segregated functions seem to be a particular case (Fox, 1993). Many conifers bear female cones on the upper part of their crown as a mechanism to enhance crossed pollination and to avoid selfing, and it is size and not height what has been related to a plastic response towards increased male allocation (Fox, 1993; Ne'eman *et al.*, 2011). Other aspects like the ecological correlates of sex allocation and gender variation (Case & Ashman, 2005; Delph & Wolf, 2005), within species genetic variation (Koelewijn & Hunscheid, 2000; Friedman & Barrett, 2011) or the existence of plastic responses triggered by cues other than size (Paquin & Aarssen, 2004; Friedman & Barrett, 2011) remain less explored.

1.2 PLASTICITY IN REPRODUCTIVE LIFE-HISTORY TRAITS

The extent to which reproductive life-history traits are influenced by environmental conditions (plasticity) has also been explored mainly in herbaceous plants (Sultan, 2000; Matesanz *et al.*, 2010). Plant reproduction has a marked allometric pattern, such that larger plant sizes are related to higher reproductive output (Niklas & Enquist, 2003). As vegetative growth is known to be highly plastic, plasticity of reproduction is typically influenced by environmentally-induced changes in vegetative size.

Even though significant, plasticity in reproduction after accounting for size effects has been considered to be minor (reviewed in Weiner *et al.*, 2009). This claim is likely to be influenced by studies on annual plants as they have simpler allocation patterns. Under predictable environments, the optimal allocation strategy for annual plants is to invest all available resources in reproduction just before death, and size usually predicts accurately reproductive allocation (Weiner *et al.*, 2009). Opposed to that, biannual and longer-lived species must also invest a certain amount of resources in maintenance and growth along at least two seasons (de Jong & Klinkhamer, 2005). Thus, reproductive allometry is likely to be determined also by the fitness returns of investment in maintenance and future growth (Zhang, 2006).

The existence of a threshold size for reproduction in many plant species is considered as evidence for such interplay between investment in several fitness-related traits, which may be environment-dependent (Kozłowski, 1992; Burd *et al.*, 2006). As a result, plasticity in this key life-history trait has been documented in several studies in short-lived plants, usually responding to resource availability and competition (de Jong *et al.*, 1998; Sultan, 2000; Bonser *et al.*, 2010; Nicholls, 2011; Anderson *et al.*, 2012). Following expectations derived from life-history theory, and backing the results found in population-differentiation studies, harsh environments are found to promote reproduction at low sizes, while competitive environments tend to reduce it. The extent to which these results similarly apply to both male and female sexual functions is currently not so well developed, although female function is considered to be more costly ant thus, perhaps, more dependent on the environment (Obeso, 2002; Case & Ashman, 2005).

1.3 ADDITIVE GENETIC VARIATION AND SHORT TERM GENETIC CHANGE IN REPRODUCTIVE LIFE-HISTORY TRAITS

Given the forecasted climate change scenarios and the sheer importance of life-history traits in adaptation, it is relevant to ask whether plants will be able to evolve at a pace fast enough as to track environmental changes. For a trait to be able to evolve in response to natural or artificial selection, first there must be an underlying heritable additive genetic basis upon which to select, and then the selective agent must be able to efficiently select genotypes based on phenotypes (Lynch & Walsh, 1998). Life-history traits were first thought to be genetically depleted, as they are closely related to fitness and are under strong natural selection (Merilä & Sheldon, 1999). It has only later been accepted that although heritabilities for fitness traits might be low, they do have a wide genetic basis (Ellegren & Sheldon, 2008). This apparent contradiction is clarified by the existence of high error variance affecting statistical inference of heritability (Houle, 1992) and multivariate genetic constraints (genetic trade-offs) (Walsh & Blows, 2009).

Rapid formation of adaptive clines in several introduced species suggests that life-history traits can evolve readily. This is even more remarkable if we consider that introduction into non-native ranges usually occurs through strong bottlenecks that narrow genetic diversity (Sakai *et al.*, 2001). There is also mounting evidence on the rapid evolution of flowering time as a consequence of climate change (Franks & Weis, 2008; Munguía-Rosas *et al.*, 2011), highlighting once again the adaptive relevance of reproductive traits. Quantitative genetic and artificial selection experiments have also documented high heritabilities and clear responses to selection under controlled conditions (Wesselingh & de Jong, 1995; Wesselingh & Klinkhamer, 1996; Matziris, 1998; Burgess *et al.*, 2007). Therefore it seems clear that plant populations can indeed cope to some extent with novel environmental conditions. But it remains still critical to document correlated responses to selection on life-history traits, most notably on forest trees.

Long life cycle of forest trees makes unfeasible for most researchers to accomplish artificial selection experiments and gather results within the duration of a research project. Forest trees are thus largely missing from the literature of artificial selection experiments although this kind of experiments constitute a powerful tool to test ecological hypothesis (Conner, 2003; Kawecki *et al.*, 2012). Collaboration between researchers and forest tree breeders is then perhaps a promising avenue to study responses to selection in trees from an evolutionary perspective.

1.3.1 Evolutionary quantitative genetics in forest trees

Statistical models commonly used to analyse data from quantitative genetic data rely on the assumption that the studied trait is expressed as the result of the additive effect of a large number of loci, and therefore follows a Gaussian distribution. Continuous traits like growth are typically normally-distributed but some key life-history traits have a discontinuous distribution. For example, reproduction itself can be considered a binary trait when estimating precocity or the threshold size for reproduction. But it can also be considered to follow a Poisson distribution when dealing with count data. Direct application of conventional quantitative genetic formulae for analysing non-Gaussian data violates model assumptions and might imply serious bias. Even though phenotypically expressed in a discontinuous fashion, several traits have been shown to be governed by an underlying additive genetic basis. It is only above a certain value of a non-observed trait, directly dependent on that additive genetic basis and known as liability, that the trait is expressed. That is why discontinuous traits are also known as threshold traits.

Generalised linear statistical models (GLZ) implicitly transform non-Gaussian data into continuous normally distributed variables by means of a link scale. GLZ are now widely used in biological sciences in the analysis on non-Gaussian data, but their use in evolutionary quantitative genetics is still scarce. A recent paper by Nakagawa and Schielzeth (2010) described statistical formulae that can be used to estimate repeatability in non-Gaussian data, of which heritability is a particular case (within-family repeatability). Even more recently, the issue of estimating quantitative genetic parameters of non-Gaussian traits by means of animal models has been addressed specifically (Holand *et al.*, 2013). When applied to non-Gaussian life-history traits, those methods can greatly help in advancing our knowledge on relevant basic and applied research questions. See appendix VI for a more comprehensive description and application of quantitative genetic parameter estimation applied to non-Gaussian data.

1.4 COSTS OF REPRODUCTION

Costs or trade-offs are defined as compromises between fitness functions, and have been fundamental in the development of life-history theory (Stearns, 1992). Among all, the costs of reproduction have been described as the *most prominent life-history trade-off* (Stearns, 1989). Their study in plants has been pursued by numerous scholars (reviewed by Obeso 2002 and by Thomas, 2011 in tree species). Overall but not overwhelming evidence confirms the existence of such costs, mainly described in terms of reduced vegetative growth (Obeso, 2002). However, depending on the methods used to describe costs of reproduction, the derived conclusions can be quite different.

Four methods have been proposed for the study of costs of reproduction (Reznick, 1992): correlational studies, manipulation experiments, genetic correlations and artificial selection experiments. The two first methods describe somatic or physiological costs of reproduction while the two latter ones describe genetic costs, and are thus the only relevant for evolutionary studies. Reviewed results, nonetheless, do not seem to greatly differ between phenotypic and genetic studies, or between correlational and experimental ones (Obeso, 2002). A non-trivial amount of non-significant or negative results should still stimulate research in the field, controlling for potentially confounding or compensatory factors and exploring the influence of additional variables. For example, studies considering the influence of additional factors have found evidence for higher costs of reproduction in harsh environments (Hansen *et al.*, 2013), for female sexual function (Montesinos *et al.*, 2006) and at small sizes (Climent *et al.*, 2008).

1.5 MEDITERRANEAN PINES AS MODEL TREE SPECIES TO STUDY REPRODUCTIVE LIFE-HISTORY TRAITS

Mediterranean pines, and specially *Pinus halepensis* Mill. And *P. pinaster* Ait., provide an excellent model to study past and future local adaptation processes, both from a basic and applied point of view (Lev-Yadun & Sederoff, 2000). This is due to several life-cycle characteristics (Ne'eman *et al.*, 2004, 2011; Tapias *et al.*, 2004): (1) short age at first reproduction that enables early measurement of reproductive traits; (2) high reproductive allocation that enhances costs of reproduction; (3) low inter-annual variability in reproduction, as opposed to masting species, which makes easier recording continuous data series with better statistical properties; (4) non-shedding of mature cones (strictly serotinous cones are only found in some *P. pinaster* populations) facilitates reconstruction of female reproduction records in past years; (5) segregation of male and female reproductive structures within a tree crown allows testing specific hypothesis about the role of each reproductive function; (6) large within-population range of sizes, adequate for testing size-dependent sex expression and sex allocation hypotheses; (7) as obligate seeder species, connections between tree parts and functions are more straightforward, increasing the adaptive value of sexual reproduction; (8) their crown architecture is relatively simple and (9) they have a relatively low life span compared to other forest species; this improves the correlation between lifetime reproductive investment and that estimated in a shorter period.

Also relevant for evolutionary ecology and sustainable forest management is the species' wide ecological niche, especially that of *P. pinaster* (Fady, 2012), that translates into a wide distribution range. This allows testing the existence of correlations between phenotypic traits and environmental conditions, indicative of local adaptation. Another important ecological factor common to both species is their relationship with disturbance regimes, namely forest fires, which act as a strong selective agent for traits like the threshold size for reproduction, bark thickness and serotiny (Tapias *et al.*, 2004; Keeley, 2012).

Finally, the vast areas occupied either by natural or planted stands along their distribution range, but notably in Spain, highlight their environmental relevance. Mediterranean pine forest stands, are suffering increasingly frequent and virulent forest fires (Pausas *et al.*, 2008), which has spurred research on their reproductive ecology, most notable in *P. halepensis* (Iraima & Espelta, 2004; Gonzalez-Ochoa *et al.*, 2004; Verkaik & Espelta, 2006; De Las Heras *et al.*, 2007; Moya *et al.*, 2007; Espelta *et al.*, 2008; Ortiz *et al.*, 2011). From a forest management point of view, these studies seek for silvicultural treatments and conditions that enhance precocious seed production. This way, assuming a high prevalence of serotinous cones in young trees (Goubitz *et al.*, 2004), a canopy seed bank is built as soon as possible, thus enabling natural regeneration after recurrent forest fires. The main conclusions drawn from these studies are that treatments that promote tree vegetative growth such as pruning or thinning (De Las Heras *et al.*, 2007; Moya *et al.*, 2007; Ruano *et al.*, 2013), fertilization (Ortiz *et al.*, 2011) or site quality (Moya *et al.*, 2007) also promote precocious reproduction. However, a higher individual reproduction in sparse stands might not be enough to achieve higher yields per hectare (Matyas & Varga, 2000; Deng *et al.*, 2012).

Despite the notable relevance of the mentioned studies for applied forest management, their discussion in the broader field of ecology is limited by two main issues. First, cone production is considered as an absolute number per tree o per surface unit, irrespective of tree size. Therefore, larger trees also commonly produce a higher number of cones, despite reproduction per unit biomass is larger in smaller trees (Ortiz *et al.*, 2011). Second, some studies directly compare natural regeneration in different populations (De Las Heras *et al.*, 2007; Moya *et al.*, 2007). This confounds genetic and environmental causes of variation when trying to explain the common link between reproduction and climatic variables. Instead, in order to test systematically whether there exists among-population genetic variation for phenotypic traits, an unbiased sample of genotypes from each study population has to be grown in environmental conditions as homogeneous as possible (White *et al.*, 2007; Salmela *et al.*, 2010).

Forest genetic trials, or common garden studies, are specifically designed to provide uniform environmental conditions under which several genetic entries can be compared. They can be classified into provenance, progeny and/or clonal trials, depending on the genetic material used to set up the common garden, and they can be carried out under greenhouse or field conditions. Progeny and clonal trials can also be used to provide estimates of quantitative genetic parameters describing the genetic architecture and genetic determination of studied phenotypic traits.

In order to be adaptive, a phenotypic trait must be expressed according to a genetic basis and must be related to fitness, such that the trait correlates with higher reproductive success (Barrett, 2010). In forest tree research, many technical handicaps hamper a direct measurement of fitness under controlled conditions such as those found in a common garden. Researchers have thus focused on traits putatively linked to fitness that are more amenable to measurement, like vegetative growth (Alía *et al.*, 1995; Chambel *et al.*, 2007), phenology (Codesido & Fernandez-Lopez, 2009), water use (Voltas *et al.*, 2008; Aranda *et al.*, 2010) and cold stress (Climent *et al.*, 2009) in order to illustrate patterns of local adaptation and population differentiation in several tree species. Life-history trait variation and evolution in Mediterranean pines has been recently studied (Grivet *et al.*, 2013) but basic account of within-species variation in reproductive traits is still largely missing, despite their relevance for understanding adaptation (but see Tapias *et al.*, 2001, 2004; Climent *et al.*, 2008).

AIMS

2. AIMS

Throughout my work I aimed to test basic evolutionary and ecological hypothesis derived from life-history theory, applied to the case of forest Mediterranean species such as *Pinus pinaster* and *P. halepensis*. I also intended that the research questions posed here could be related to practical issues regarding forest management and conservation of genetic resources. Specifically, my aims were:

- Document population differentiation in reproductive life-history traits such as the threshold size for reproduction and reproductive allometry (I, II, III, VI, VII)

- Test the hypothesis that reproductive life-history traits, as closely linked to fitness, are related to and modulated by the environment by means of plasticity (III) and natural selection (II, III, VII)

- Estimate quantitative genetic parameters (additive genetic variance, heritability, Qst) of reproductive life-history traits (I, IV, VII)

- Test the hypothesis that fitness components are negatively correlated by means of trade-offs, specifically between reproductive and vegetative growth traits by means of phenotypic correlations (I, V, VII), manipulation experiments (V), genetic correlations (I, II, III, IV, V, VII) and selection experiments (IV)

MATERIAL AND METHODS

3. MATERIAL AND METHODS

3.1 STUDY SPECIES

Pinus pinaster Ait. (maritime pine) and *Pinus halepensis* Mill. (Aleppo pine) are two Mediterranean pine species belonging to section *Pinus*, subsection *Pinaster*. *P. pinaster* occurs in Southern France, Northern Africa and the Iberian Peninsula, where glacial refugia have been suggested (Bucci *et al.*, 2007; de Lucas *et al.*, 2009). It has a very broad ecological niche, spanning from Atlantic to continental and semi-arid climates, and occurs at elevations ranging from sea level to 2000 m a.s.l. in Morocco.

P. halepensis has a broader geographical distribution, from Syria and Turkey in the East to the Eastern Iberian Peninsula in the West. Despite its common name, Aleppo pine, populations in the Eastern-most range are scarce and isolated, being more frequent a closely related species, *P. brutia* Ten.. *P. halepensis* also occurs around the Mediterranean basin in Greece, the Balkans, Italy, France, Israel, Tunisia, Libya, Algeria and Morocco (Chambel *et al.*, 2013). Present-day distribution is thought to be derived from a range expansion from Eastern populations towards the West (Grivet *et al.*, 2009b). Its ecological niche, though also broad, is more restricted to drier and warmer areas and has a greater tolerance to drought stress than *P. pinaster*. Both species coexist in Eastern and Southern Iberia along a coastal cline as well as in Northern Morocco (Soto *et al.*, 2010).

In Spain, the two species are found in vast areas in both natural and planted stands, as they have been extensively used in afforestation (Alía *et al.*, 1996; Gil *et al.*, 1996). *P. pinaster* is harvested for timber production mostly in the Atlantic region, and used to be an important species for resin production in Portugal and Central Spain (Perez *et al.*, 2013), an economic activity that is regaining importance. Recently, *P. pinaster* forests have also received attention as important producers of edible wild mushrooms (Gassibe *et al.*, 2011). Apart from this economic interest, both species are widely used for ecological restoration after forest fires and for former agricultural land conversion into forests, although their suitability for this objective has been largely debated during the last decades (Bellot *et al.*, 2004; Maestre & Cortina, 2004).

With such a high relevance, a comprehensive series of common garden provenance and provenance-progeny experiments of both species was implemented in Spain as well as in other Mediterranean countries (Chambel *et al.*, 2013). Rather than breeding, the main purpose of those experiments was to determine among-population ecotypic variation and to estimate quantitative genetic parameters for adaptive traits. This information is key to guide the use of forest reproductive materials and to infer the possible sources of adaptation to climate change. When replicated at contrasted sites, these series of common garden experiments are valuable to estimate phenotypic plasticity and genotype x environment interaction.

3.2 COMMON GARDEN EXPERIMENTS

Data presented in this study are derived from several common garden experiments installed in Spain and, in the most part, belonging to the Spanish National Forest Genetic Trial Network (www.genfored.es) (Figure 1). Plantation, maintenance and measurement of the common gardens has been mostly accomplished by the Centro de Investigación Forestal belonging to the Instituto Nacional de Investigación Agraria (INIA-CIFOR), in collaboration with the Spanish Government (former MMA, currently MMARM) as well as other regional institutions (CITA-Aragón, Junta de Castilla y León, Banc de Llavors). Collaboration from local forest services has been fundamental for the maintenance of such long-lasting experiments.

Except for trials F26MON (II), PREB (I) and PRIA (I), which are progeny tests from breeding programs, sampling was done randomly in wild populations. For each wild population, seeds were collected from about 30 trees at least 100 m apart from each other. Then, seeds were either bulked (provenance trials) or kept separate in families (progeny trials). Seedlings derived from those seeds were produced under identical conditions in a nursery and planted into the final common garden site after one or two years.

For *Pinus halepensis*, I used both provenance and provenance-progeny common garden experiments. The provenance common garden was installed in 1997, comprising 52 native range-wide provenances, originated in continental Spain, Balearic Islands (Spain), Italy, France, Tunisia and Greece. It consists of six replicates placed in contrasting environments. In the present study (III) I used data from only two of those sites (Valdeolmos, Madrid –P24OLM-, and Rincón de Ademuz, Valencia –P24ADE-), selected according to data availability and contrasting environmental conditions. P24OLM study site has a deep alluvial soil and the area has warm summers and mild winters. By contrast, P24ADE has a rocky and shallow soil at a windy slope with colder winters. This combination of soil and climate factors makes P24ADE site much more limiting for vegetative growth than P24OLM. Further details can be accessed in (III).

The *P. halepensis* provenance-progeny common garden was installed in 1995, being replicated in two sites, both included in the present study (I) (Megeces, Valladolid –F24MEG- and Montañana, Zaragoza, F24MON-). They comprise open pollinated families from 28 populations spanning the species distribution range in continental Spain and Balearic Islands, as well as progeny from three planted stands from inland Spain. Both trial sites also show a sharp environmental contrast. F24MEG is placed on a calcareous soil on a gentle slope, while F24MON is placed on a fertile alluvial soil with comparatively lower summer drought stress. Further details can be accessed in (I). In 2010, a subset of 110 trees in P24MEG common garden was also used in a manipulative experiment aiming at detecting somatic costs of reproduction (IV). First and second year developing female cones were carefully removed from half of the trees, remaining the other half as a control group. Vegetative growth and female reproduction were recorded during two years after treatment, and values for trees subject to treatment and control trees were compared. See (IV) for further details.

For *Pinus pinaster*, I used one provenance-progeny and three progeny common garden experiments, two of them not included in Genfored, but carried out by the Lourizán Forest Research Centre (Xunta de Galicia) (I). The provenance-progeny trial was installed in 2005, being replicated at five different sites, only two of them used in the present study (I,II) (A Merca, Orense –F26MER- and Cavada, Asturias –F26CAV-). They comprise open-pollinated families from 23 native populations spanning most of the species distribution range, including Atlantic Iberian Peninsula, Atlantic France, Corsica, Mediterranean Spain and Morocco. The ecological conditions of F26MER trial site can be considered intermediate respect to the species ecological niche, as it is placed in inland north-western Spain at a transition zone between Atlantic and Mediterranean climates. Further details can be accessed in (II). Ecological conditions of F26CAV are typically Atlantic, with higher summer rainfall than F26MER (I).

Figure 1. Location of *Pinus pinaster* and *P. halepensis* common gardens used in the present study. Red icons, *P. pinaster*; blue icons, *P. halepensis*. Check common garden code for a more detailed description in the text.

The two *P. pinaster* progeny trials reported in (I) (PREB and PRIA) were installed in 2003 with 28 progenies from selected plus trees from the Atlantic coast of Galicia (NW Spain). The *P. pinaster* progeny trial (F26MON) reported in (IV) was installed in 2001 at a highly continental area in the northern Iberian Range (Central-North Spain) as part of a breeding program in this Region of Provenance. It comprises open-pollinated families from phenotypically selected trees in natural stands neighbouring the trial site. It also contains the bulked progeny of a group of randomly selected trees from the same stands. The trial site has limiting conditions for vegetative growth due to cold winters and short summers plus a shallow and unfertile soil. Further details can be accessed in (IV).

3.3 MEASUREMENTS

All common gardens were measured periodically since their establishment so that wide data series were already available prior to this work. This was possible thanks to the above-mentioned cooperative work in the framework of Genfored. Recorded variables were related to vegetative growth and more recently to reproduction (Table 1). Total height was measured with a telescopic pole to the nearest cm and diameter at breast height with a calliper to the nearest mm. When possible, female cones were counted according to cohorts, attending to their size and colour. This was possible for most of the common gardens (F26MER, F26CAV, PREB, PRIA, F26MON, F24MEG, P24OLM, P24ADE), given the relatively small size of the trees. Female strobili are formed during spring and are red and small sized (approx. 1 cm long) (Figure 2). By the end of the first season their colour turns green and their size increases (2-3 cm long). During the second season they attain their final size (10-20 cm long *P. pinaster*, 10 cm *P. halepensis*) but remain green. Then they gradually change their colour into bright brown between the autumn of the second season and the beginning of the third season. From that moment, the colour of the zones of the cone most exposed to the sun starts fading, turning into pale grey (Gil *et al.*, 2009) (Figure 2). In F24MON, trees were large and crowns were close to each other, making it difficult to discriminate between cohorts. Instead, total female cones per tree were estimated from counts during 15 seconds (Knops & Koenig, 2012). This time interval was chosen after several tentative counts by different observers, aiming at combining a reasonable correlation with full cone count and a fast speed needed for measuring hundreds of trees with affordable field work schedules.

Figure 2. *Pinus pinaster* (a-e) and *P. halepensis* (f-j) female cone developmental stages and male cone clusters. a,f, female strobili emerged in spring; b,g one-year old female conelets; c, h, two-year old female cones; d, i, serotinous cones; e, j, male cone clusters.

Along ontogeny, male reproduction follows female reproduction in *P. halepensis* (Ne'eman *et al.*, 2004) but not in *P. pinaster*, indicating a more prominent role of early male reproduction in this species. Semi-quantitative data on male reproduction was recorded in F26MER common garden. Male pollen cone clusters were counted in 15 seconds and a qualitative assessment was used to estimate their size and density. Then, quantitative count data were weighed according to qualitative estimates.

As derived variables, stem volume over bark was used as an indicator of available resources in (I). Total biomass was estimated from allometric formulae, using diameter at breast height data (Montero *et al.*, 2005). The threshold size for reproduction was derived from binary models combining vegetative growth data and male or female binary reproduction data (presence / absence) (Wesselingh *et al.*, 1997) (I, II, III, IV, VII). Correlations between several directly measured and derived traits, indicative of phenotypic or genetic trade-offs were performed at several levels (individual, family or population) (I, II, III, IV, VII).

Table 1. Summary of reported *Pinus pinaster* and *P. halepensis* traits recorded in several common garden experiments (see corresponding appendix).

Species	**Trial**	**Appendix**	**Recorded trait**
P. pinaster	F26MER	I, II, VI, VII	Total height, diameter at breast height, quantitative female reproduction by cohorts and semi-quantitative male reproduction
	F26CAV	I	
	PREB PRIA	I	Total height, basal diameter and quantitative female reproduction by cohorts
	F26MON	IV	Total height, diameter at breast, qualitative stem form, quantitative female reproduction by cohorts and qualitative male reproduction
P. halepensis	F24MEG	I, V, VII	Total height, diameter at breast height, and female quantitative female by cohorts
	F24MON	I, VII	Total height, diameter at breast height, and total quantitative female reproduction
	P24OLM	III, VII	Total height, diameter at breast height, and quantitative female reproduction by cohorts
	P24ADE	III, VII	Total height, diameter at breast height, and quantitative female reproduction by cohorts

Environmental conditions (temperatures and rainfall) from the areas where sampled populations had evolved, as well as from the common garden sites, were obtained from the climatic models by Gonzalo-Jiménez (2010) for Iberian populations and by Hijmans *et al.* (2005) for non-Iberian populations (II, III). Two models were used because Gonzalo-Jiménez's model provides better estimates than others mostly due to the use of a denser data network, but it is only available for the Iberian Peninsula. Otherwise, both models are highly compatible. These data were used to test for the existence of ecotypic trends in phenotypic traits measured in the common gardens. Environmental conditions in the common garden were also used to compute ecological distances (Gower's distance) between the conditions of origin of the sampled populations and those at the common garden (II, III).

3.4 MOLECULAR DATA

Molecular marker data was used in (II) in order to control for strong neutral population genetic structure in *P. pinaster*, when testing for local adaptation patterns. Twelve nuclear microsatellite markers were genotyped in an average of 16 trees per population (range 6 - 30). Genotypic data was used to compute neutral genetic structure with the software STRUCTURE v2.2 (Pritchard *et al.*, 2000) (Figure 3). More details can be accessed in (II).

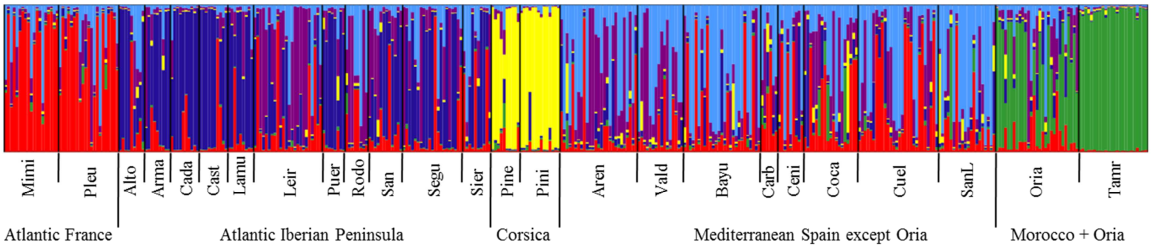


Figure 3. Barplot of individual-tree assignment probability to each of the optimal K = 6 clusters representing five geographical genetic groups in *Pinus pinaster* as produced by STRUCTURE 2.2 software. Each tree is represented as a line segment and vertically partitioned into K-coloured components, representing the individual's estimated proportions of ancestry in the K clusters. Population abbreviations are given in appendix II.

3.5 DATA ANALISYS

Gaussian variables were fitted with Linear Mixed Models (LMM) while Generalised Linear Mixed Models (GLMM) were used for non-Gaussian variables. Population and/or family within population were included as random factors and informed about population differentiation or additive genetic variance at the species level. In IV, a two-level fixed factor (selected and non-selected) was included in the models in order to test for selection effects. Adjusted population and family estimates were derived from those models and used in correlations with other traits and climatic variables.

Block structure from the experimental design was included in the models when analysing growth variables in order to minimise environmental noise. In some cases, block structure was not considered when estimating quantitative genetic parameters. This yields results closer to those expected in nature, which might be more meaningful for evolutionary studies (Hadfield *et al.*, 2010). Models fitted to reproductive trait data (threshold size for reproduction, cumulative reproductive investment and reproductive allometry) could include tree height, volume over bark or biomass as a covariate. Thus, results reflect variation in reproductive traits not merely due to size effects (Weiner *et al.*, 2009). Several quantitative genetic parameters were computed from variance components derived from mixed models in provenance-progenies or progenies trials, namely narrow-sense heritability (h^2), population differentiation (Q_{ST}) and coefficient of additive genetic variance (CV_A) (Falconer, 1989). Specific formulae to compute quantitative genetic parameters derived from Generalised Linear Mixed Models are described in IV and VI after (Nakagawa & Schielzeth, 2010).

RESULTS

4. RESULTS

4.1 POPULATION DIFFERENTIATION AND LOCAL ADAPTATION

Significant population differentiation for vegetative and reproductive traits both in *P. halepensis* and *P. pinaster* has been a common finding throughout this work (I, II, III, VI, VII), as indicated by significant population effects in statistical analyses (Table 2). In F26MER common garden, *P. pinaster* male and female sexual functions were analysed separately in the same individuals, allowing the study of early sex allocation patterns. Despite significant genetic differentiation for both sexual functions, male threshold size for reproduction was much less variable than female threshold size for reproduction, leading to variation in early sex allocation mainly due to variation in female function (Fig. 4).

Significant correlations between environmental variables and phenotypic traits, likely indicative of local adaptation were found for *P. pinaster* (II) and *P. halepensis* (III). In the case of *P. pinaster*, correlations with environmental variables were still significant after correcting for neutral genetic structure, a potentially important confounding factor. Equivalent molecular data for *P. halepensis* were not available and correlations were not corrected, but in this species neutral genetic structure is much weaker than in *P. pinaster*. For both species, results were remarkably similar, with variables representing enhanced and early reproduction being negatively correlated with environmental variables indicative of favourable growth conditions, mostly higher winter temperatures and lower summer temperatures. Surprisingly, rainfall regimes were little correlated with the variation patterns found.

Table 2. Vegetative and reproductive traits for which evidence of population differentiation was found in several *Pinus pinaster* and *P. halepensis* common gardens.

	Trait	**Trial**	**Species**	**Appendix**
Vegetative	Height	F24MEG, F24MON, P24ADE, P24OLM F26MER	*P. halepensis*, *P. pinaster*	III, VII, II, VII
	Volume over bark	F24MEG, F24MON, PREB, PRIA	*P. halepensis*, *P. pinaster*	I
	Biomass	F24MEG, F24MON, P24ADE, P24OLM, F26MER	*P. halepensis*, *P. pinaster*	III, VII, VII
Reproductive	Female cumulative repr. investment	F24MEG, F24MON, P24ADE, P24OLM, F26MER	*P. halepensis*, *P. pinaster*	III, VII, VII
	Female repr. allocation	F24MEG, F24MON, P24ADE, P24AOLM PREB, PRIA	*P. halepensis*, *P. pinaster*	I, III
	Repr.allometry	P24ADE, P24OLM, F26MER	*P. halepensis*, *P. pinaster*	III, VII
	Female threshold size for reproduction	F24MEG, F24MON, P24ADE, P24OLM, F26MER, F26CAV	*P. halepensis*, *P. pinaster*	I, III, VII, VII
	Male threshold size for reproduction	F24MEG, F26MER	*P. halepensis* *P. pinaster*	II, VI, VII

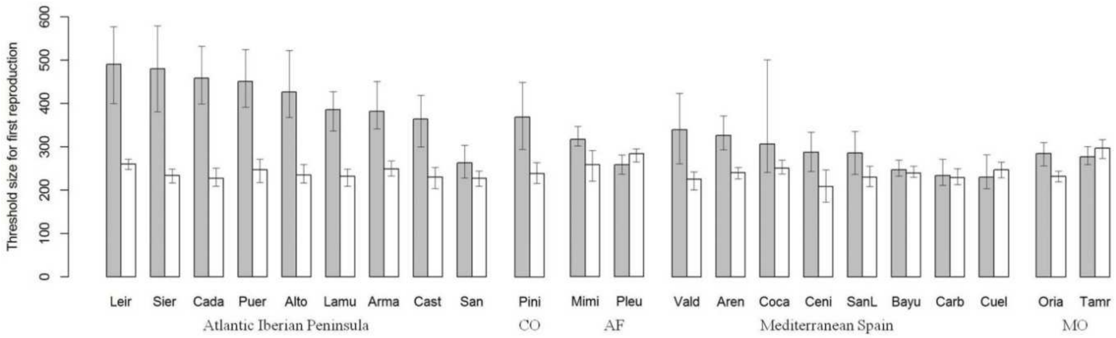


Figure 4. Median threshold size for first reproduction through male (white bars) and female (grey bars) functions in *Pinus pinaster* populations grown in F26MER common garden in north-west Spain. Bars represent the posterior mode of Bayesian estimates, with lower and upper 95% credible intervals. Population abbreviations are given in appendix II.

4.2 PHENOTYPIC PLASTICITY

The study of phenotypic plasticity and genotype by environment patterns for reproductive traits was first addressed in (I) and then more explicitly in (III). The allometry of reproduction in *P. halepensis* responded plastically to environmental conditions, expressing an advanced ontogeny (enhanced reproduction) when grown under environmental stress (III) (Figure 5). Interestingly, cumulative reproductive investment was similar between two contrasted sites (III, Figure 5). A consistent finding across species and sites was the absence of relevant genotype by environment interaction for reproductive traits (threshold size for reproduction, reproductive allometry or cumulative reproductive investment) indicated either by high site-to-site correlations (I, III) or non-significant site by populations terms (III). This result contrasted with that found for vegetative traits (height, biomass, volume over bark) where site-to-site correlations were low and site by population terms were highly significant.

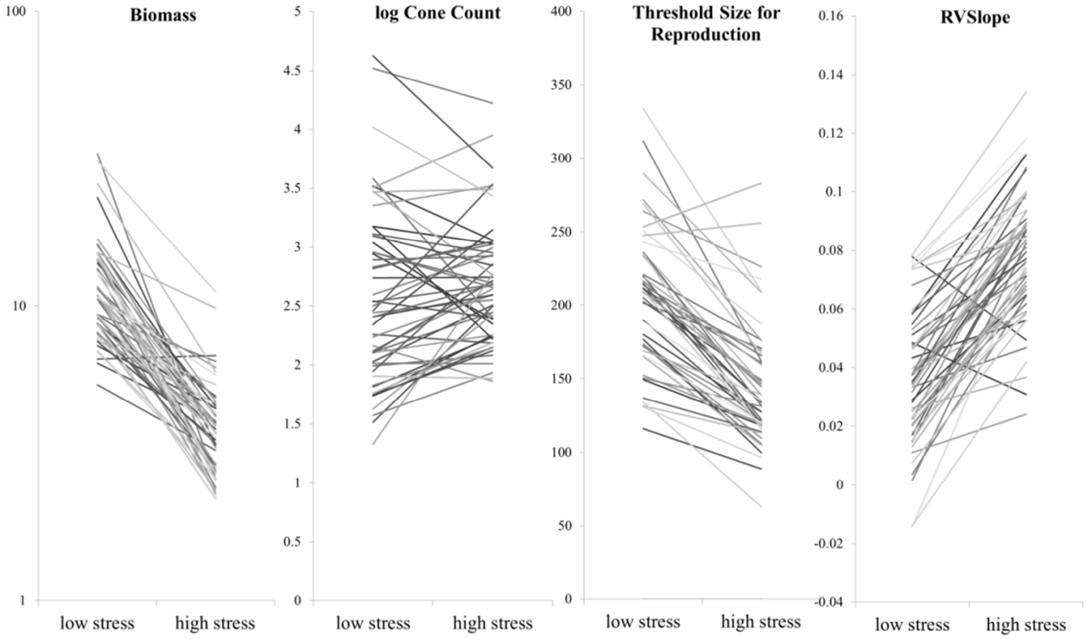


Figure 5. Reaction norms for vegetative and reproductive traits measured in two *Pinus halepensis* provenance common gardens subject to contrasting environmental conditions (P24OLM low and P24ADE high environmental stress). Lines connect mean values per source population at each common garden. Variables and units are as defined in appendix III.

4.3 ADDITIVE GENETIC VARIATION AND SHORT TERM GENETIC CHANGE IN REPRODUCTIVE LIFE-HISTORY TRAITS

Heritabilities for vegetative and reproductive traits are reported in (I, IV, VI and VII). Heritabilities for vegetative traits were lower than those for vegetative traits in both species, except for male function in IV, which was bound to cero. When comparing male and female function, no patterns emerged for range-wide populations in *P. pinaster*, with moderate to high heritabilities (0.27-0.73) (VI).

A single event of artificial selection was sufficient to produce genetic changes in all recorded traits at a young age, except diameter at breast height and biomass (IV). Meaningfully, direct response to selection in vegetative traits was much weaker than the indirect response for both male and female reproductive traits.

4.4 COSTS OF REPRODUCTION

The study of costs of reproduction, either phenotypic or genetic, was present all along this work (I, II, III, IV, VII) and was specifically addressed in V. Altogether, all four approaches to the description of costs of reproduction were explored: phenotypic correlations (I, V), manipulation experiments (V), genetic correlations (I, V) and selection experiments (IV).

Phenotypic correlations between variables relating vegetative size and absolute female reproductive size were invariably positive, and ranged from a minimum of 0.12 in *Pinus pinaster* (I) to a maximum of 0.56 in *P. halepensis* (I), indicating that larger trees tended to produce more seed cones (Figure 6). When vegetative size was compared with reproductive allocation, correlations tended to be negative [-0.12 – -0.18 *P. pinaster* (I), -0.40 – 0.11 *P. halepensis* (I, V)], indicating that small trees tended to produce a proportionally higher number of seed cones. In *P. pinaster*, trees that started their reproductive phase producing only females cones were smaller than those that started producing only male cones, while cosexuals were the largest reproductive class (I, II, IV). In *P. halepensis*, there were virtually no trees producing only male cones (I).

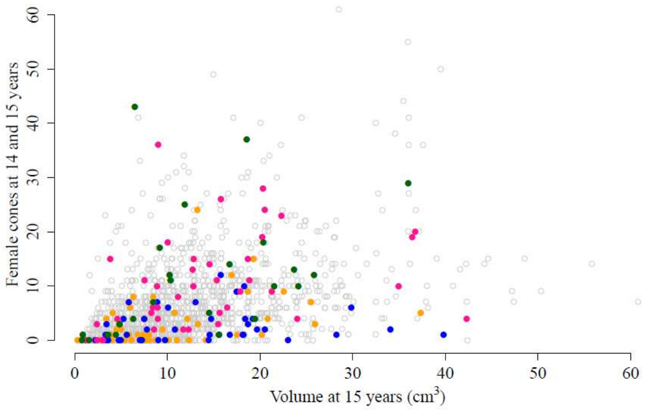


Figure 6. Scatterplot of the number of developing female cones (emerged in the 14th and 15th year, respectively) versus tree size at 15 years, as estimated by the stem volume over bark, in several provenances of *Pinus halepensis* trees grown in F24MEG common garden. Blue dots, Cabanellas population; yellow dots, Santany population; pink dots, Cazorla population; green dots, Villavieja de Tordesillas population. $r = 0.43$; 95% confidence interval 0.39-0.47; p-value <0.001; $n = 1385$.

The results from the manipulation experiment (V) indicated that removal of female cones prior to their development resulted in a marginally significant ($P < 0.07$) increment of growth the same year after treatment, but the effect vanished one year after $P = 0.77$). In contrast, there was a more significant increase of female reproduction one year after cone removal ($P < 0.01$) (Figure 7).

Genetic correlations between vegetative size and absolute female reproductive size yielded contrasting results. Strong negative genetic correlations were found for *P. halepensis* in F24MON (I) and weak negative for *P. pinaster* in PREB and PRIA, opposing phenotypic correlations in those three cases and thus implying strong positive environmental correlations. Genetic correlations in F24MEG (I, V) were also lower than phenotypic correlations, but remained positive. Genetic correlations between vegetative size and reproductive allocation were all negative and commonly strong in both species (I, II). Among-population correlations, indicative of genetically-based trade-offs were also reported in II and III for *P. pinaster* and *P. halepensis*, respectively. A positive correlation between size and threshold size for female reproduction (i.e. delayed reproduction at bigger sizes) was found, although only under a stressful environment for *P. halepensis*.

Results from the selection experiment (IV) provided stronger evidence for genetically-based costs of reproduction in *P. pinaster*, as progeny from trees selected for increased growth also showed a reduced investment in both male and female reproduction.

This was indicated by increased threshold sizes and enhanced size-corrected reproduction for male and female reproductive functions (Figure 8).

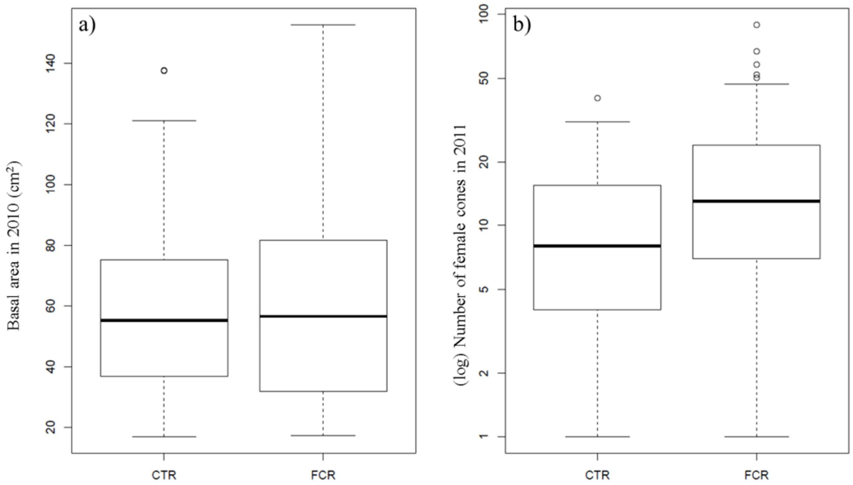


Figure 7. Boxplots of a) basal area in 2010, one growing season after experimental manipulation, and b) number of female cones in 2011, one reproductive season after experimental manipulation comparing control *Pinus halepensis* individuals (CTR) with others subjected to experimental removal of developing female cones (FCR). Appendix V.

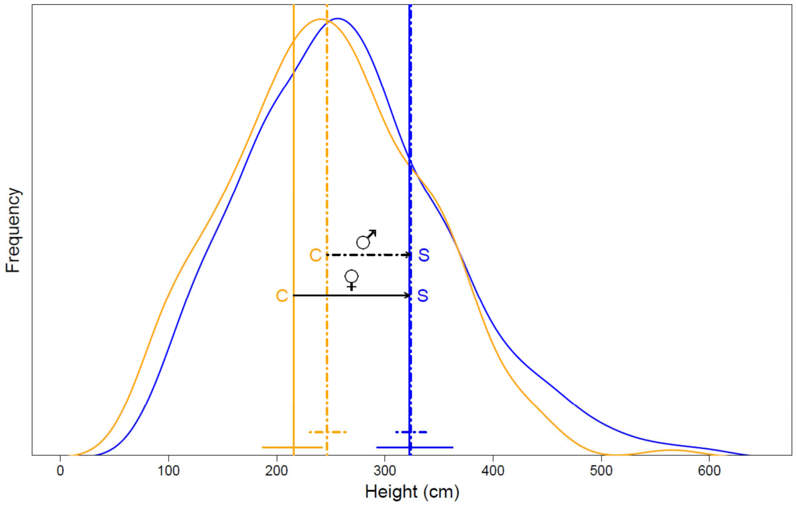


Figure 8. Comparison of height density distribution and threshold size (height) for first reproduction between *Pinus pinaster* progenies of trees either selected (S) or not selected (C, control) for timber production after one generation. F26MON common garden. Bell-shaped lines represent height probability distribution at age 10 yrs. Control group, orange lines; selected group, blue lines. Vertical lines represent threshold sizes for reproduction. Solid line, female function; broken line, male function. 95% Credible intervals for threshold sizes are represented by horizontal lines. Arrows show changes in threshold size for reproduction in male and female function due to selection. Control group, left; selected group, right. Appendix VII

DISCUSSION

5. DISCUSSION

Results presented throughout this study based on data from range-wide *P. pinaster* and *P. halepensis* common garden experiments have revealed significant population variation for several key reproductive traits. These findings, that build up on previous work in *P.halepensis* (Climent *et al.*, 2008) and *P. pinaster* (Tapias *et al.*, 2004), are among the first reports in the forestry literature on this topic. Results also highlight a close relationship between reproductive traits and provenance environmental variables i.e. those found where populations had evolved, as well as between reproductive and growth traits.

5.1 POPULATION DIFFERENTIATION AND LOCAL ADAPTATION

The existence of within-species population variation for adaptive traits is a well-established fact for species with extensive distribution ranges e.g. *P. contorta* (Rehfeldt *et al.*, 1999) and *P. sylvestris* (Rehfeldt *et al.*, 2002), or for those showing a wide neutral genetic differentiation between populations e.g. *P. pinaster* (Alía *et al.*, 1995). This is less acknowledged for species that do not meet those conditions, like *P. halepensis*. But even within the Iberian Peninsula, where neutral genetic diversity is lowest due to recent migration (Grivet *et al.*, 2009b), population differentiation is also present in this species. So, barring some exceptional cases like *P. pinea* (Mutke *et al.*, 2010), within-species variation is a frequent finding (Alberto *et al.*, 2013).

Local adaptation in plants is commonly tested by comparing fitness of local versus non-local populations grown in reciprocal transplant experiments (Leimu & Fischer, 2008). Unfortunately, in tree studies such comprehensive tests are often unaffordable and usually, many populations are tested in only one or few common gardens. Evidence of local adaptation can nonetheless be inferred from the relationship between phenotypic trait values measured at the common garden, and environmental descriptors of the areas where populations have evolved. Also, under the hypothesis of local adaptation, it is expected a positive correlation between fitness and a measure of how similar is the common garden environment compared to that where populations have evolved e.g. Gower's environmental distance. In the present study, the aforementioned population variation found both for vegetative and reproductive traits in *P. pinaster* (I, II, VII) and *P.*

halepensis (I, III, VII), did not occur haphazardly. Instead, mounting evidence suggests that indeed adaptive phenotypic variation is related to environmental conditions where populations have evolved (reviewed in Alberto *et al.*, 2013) suggesting that studied traits have adaptive value and that local adaptation processes have occurred and might still be occurring. Additionally, when phenotypic traits are measured directly in native populations rather than in common gardens, population differentiation tends to be much larger, given synergistic phenotypic and genetic variation i.e. co-gradient variation (Kremer *et al.*, 2013). Phenological traits related to cold damage like bud burst and growth cessation, are commonly used as examples of traits with adaptive clinal variation (Savolainen *et al.*, 2007; Neale & Kremer, 2011). The results presented in this study (II, III), add reproductive traits to that list.

Drought is thought to be an important limiting factor for Mediterranean species (Baquedano *et al.*, 2008), and thus a correlation between rainfall and life-history traits was expected. Also, summer rainfall is correlated with area burnt in forest fires (Pausas, 2004; Pausas & Paula, 2012), and can potentially be linked to fire-related traits such as serotiny (Hernández-Serrano *et al.* in prep). However, it was the continentality index (annual temperature range) that lied among the climatic variables that best correlated with reproductive traits. Precocious populations tended to originate in areas with low winter and high summer temperatures, i.e. large continentality index, that define short growing seasons. Populations with delayed reproductive ontogeny tended to originate in areas with mild winter and summer temperatures, i.e. low continentality index that define long growing seasons. This pattern was stronger in *P. pinaster* (II) than in *P. halepensis* (III). These results largely agree with assumptions derived from life history theory, predicting a delayed reproduction (both in age and size terms) in those environments that favour vegetative growth (Roff, 1992b). The extent to which genetically-based patterns are enhanced or counteracted by phenotypic plasticity in natural *P. pinaster* or *P. halepensis* populations, resulting in co-gradient or counter-gradient variation remains unexplored. Results obtained in a replicated common garden experiment (III, see below), point towards co-gradient variation for genetic and environmental effects.

The study of population variation in early male reproductive function, addressed for *P. pinaster*, provided a different picture. Among-population differences in male threshold size for reproduction (TSR), despite significant, varied much less than those for female TSR, and they were not correlated with any environmental variable. A likely explanation for these results can be derived from size-dependent sex allocation theory applied to wind-pollinated plants (Klinkhamer *et al.*, 1997), and specifically to monoecious trees (Fox, 1993). It is believed that fitness gain curves in wind pollinated species will be saturating for female function, but not or to a much lesser extent for male function. Also, male function is likely to benefit more from indirect size effects (de Jong & Klinkhamer, 1994). This seems to be corroborated by parentage studies in wind pollinated trees, as the number of sires and male migrants contributing to the next generation is much higher than that of females (Bittencourt & Sebbenn, 2007; Grivet *et al.*, 2009a; Robledo-Arnuncio, 2011). Such combination of male and female fitness gain curves theoretically leads to increased male sex allocation with size, a pattern confirmed in this study in *P. pinaster* (VI) and independently also in *P. halepensis* (Ne'eman *et al.*, 2011). Here, the role of height, although highly correlated with total size, seems to be indirect for the explanation of size-dependent sex allocation. This is because in conifer species like *P. pinaster* (II) and *P. halepensis* (Shmida *et al.*, 2000) gender is segregated in tree crowns, with female cones placed on top of the tree. The existence of low variability in the male TSR can then be related to uniform conditions across populations for pollen dispersal, depending on tree height. This would set a relatively narrow threshold from which release of pollen is efficient, but above which no major increases in efficiency are gained (II). These results will also need to be contrasted with quantitative male reproduction at a more advanced age.

5.2 ADAPTIVE PROCESSES WITHIN POPULATIONS

The vision that reproductive traits are important targets of natural selection is widely supported in plants by increasing evidence (Munguía-Rosas *et al.*, 2011; Shaw & Etterson, 2012): first, genetically-based clinal variation in reproductive traits has been commonly reported for short lived plants, interestingly, also for species in introduced ranges (Colautti *et al.*, 2010; Lachmuth *et al.*, 2011). Second, variation in reproductive traits has been related to species persistence in native plant communities (Willis *et al.*, 2008; Dante *et al.*, 2013). And third, microevolutionary processes in annual species can produce significant changes in reproductive traits in only few generations (Franks *et al.*, 2007; Sultan *et al.*, 2013). The question seems solved for short lived plants species, but will forest trees be able to respond to selective pressures if reproductive traits are targeted? (Davis *et al.*, 2005)

Selection acts on phenotypes, which are the result of the interaction between particular genotypes and particular environments. Local-scale environmental influence varies on different traits, being the expression of vegetative growth commonly affected by small-scale spatial heterogeneity (Zas, 2006) as opposed to the allometry of reproduction (II, III). Similarly to phenotypic variation, genetic variance of plastic traits is also dependent on the environment (Sgrò & Hoffmann, 2004; Matesanz *et al.*, 2013). The present study suggests that the expression of growth traits under unfavourable conditions can be more constrained than that of reproductive traits, despite both exhibit plasticity (III). Also, significant genotype by environment interaction for growth traits but not for reproductive traits (I, III) may be related to the spatial scale at which the environment influences those traits (van Kleunen & Fischer, 2005; Rodríguez, 2012).

A factor known to lower the effectiveness of selective processes is the expression of adaptive phenotypic plasticity (static adaptation) (van Kleunen & Fischer, 2005). Reproductive allocation in short lived plants had been thought to be one of the less plastic traits (Weiner *et al.*, 2009). Nonetheless, it has been suggested that plasticity of reproductive allocation in long-lived sessile species like trees should be relevant (Burd *et al.*, 2006), but there is a scarcity of studies reporting on this subject. The case of adaptive plasticity of reproductive allocation in range-wide *P. halepensis* populations described in this study is therefore particularly important (III). Trees grown under a more stressful environment reached sexual maturity earlier, both in time and in size, than under a favourable environment. Notably, this response matches genetic responses to selection in climatic clines (II, VII).

Given that only genetic differences can promote evolutionary change, and considering single traits, a higher correlation between genotype and phenotype implies a higher efficiency in selective processes, as defined in the classical breeder's equation (Cornelius, 1994; Lefèvre *et al.*, 2013). In the present study, higher heritability values for reproductive versus growth traits (I, VI) suggest a more efficient selection for reproductive traits. A greater change due to artificial selection in reproductive traits compared to growth traits (IV) supports this idea. Interestingly, other phenotypic traits that also show clinal variation like bud burst and bud set also have high heritabilities (>0.5) (Neale & Kremer, 2011). Still, the only currency of natural selection is fitness, and only those phenotypic traits genetically correlated with fitness (either positively or negatively) will experience genetic changes after selection. This is known as the Robertson-Price identity (Robertson, 1966; Price, 1970), and currently there is a lack of studies applying it to trees. High within-population genetic variation and its phenotypic expression, likely indicate that reproductive life-history traits will indeed respond to natural selective forces. This response might be delayed by the expression of adaptive phenotypic plasticity (Matesanz *et al.*, 2012; Anderson *et al.*, 2012), although considering large time scales, early reproduction has also been related to increased evolutionary rates (Verdú, 2002).

5.3 COSTS OF REPRODUCTION AND TRADE-OFFS

Considering the principles of allocation and life-history theory (Stearns, 1989), for a given amount of resources an increase in reproduction is typically related to a decrease in growth (Roff, 2000; Obeso, 2002; Thomas, 2011). At the individual level, trade-offs might not be evident given that not all trees have access to the same level of resources (Reznick *et al.*, 2000). Within a population, a tree with access to more resources e.g. deeper soil, can attain a larger size and at the same time produce a higher number of seeds than other i.e. a positive environmental correlation. This, in turn, creates a positive relationship between growth and reproduction for the whole population (de Jong & Klinkhamer, 2005). Forest management practices aimed at increasing cone production in a given forest stand (thinning, pruning, fertilization), exploit this phenotypic relationship between large size and large seed production (Gonzalez-Ochoa *et al.*, 2004; De Las Heras *et al.*, 2007; Ortiz *et al.*, 2011; Ruano *et al.*, 2013).

Potentially confounding factors can be controlled, for example by submitting to experimental manipulation a given group of trees and comparing the response to an equivalent control group (Reznick, 1992). The results of such an experiment in young *P. halepensis* trees provided some insight into the expression of somatic costs of reproduction in this species (III). Removal of developing female reproductive structures was related to a 70% increase in reproduction the following season, compared to the control group. Contrarily, the response in terms of vegetative growth was weak. This suggests that costs of reproduction in this species might be more relevant in terms of future reproduction, rather than just considering vegetative growth. A cost of current reproduction paid in future reproduction has indeed a deeper evolutionary significance (Reznick, 1985; Stearns, 1989) but evidence in plants is not overwhelming (Obeso, 2002) and has been rarely considered in tree species (Thomas, 2011).

Evidence of somatic costs of reproduction is also inferred from differences in average size for trees starting their reproductive phase as males, females or cosexuals (I, II and VI). Early females were smaller than early males and cosexuals, suggesting a higher cost of reproduction for female function (Montesinos *et al.*, 2006; Verdú *et al.*, 2007). Nonetheless, the pattern is somewhat blurred given that cosexuals, bearing both female and male reproductive structures, are among the largest individuals. It is therefore difficult to separate to what extent reproduction is dragging growth, and in turn, to what extent vigour can help overcome those costs of reproduction. Perhaps, a more direct approach of female versus male costs of reproduction could be achieved in dioecious species (Montesinos *et al.*, 2012), which in conifers tend to be dispersed by animals (Givnish, 1980). Also, in mature cosexual pine trees investment in male function is typically very high, and likely to be costly. The hypothesis that male sexual function in conifers has been enhanced by sexual selection remains unexplored (Moore & Pannell, 2011; Pannell & Labouche, 2013).

Phenotypic correlations between size and reproduction where invariably positive for all datasets analysed in this study. In fact, positive phenotypic vegetative-reproductive size correlations, are the base for general allometric models (Niklas & Enquist, 2003), and have been rightly considered as proof for the adaptive value of size. A few studies on parentage analysis have also been able to find a positive correlation between tree size and the number of seedlings established in a forest stand (González-Martínez *et al.*, 2006; Moran & Clark, 2012). But this does not necessarily mean that size is an accurate predictor of reproduction. Taking the relationship one step further, and considering it strong and invariable, growth traits have been repeatedly used as surrogates of fitness in forest trees (Wu & Ying, 2004; Savolainen *et al.*, 2007; Oddou-Muratorio & Klein, 2008; Ramírez-Valiente *et al.*, 2010). The allometry of reproduction has been studied most extensively in short lived plants, where statistical models with plant size as an explanatory variable typically explain 90% of reproductive variance (Samson & Werk, 1986; Weiner *et al.*, 2009). Instead, the relationship between vegetative and reproductive size in trees is far from being tight, and models with highly significant correlations usually only explain a low proportion of variance e.g. (House, 1992; Haymes & Fox, 2012; Granado-Yela *et al.*, 2013). For long lived species, relevant variation of reproduction after accounting for size effects is left to be explained by additional factors like the environment or the genetic background (III).

An interesting case of loose allometric relationships between vegetative and reproductive size is described in *P. halepensis*, where some large trees, well beyond their threshold size for reproduction produce none or very few cones (Climent *et al.*, 2008 and III). Under the assumption of a deterministic allometric model, those trees will eventually outperform smaller trees in terms of reproductive output. This is known as a bang-bang strategy (Falster & Westoby, 2003; Weiner *et al.*, 2009). Despite this assumptions seems reasonable, fuzzy vegetative-reproductive size relationships are common, and the fate of those trees is nonetheless uncertain. Could it be possible that trees with low reproductive allocation are also over-represented in the largest tree classes in forest stands? In such case, this could be the outcome of senescence (Genet *et al.*, 2010; Moran & Clark, 2012), an invalid explanation for young trees. Another option is that large trees with low or no reproductive investment could also arise merely due to (lack of) phenotypic costs of reproduction, as attaining large sizes may only be possible if investment in reproduction is low (Moran & Clark, 2012). Also, besides environmental plastic effects (III), and given within-population genetic variability for the allometry of reproduction (I, VI), the occurrence of such phenotypes can have a genetic basis.

As mentioned above, a genetic trade-off between investment in growth and reproduction is in fact a general expectation derived from life-history theory (Roff, 1992a), provided that no third variables interact (Roff, 2000). Under this assumption, a high genetic breeding value for growth implies a lower investment in reproduction, a possibility already mentioned in the forestry literature (Loehle *et al.*, 1987). Genetic correlations between reproduction and growth reported in this study did not support this view unambiguously (I, III, V, VI and VII). It seems clear however, that both phenotypic and environmental correlations are positive, and that genetic correlations are smaller even though not always negative. As genetic correlations are known to be environmental-dependent (Sgrò & Hoffmann, 2004 and III), a meta-analytical approach will be useful to shed light on the issue. Also, studies that integrate a comprehensive set of fitness-related traits will provide a more realistic picture of adaptive strategies (Pigliucci, 2004). An integrated analysis of further traits such as constitutive and induced defences (Sampedro *et al.*, 2011), drought resistance (Voltas *et al.*, 2008), cold tolerance (Climent *et al.*, 2009), phenology (Salmela *et al.*, 2013), serotiny (Hernández-Serrano *et al.* in prep), stem architecture (Sierra-de-Grado *et al.*, 1999), ontogenetic heteroblasty (Climent *et al.*, 2013), bark thickness (Tapias *et al.*, 2004) and seed size and dispersal ability (Santos-del-Blanco & Climent, 2011) will be highly valuable.

Despite inconsistencies in genetic correlations, empirical evidence for within-population genetic variation in allocation to reproduction trading-off with variation in size is provided in (IV). Progeny of trees selected under natural conditions for good growth and stem form did also show reduced allocation to reproduction during their early developmental stages. It can be argued that those trees will also end up producing large amounts of cones later in development (bang-bang strategy), but the example at least provides evidence for the relevance of the genetic basis of reproduction, even under natural conditions where environmental variation is high (Wilson *et al.*, 2010). Other studies have also revealed negative genetic correlations between growth and other adaptive traits like drought resistance (Kaya *et al.*, 1994) and cold hardiness (Howe & Aitken, 2003), which altogether add to the importance of recording and integrating traits other than vegetative growth in order to define adaptation.

5.4 FITNESS TRAITS AND ADAPTIVE FOREST MANAGEMENT

The present study raises concern about considering size solely as a surrogate for fitness (III), even more if size is taken as a criterion to select trees to produce seed for regeneration in managed stands (Lefèvre *et al.*, 2013). Measuring fitness is a gigantic and mostly unreachable task in forest trees (Shaw & Etterson, 2012). Although seedling paternity analysis in forest stands can be used to relate realised fitness with certain phenotypic traits of parent trees (Burczyk *et al.*, 2006), large population sizes, large distribution areas and extensive gene flow distances together with changing selective pressures along development make the results only partially realistic.

Still, this kind of studies is hugely valuable to assign weighed importance to specific phenotypic traits such as size, and justify their use as surrogates of fitness (Moran & Clark, 2012). As expected, the few examples available to date do find a positive relationship between size and reproductive success (Oddou-Muratorio & Klein, 2008; Oddou-Muratorio *et al.*, 2010), but on top of that, also one between reproduction and reproductive success (González-Martínez *et al.*, 2006; Piotti *et al.*, 2009). Several studies in seed orchards have also shown a correlation between pollen production and reproductive success (Schoen & Stewart, 1986; Goto *et al.*, 2005; Moriguchi *et al.*, 2007; Hansen & Nielsen, 2010; Doerksen *et al.*, 2011).

Importantly, under a scenario of increased disturbance events such as forest fires and extreme drought (Lindner *et al.*, 2010), life expectancy of trees might be lower than in the present –particularly in the western Mediterranean basin, a hotspot of predicted climate change. Such scenario is likely to select for early reproduction, with trees investing resources in reproduction early in life (Kozłowski, 1992; Munguía-Rosas *et al.*, 2011) rather than expecting future unlikely rewards in terms of increased reproductive output.

The use of size as a surrogate for fitness is also frequently justified as height and diameter at breast height are much easier to estimate than reproductive output (Wu & Ying, 2004). It seems necessary then to improve and develop methods to estimate reproductive output fast and accurately (Koenig *et al.*, 1994, 2013; LaMontagne *et al.*, 2005) (I, III, VI). Though, for the same reasons stated above, namely negative genetic correlations, it will not be desirable to rely solely on reproductive output as a criterion for selection (Varghese *et al.*, 2009).

Recently a new paradigm of adaptive forest management has emerged, aiming to maintain evolutionary processes and adaptive potential of managed populations (Koskela *et al.*, 2013; Lefèvre *et al.*, 2013). In order to achieve it, a better understanding of evolutionary processes shaping fitness traits will be necessary. Also, as exposed throughout this study, phenotypic data obtained from common garden experiments will be relevant in order to characterise forest reproductive materials and to aid in sustainable forest management.

CONCLUSIONS

6. CONCLUSIONS

I. *Pinus pinaster* and *P. halepensis* range-wide populations vary significantly in terms of reproductive life-history traits like the threshold size for reproduction, reproductive output and reproductive allometry.

II. Genetically-based population mean values for reproductive life-history traits tend to vary clinally according to environmental factors, particularly those determining the length of the growing season.

III. In *Pinus pinaster*, clinal variation was not found for the threshold size for male reproduction. This result could be explained by environment-independent male fitness gain curves, as conditions for pollen dispersal in this wind-pollinated species seem to be uniform across populations.

IV. The allometry of reproduction shows putatively adaptive plasticity in *Pinus halepensis* as trees accelerate their sexual development under stressful conditions. This is a likely scenario to be found under climate change, and may provide tree populations with enhanced resilience.

V. There is substantial within-population genetic variation for reproductive life-history traits, and these show higher heritabilities than growth traits, allowing for steady adaptive processes.

VI. Somatic costs of reproduction in *Pinus halepensis* were detected by means of a manipulation experiment but not by a correlational study. In this species, reproduction had a greater effect in terms of future reproduction than in terms of growth.

VII. Genetic costs of reproduction in terms of growth were more elusive to be detected and might be species- and environment-dependent. However, a selection experiment in *Pinus pinaster* aimed at increasing growth yield, resulted in a strong correlated negative response in reproductive traits, indicating a genetically based trade-off between growth and reproduction.

VIII. Considering the prevalence of a loose relationship between reproduction and vegetative size in trees, and the likely existence of a genetic trade-off between reproduction and growth, it is advised against the use of size as a single surrogate of fitness. Instead, obtaining reproductive data is encouraged if criteria for achieving adaptive forest management are to be met.

IX. To the extent that the described population variation is relevant for (static) adaptation, population characterization will be valuable for conservation and sustainable management of forest genetic resources. Standing genetic variation and differentiation also provides insight into past adaptive processes and inform about likely future evolutionary responses.

REFERENCES

7. REFERENCES

Alberto FJ, Aitken SN, Alía R, González-Martínez SC, Hänninen H, Kremer A, Lefèvre F, Lenormand T, Yeaman S, Whetten R, *et al.* 2013. Potential for evolutionary responses to climate change - evidence from tree populations. *Global Change Biology* **19**: 1645–1661.

Alexander JM, Edwards PJ, Poll M, Parks CG, Dietz H. **2009**. Establishment of parallel altitudinal clines in traits of native and introduced forbs. *Ecology* **90**: 612–622.

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

Alía R, Martín S, de Miguel J, Galera R, Agúndez D, Gordo J, Salvador L, Catalán G, Gil L. **1996**. *Las regiones de procedencia de* Pinus pinaster *Aiton.* Ministerio de Medio Ambiente, Madrid, Spain.

Anderson JT, Inouye DW, McKinney AM, Colautti RI, Mitchell-Olds T. **2012**. Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. *Proceedings of the Royal Society of London. Series B. Biological Sciences* **279**: 3843–3852.

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

Ashman T-L. **2006**. The evolution of separate sexes: a focus on the ecological context. In: Harder LD, Barrett SCH, eds. Ecology and evolution of flowers. Oxford University Press, USA, 204–222.

Baquedano FJ, Valladares F, Castillo FJ. **2008**. Phenotypic plasticity blurs ecotypic divergence in the response of *Quercus coccifera* and *Pinus halepensis* to water stress. *European Journal of Forest Research* **127**: 495–506.

Barrett SCH. **2010**. Understanding plant reproductive diversity. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**: 99–109.

Barrett SCH, Yakimowski S. **2010**. Ecological genetics of sex ratios in plant populations. *Proceedings of the Royal Society of London. Series B. Biological Sciences* **365**: 2549–2557.

Bellot J, Maestre FT, Chirino E, Hernández N, de Urbina JO. **2004**. Afforestation with *Pinus halepensis* reduces native shrub performance in a Mediterranean semiarid area. *Acta Oecologica* **25**: 7–15.

Bittencourt JVM, Sebbenn AM. **2007**. Patterns of pollen and seed dispersal in a small, fragmented population of the wind-pollinated tree *Araucaria angustifolia* in southern Brazil. *Heredity* **99**: 580–591.

Bonser SP, Ladd B, Monro K, Hall MD, Forster MA. **2010**. The adaptive value of functional and life-history traits across fertility treatments in an annual plant. *Annals of Botany* **106**: 979–988.

Brys R, Shefferson R, Jacquemyn H. **2011**. Impact of herbivory on flowering behaviour and life history trade-offs in a polycarpic herb: a 10-year experiment. *Oecologia* **166**: 293–309.

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

Burczyk J, Adams WT, Birkes DS, Chybicki IJ. **2006**. Using genetic markers to directly estimate gene flow and reproductive success parameters in plants on the basis of naturally regenerated seedlings. *Genetics* **173**: 363–372.

Burd M, Allen TFH. **1988**. Sexual allocation strategy in wind-pollinated plants. *Evolution* **42**: 403–407.

Burd M, Read J, Sanson GD, Jaffré T. **2006**. Age-size plasticity for reproduction in monocarpic plants. *Ecology* **87**: 2755–2764.

Burgess KS, Etterson JR, Galloway LF. **2007**. Artificial selection shifts flowering phenology and other correlated traits in an autotetraploid herb. *Heredity* **99**: 641–648.

Callahan HS, Pigliucci M. **2002**. Shade-induced plasticity and its ecological significance in wild populations of *Arabidopsis thaliana*. *Ecology* **83**: 1965–1980.

Case A, Ashman T-L. **2005**. Sex-specific physiology and its implications for the cost of reproduction. *Reproductive Allocation in Plants*.

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

Chambel MR, Climent J, Pichot C, Ducci F. **2013**. Mediterranean Pines (*Pinus halepensis* Mill. and *brutia* Ten.). In: Pâques LE, ed. Forest Tree Breeding in Europe: Current State-of-the-Art and Perspectives. Dordrecht: Springer Netherlands, 229–265.

Charnov EL. **1982**. *The theory of sex allocation*. Princeton, NJ: Princeton University Press.

Childs DZ, Metcalf CJE, Rees M. **2010**. Evolutionary bet-hedging in the real world: empirical evidence and challenges revealed by plants. *Proceedings of the Royal Society of London. Series B. Biological Sciences* **277**: 3055–3064.

Climent J, Costa e Silva F, Chambel MR, Pardos M, Almeida MH. **2009**. Freezing injury in primary and secondary needles of Mediterranean pine species of contrasting ecological niches. *Annals of Forest Science* **66**: 407–407.

Climent J, Dantas AK, Alía R, Majada J. **2013**. Clonal variation for shoot ontogenetic heteroblasty in maritime pine (*Pinus pinaster* Ait.). *Trees*: d.o.i.:10.1007/s00468–013–0901–1.

Climent J, Prada MA, Calama R, Chambel MR, de Ron DS, Alía R. **2008**. To grow or to seed: ecotypic variation in reproductive allocation and cone production by young female Aleppo pine (*Pinus halepensis, Pinaceae*). *American Journal of Botany* **95**: 833–842.

Codesido V, Fernandez-Lopez J. **2009**. Genetic variation in seasonal growth patterns in radiata pine in Galicia (northern Spain). *Forest Ecology and Management* **257**: 518–526.

Colautti RI, Eckert CG, Barrett SCH. **2010**. Evolutionary constraints on adaptive evolution during range expansion in an invasive plant. *Proceedings of the Royal Society of London. Series B. Biological Sciences* **277**: 1799–1806.

Conner JK. **2003**. Artificial selection: a powerful tool for ecologists. *Ecology* **84**: 1650–1660.

Cornelius JP. **1994**. The effectiveness of plus-tree selection for yield. *Forest Ecology and Management* **1127**: 23–34.

Dante SK, Schamp BS, Aarssen LW. **2013**. Evidence of deterministic assembly according to flowering time in an old-field plant community. *Functional Ecology* **27**: 555–564.

Darwin C. **1859**. *On the origin of species, or the preservation of favoured races in the struggle for life*. London, UK: Murray, J.

Davis MB, Shaw RG, Etterson JR. **2005**. Evolutionary responses to changing climate. *Ecology* **86**: 1704–1714.

Delph LF, Wolf DE. **2005**. Evolutionary consequences of gender plasticity in genetically dimorphic breeding systems. *New Phytologist* **166**: 119–128.

Deng J, Ran J, Wang Z, Fan Z, Wang G, Ji M, Liu J, Wang Y, Liu J, Brown JH. **2012**. Models and tests of optimal density and maximal yield for crop plants. *Proceedings of the National Academy of Sciences of the United States of America* **109**: 15823–15828.

Van Dijk H, Boudry P, McCombre H, Vernet P. **1997**. Flowering time in wild beet (*Beta vulgaris* ssp. *maritima*) along a latitudinal cline. *Acta Oecologica* **18**: 47–60.

Dodd ME, Silvertown J. **2000**. Size-specific fecundity and the influence of lifetime size variation upon effective population size in *Abies balsamea*. *Heredity* **85**: 604–609.

Doerksen T, Deslauriers M, Beaulieu J. **2011**. Ecological and biological information improves inferred paternity in a white spruce breeding orchard. *Canadian Journal of Forest Research* **41**: 1344–1351.

Ellegren HH, Sheldon BC. **2008**. Genetic basis of fitness differences in natural populations. *Nature* **452**: 169–175.

Espelta JM, Verkaik I, Màrcia E, Lloret F. **2008**. Recurrent wildfires constrain long-term reproduction ability in *Pinus halepensis* Mill. *International Journal of Wildland Fire* **17**: 579–585.

Fady B. **2012**. Biogeography of neutral genes and recent evolutionary history of pines in the Mediterranean Basin. *Annals of Forest Science*: 421–428.

Falconer D. **1989**. *Introduction to quantitative genetics.* New York. Longman.

Falster DS, Westoby M. **2003**. Plant height and evolutionary games. *Trends in Ecology & Evolution* **18**: 337–343.

Fox JJ. **1993**. Size and sex allocation in monoecious woody plants. *Oecologia* **94**: 110–113.

Franks SJ, Sim S, Weis AE. **2007**. Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 1278–1282.

Franks SJ, Weis AE. **2008**. A change in climate causes rapid evolution of multiple life-history traits and their interactions in an annual plant. *Journal of Evolutionary Biology* **21**: 1321–1334.

Friedman J, Barrett SCH. **2011**. Genetic and environmental control of temporal and size-dependent sex allocation in a wind-pollinated plant. *Evolution* **65**: 2061–2074.

Gassibe PV, Fabero RF, Hernández-Rodríguez M, Oria-de-Rueda JA, Martín-Pinto P. **2011**. Fungal community succession following wildfire in a Mediterranean vegetation type dominated by *Pinus pinaster* in Northwest Spain. *Forest Ecology and Management* **262**: 655–662.

Genet H, Bréda N, Dufrêne E. **2010**. Age-related variation in carbon allocation at tree and stand scales in beech (*Fagus sylvatica* L.) and sessile oak (*Quercus petraea* (Matt.) Liebl.) using a chronosequence approach. *Tree Physiology* **30**: 177–192.

Gil L, Díaz P, Jiménez P, Roldán M, Alía R, Agúndez D, de Miguel J, Martín S, Tuero M. **1996**. *Regiones de procedencia de Pinus hapelensis Mill. en España*. Madrid, Spain: Ministerio de Medio Ambiente.

Gil L, López R, García-Mateos AA, González-Doncel I. **2009**. Seed provenance and fire-related reproductive traits of *Pinus pinaster* in central Spain. *International Journal of Wildland Fire* **18**: 1003–1009.

Givnish TJ. **1980**. Ecological constraints on the evolution of breeding systems in seed plants: dioecy and dispersal in gymnosperms. *Evolution* **34**: 959–972.

Goldman DA, Willson MF. **1986**. Sex allocation in functionally hermaphroditic plants: A review and critique. *The Botanical Review* **52**: 157–194.

González-Martínez SC, Burczyk J, Nathan R, Nanos N, Gil L, Alía R. **2006**. Effective gene dispersal and female reproductive success in Mediterranean maritime pine (*Pinus pinaster* Aiton). *Molecular ecology* **15**: 4577–4588.

Gonzalez-Ochoa AI, López-Serrano FR, de las Heras J. **2004**. Does post-fire forest management increase tree growth and cone production in *Pinus halepensis*? *Forest Ecology and Management* **188**: 235–247.

Gonzalo-Jiménez J. **2010**. *Diagnosis fitoclimática de la España Peninsular: hacia un modelo de clasificación funcional de la vegetación y de los ecosistemas peninsulares españoles*. Organismo Autónomo de Parques Nacionales.

Goto S, Watanabe A, Miyahara F, Mori Y. **2005**. Reproductive success of pollen derived from selected and non-selected sources and its impact on the performance of crops in a nematode-resistant Japanese black pine seed orchard. *Silvae genetica* **18**: 95–98.

Goubitz S, Nathan R, Roitemberg R, Shmida A, Ne'eman G. **2004**. Canopy seed bank structure in relation to: fire, tree size and density. *Plant Ecology* **173**: 191–201.

Granado-Yela C, Balaguer L, García-Verdugo C, Carrillo K, Méndez M. **2013**. Thriving at the limit: Differential reproductive performance in range-edge populations of a Mediterranean sclerophyll (*Olea europaea*). *Acta Oecologica* **52**: 29–37.

Grime JP. **1977**. Evidence for the existence of three primary strategies in plants and its relevance to ecological and Evolutionary Theory. *The American Naturalist* **111**: 1169–1194.

Grivet D, Climent J, Zabal-Aguirre M, Neale DB, Vendramin GG, González-Martínez SC. **2013**. Adaptive evolution of Mediterranean pines. *Molecular Phylogenetics and Evolution* **68**: 555–566.

Grivet D, Robledo-Arnuncio JJ, Smouse PE, Sork VL. **2009a**. Relative contribution of contemporary pollen and seed dispersal to the effective parental size of seedling population of California valley oak (Quercus lobata, Née). *Molecular Ecology* **18**: 3967–3979.

Grivet D, Sebastiani F, González-Martínez SC, Vendramin GG. **2009b**. Patterns of polymorphism resulting from long-range colonization in the Mediterranean conifer Aleppo pine. *New Phytologist* **184**: 1016–1028.

Guo H, Weiner J, Mazer SJ, Zhao Z, Du G, Li B. **2012**. Reproductive allometry in *Pedicularis* species changes with elevation. *Journal of Ecology* **100**: 452–458.

Hadfield JD, Wilson AJ, Garant D, Sheldon BC, Kruuk LEB. **2010**. The misuse of BLUP in ecology and evolution. *The American Naturalist* **175**: 116–125.

Hansen CF, García MB, Ehlers BK. **2013**. Water availability and population origin affect the expression of the tradeoff between reproduction and growth in *Plantago coronopus*. *Journal of Evolutionary Biology* **26**: 993–1002.

Hansen OK, Nielsen UB. **2010**. Microsatellites used to establish full pedigree in a half-sib trial and correlation between number of male strobili and paternal success. *Annals of Forest Science* **67**: 703.

Hardy ICW. **2002**. *Sex ratios: concepts and research methods*.

Haymes KL, Fox GA. **2012**. Variation among individuals in cone production in *Pinus palustris* (*Pinaceae*). *American Journal of Botany* **99**: 640–645.

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

Holand AM, Steinsland I, Martino S, Jensen H. **2013**. Animal Models and Integrated Nested Laplace Approximations. *G3*: d.o.i.:10.1534/g3.113.006700.

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

House S. **1992**. Population density and fruit set in three dioecious tree species in Australian tropical rain forest. *Journal of Ecology* **80**: 57–69.

Howe GT, Aitken SN. **2003**. From genotype to phenotype: unraveling the complexities of cold adaptation in forest trees. *Canadian Journal of Botany* **1266**: 1247–1266.

Iraima V, Espelta JM. **2004**. Effect of thinning and post-fire regeneration age on the reproductive characteristics of *Pinus halepensis* Mill. forests. *Journal of Ecology* **2**: 1–7.

De Jong TJ, Goosen de Roo L, Klinkhamer PGL. **1998**. Is the threshold size for flowering in *Cynoglossum officinale* fixed or dependent on environment? *New Phytologist* **138**: 489–496.

De Jong TJ, Klinkhamer PGL. **1994**. Plant size and reproductive success through female and male function. *Journal of Ecology*: 399–402.

De Jong TJ, Klinkhamer PGL. **2005**. *Evolutionary ecology of plant reproductive strategies*. Cambridge: Cambridge University Press.

Kagaya M, Tani T, Kachi N. **2009**. Variation in flowering size and age of a facultative biennial, *Aster kantoensis* (*Compositae*), in response to nutrient availability. *American Journal of Botany* **96**: 1808–1813.

Kawecki TJ, Lenski RE, Ebert D, Hollis B, Olivieri I, Whitlock MC. **2012**. Experimental evolution. *Trends in Ecology & Evolution* **27**: 547–560.

Kaya Z, Adams WT, Campbell RK. **1994**. Adaptive significance of intermittent shoot growth in Douglas-fir seedlings. *Tree Physiology* **14**: 1277–1289.

Keeley JE. **2012**. Ecology and evolution of pine life histories. *Annals of Forest Science*: 445–453.

Van Kleunen M, Fischer M. **2005**. Constraints on the evolution of adaptive phenotypic plasticity in plants. *New Phytologist* **166**: 49–60.

Klinkhamer PGL, de Jong TJ, Metz H. **1997**. Sex and size in cosexual plants. *Trends in Ecology and Evolution* **12**: 260–265.

Knops JMH, Koenig WD. **2012**. Sex allocation in california oaks: trade-offs or resource tracking? *PloS one* **7**: e43492.

Koelewijn HP, Hunscheid MPH. **2000**. Intraspecific variation in sex allocation in hermaphroditic *Plantago coronopus* (L.). *Journal of Evolutionary Biology* **13**: 302–315.

Koenig WD, Díaz M, Pulido F, Alejano R, Beamonte E, Knops JMH. **2013**. Acorn production patterns. In: Campos P, Huntsinger L, Oviedo Pro JL, Starrs PF, Diaz M, Standiford RB, Montero G, eds. Mediterranean Oak Woodland Working Landscapes. Dordrecht: Springer Netherlands, 181–209.

Koenig WD, Knops JMH, Carmen WJ, Stanback MT, Mumme RL. **1994**. Estimating acorn crops using visual surveys. *Canadian Journal of Forest Research* **24**: 2105–2112.

Kollmann J, Bañuelos MJ. **2004**. Latitudinal trends in growth and phenology of the invasive alien plant *Impatiens glandulifera* (*Balsaminaceae*). *Diversity and Distributions* **10**: 377–385.

Koskela J, Lefèvre F, Schueler S, Kraigher H, Olrik DC, Hubert J, Longauer R, Bozzano M, Yrjänä L, Alizoti P, *et al.* 2013. Translating conservation genetics into management: Pan-European minimum requirements for dynamic conservation units of forest tree genetic diversity. *Biological Conservation* **157**: 39–49.

Kozłowski J. **1992**. Optimal allocation of resources to growth and reproduction: implications for age and size at maturity. *Trends in Ecology and Evolution* **7**: 15–19.

Kremer A, Potts BM, Delzon S. **2013**. Genetic divergence in forest trees: understanding the consequences of climate change. *Functional Ecology*: d.o.i.:10.1111/1365–2435.12169.

Kuss P, Rees M, Ægisdóttir HH, H Ellner SP, P Stöcklin J. **2008**. Evolutionary demography of long lived monocarpic perennials: a time lagged integral projection model. *Journal of Ecology* **96**: 821–832.

Lacey E. **1988**. Latitudinal variation in reproductive timing of a short-lived monocarp, *Daucus carota* (*Apiaceae*). *Ecology* **69**: 220–232.

Lachmuth S, Durka W, Schurr FM. **2011**. Differentiation of reproductive and competitive ability in the invaded range of *Senecio inaequidens*: the role of genetic Allee effects, adaptive and nonadaptive evolution. *New Phytologist* **192**: 529–541.

LaMontagne JM, Peters S, Boutin S. **2005**. A visual index for estimating cone production for individual white spruce trees. *Canadian Journal of Forest Research* **35**: 3020–3026.

De Las Heras J, Moya D, López-Serrano FR, Condés S. **2007**. Reproduction of postfire *Pinus halepensis* Mill. stands six years after silvicultural treatments. *Annals of Forest Science* **64**: 59–66.

Lefèvre F, Boivin T, Bontemps A, Courbet F, Davi H, Durand-Gillmann M, Fady B, Gauzere J, Gidoin C, Karam M-J, *et al.* 2013. Considering evolutionary processes in adaptive forestry. *Annals of Forest Science*: d.o.i. 10.1007/s13595–013–0272–1.

Leimu R, Fischer M. **2008**. A meta-analysis of local adaptation in plants. *PloS one* **3**: e4010.

Lev-Yadun S, Sederoff R. **2000**. Pines as model gymnosperms to study evolution, wood formation, and perennial growth. *Journal of Plant Growth Regulation* **19**: 290–305.

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

Loehle C, Namkoong G, Burdon RD, Collins B, William J, Scott M, Stangel P. **1987**. Constraints on tree breeding : growth tradeoffs, growth strategies, and defensive investments. *Forest Science* **33**: 1089–1097.

De Lucas A, González-Martínez SC, Hidalgo E, Bravo F, Heuertz M. **2009**. Admixture, one-source colonization or long-term persistence of maritime pine in the Castilian Plateau? Insights from nuclear microsatellite markers. *Investigación Agraria, Sistemas y Recursos Forestales* **18**: 3–12.

Lynch M, Walsh B. **1998**. *Genetics and Analysis of Quantitative Traits*. Sinauer Associates, Incorporated.

Maestre FT, Cortina J. **2004**. Are *Pinus halepensis* plantations useful as a restoration tool in semiarid Mediterranean areas? *Forest Ecology and Management* **198**: 303–317.

Matesanz S, Gianoli E, Valladares F. **2010**. Global change and the evolution of phenotypic plasticity in plants. *Annals of the New York Academy of Sciences* **1206**: 35–55.

Matesanz S, Horgan-Kobelski T, Sultan SE. **2012**. Phenotypic plasticity and population differentiation in an ongoing species invasion. *PLoS ONE*: e44955.

Matesanz S, Horgan-Kobelski T, Sultan SE. **2013**. Contrasting levels of evolutionary potential in populations of the invasive plant *Polygonum cespitosum*. *Biological Invasions*: d.o.i.:10.1007/s10530–013–0533–9.

Matyas C, Varga G. **2000**. Effect of intra-specific competition on tree architecture and aboveground dry matter allocation in Scots pine. *Forest Systems* **9**: 111–119.

Matziris D. **1998**. Genetic variation in cone and seed characteristics in a clonal seed orchard of Aleppo pine grown in Greece. *Silvae Genetica* **347**: 37–41.

Mendez M, Karlsson PS. **2004**. Between-population variation in size-dependent reproduction and reproductive allocation in *Pinguicula vulgaris* (*Lentibulariaceae*) and its environmental correlates. *Oikos* **104**: 59–70.

Merilä J, Sheldon BC. **1999**. Genetic architecture of fittness and non fittness traits : empirical patterns and development of ideas. *Heredity* **83**: 103–109.

Montero G, Ruiz-Peinado R, Muñoz M. **2005**. *Producción de biomasa y fijación de CO2 por los bosques españoles*. Madrid, Spain: Instituto Nacional de Investigacion y Tecnología Agraria y Alimentaria.

Montesinos D, de Luís M, Verdú M, Raventós J, García-Fayos P. **2006**. When, how and how much: gender-specific resource-use strategies in the dioecious tree *Juniperus thurifera*. *Annals of Botany* **98**: 885–889.

Montesinos D, Villar-Salvador P, García-Fayos P, Verdú M. **2012**. Genders in J*uniperus thurifera* have different functional responses to variations in nutrient availability. *New Phytologist* **193**: 705–712.

Moore JC, Pannell JR. **2011**. Sexual selection in plants. *Current biology* **21**: R176–R182.

Moran E V, Clark JS. **2012**. Causes and consequences of unequal seedling production in forest trees: a case study in red oaks. *Ecology* **93**: 1082–1094.

Moriguchi Y, Tsuchiya S, Iwata H, Itoo S, Tani N, Taira H, Tsumura Y. **2007**. Factors influencing male reproductive success in a *Cryptomeria japonica* seed orchard revealed by microsatellite marker analysis. *Silvae Genetica* **5**: 207–214.

Moya D, Espelta JM, Verkaik I, López-Serrano FR, de las Heras J. **2007**. Tree density and site quality influence on *Pinus halepensis* Mill. reproductive characteristics after large fires. *Annals of Forest Science* **64**: 649–656.

Munguía-Rosas MA, Ollerton J, Parra-Tabla V, De-Nova JA. **2011**. Meta-analysis of phenotypic selection on flowering phenology suggests that early flowering plants are favoured. *Ecology letters* **14**: 511–521.

Mutke S, Gordo J, Chambel MR, Prada MA, Álvarez D, Iglesias S, Gil L. **2010**. Phenotypic plasticity is stronger than adaptative differentiation among Mediterranean stone pine provenances. *Forest Systems* **19**: 354–366.

Nakagawa S, Schielzeth H. **2010**. Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biological Reviews of the Cambridge Philosophical Society* **85**: 935–956.

Ne'eman G, Gidi Goubitz S, Shirrinka Nathan R. **2004**. Reproductive traits of *Pinus halepensis* in the light of fire–a critical review. *Plant Ecology* **171**: 69–79.

Ne'eman G, Goubitz S, Werger MJA, Shmida A. **2011**. Relationships between tree size, crown shape, gender segregation and sex allocation in *Pinus halepensis*, a Mediterranean pine tree. *Annals of Botany* **108**: 197–206.

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

Nicholls AM. **2011**. Size-dependent analysis of allocation to sexual and clonal reproduction in *Penthorum sedoides* under contrasting nutrient levels. *International Journal of Plant Sciences* **172**: 1077–1086.

Niklas KJ, Enquist BJ. **2003**. An allometric model for seed plant reproduction. *Evolutionary Ecology Research* **5**: 79–88.

Obeso JR. **2002**. The costs of reproduction in plants. *New Phytologist* **155**: 321–348.

Oddou-Muratorio S, Bontemps A, Klein EK, Chybicki I, Vendramin GG, Suyama Y. **2010**. Comparison of direct and indirect genetic methods for estimating seed and pollen dispersal in *Fagus sylvatica* and *Fagus crenata*. *Forest Ecology and Management* **259**: 2151–2159.

Oddou-Muratorio S, Klein EK. **2008**. Comparing direct vs. indirect estimates of gene flow within a population of a scattered tree species. *Molecular Ecology* **17**: 2743–2754.

Ortiz O, Ojeda G, Espelta JM, Alcaniz JM. **2011**. Improving substrate fertility to enhance growth and reproductive ability of a *Pinus halepensis* Mill. afforestation in a restored limestone quarry. *New Forests* **43**: 365–381.

Pannell JR, Labouche A-M. **2013**. The incidence and selection of multiple mating in plants. *Proceedings of the Royal Society B: Biological Sciences* **368**: 1613.

Paquin V, Aarssen LW. **2004**. Allometric gender allocation in *Ambrosia artemisiifolia* (*Asteraceae*) has adaptive plasticity. *American Journal of Botany* **91**: 430–438.

Pausas J. **2004**. Changes in fire and climate in the eastern Iberian Peninsula (Mediterranean basin). *Climatic change* **63**: 337–350.

Pausas JG, Llovet J, Rodrigo A, Vallejo R. **2008**. Are wildfires a disaster in the Mediterranean basin?–A review. *International Journal of Wildland Fire* **17**: 713–723.

Pausas JG, Paula S. **2012**. Fuel shapes the fire-climate relationship: evidence from Mediterranean ecosystems. *Global Ecology and Biogeography* **21**: 1074–1082.

Perez SO, Robredo FG, Tellez EA, Belda CF. **2013**. Effects of the crisis in the resin sector on the demography of rural municipalities in Spain. *Forest Systems* **22**: 39–46.

Petit RJ, Hampe A. **2006**. Some evolutionary consequences of being a tree. *Annual Review of Ecology, Evolution, and Systematics* **37**: 187–214.

Pigliucci M. **2004**. *Phenotypic integration: Studying the Ecology and Evolution of complex phenotypes*. Wiley Online Library.

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

Price G. **1970**. Selection and covariance. *Nature* **227**: 520–521.

Pritchard JK, Stephens M, Donnelly P. **2000**. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959.

Ramírez-Valiente JA, Valladares F, Delgado Huertas A, Granados S, Aranda I. **2010**. Factors affecting cork oak growth under dry conditions: local adaptation and contrasting additive genetic variance within populations. *Tree Genetics & Genomes* **7**: 285–295.

Rees M, Sheppard A, Briese D, Mangel M. **1999**. Evolution of size-dependent flowering in *Onopordum illyricum*: A quantitative assessment of the role of stochastic selection pressures. *American Naturalist* **154**: 628–651.

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

Rehfeldt GE, Ying CC, Spittlehouse DL, Hamilton DA, Hamilton Jr DA. **1999**. Genetic responses to climate in *Pinus contorta*: niche breadth, climate change, and reforestation. *Ecological Monographs* **69**: 375–407.

Reznick D. **1985**. Costs of reproduction: an evaluation of the empirical evidence. *Oikos* **44**: 257–267.

Reznick D. **1992**. Measuring the costs of reproduction. *Trends in Ecology & Evolution* **7**: 1990–1993.

Reznick D, Nunney L, Tessier A. **2000**. Big houses, big cars, superfleas and the costs of reproduction. *Trends in Ecology & Evolution* **15**: 421–425.

Robertson A. **1966**. A mathematical model of the culling process in dairy cattle. *Animal Production* **8**: 95–108.

Robledo-Arnuncio JJ. **2011**. Wind pollination over mesoscale distances: an investigation with Scots pine. *New Phytologist* **190**: 222–233.

Rodríguez RL. **2012**. Grain of environment explains variation in the strength of genotype × environment interaction. *Journal of Evolutionary Biology* **25**: 1897–1901.

Roff DA. **1992a**. *The evolution of life histories: theory and analysis*. New York, USA: Chapman and Hall.

Roff DA. **1992b**. Age and size at maturity. In: Roff DA, ed. The evolution of life histories: theory and analysis. New York: Chapman and Hall, 179–241.

Roff DA. **2000**. Trade-offs between growth and reproduction: an analysis of the quantitative genetic evidence. *Journal of Evolutionary Biology* **13**: 434–445.

Ruano I, Rodríguez-García E, Bravo F. **2013**. Effects of pre-commercial thinning on growth and reproduction in post-fire regeneration of *Pinus halepensis* Mill. *Annals of Forest Science* **70**: 357–366.

Sakai A, Allendorf FW, Holt J, Lodge D. **2001**. The population biology of invasive species. *Annual Review of Ecology, Evolution, and Systematics* **32**: 305–332.

Salmela MJ, Cavers S, Cottrell JE, Iason GR, Ennos RA. **2013**. Spring phenology shows genetic variation among and within populations in seedlings of Scots pine (*Pinus sylvestris* L.) in the Scottish Highlands. *Plant Ecology & Diversity*: d.o.i.:10.1080/17550874.2013.795627.

Salmela MJ, Cavers S, Wachowiak W, Cottrell JE, Iason GR, Ennos RA. **2010**. Understanding the evolution of native pinewoods in Scotland will benefit their future management and conservation. *Forestry* **83**: 535–545.

Sampedro L, Moreira X, Zas R. **2011**. Costs of constitutive and herbivore-induced chemical defences in pine trees emerge only under low nutrient availability. *Journal of Ecology* **99**: 818–827.

Samson D, Werk K. **1986**. Size-dependent effects in the analysis of reproductive effort in plants. *American Naturalist* **127**: 667–680.

Santos-del-Blanco L, Climent J. **2011**. Just quality vs. quantity? Trade-offs between reproductive allocation, seed weight and dispersal ability in mediterranean pines. 12th European Ecological Federation Congress. Ávila, Spain: AEET, 382.

Savolainen O, Pyhäjärvi T, Knürr T. **2007**. Gene flow and local adaptation in trees. *Annual Reviews of Ecology, Evolution and Systematics* **38**: 595–619.

Schoen DJ, Stewart SC. **1986**. Variation in male reproductive investment and male reproductive success in white spruce. *Evolution* **40**: 1109–1120.

Sgrò CM, Hoffmann AA. **2004**. Genetic correlations, tradeoffs and environmental variation. *Heredity* **93**: 241–248.

Shaw RG, Etterson JR. **2012**. Rapid climate change and the rate of adaptation: insight from experimental quantitative genetics. *New Phytologist* **195**: 752–765.

Sierra-de-Grado R, Diez-Barra R, Alía R. **1999**. Evaluacion de la rectitud del fuste en seis procedencias de *Pinus pinaster* Ait. *Investigación Agraria, Sistemas y Recursos Forestales* **8**: 263–278.

Soto A, Robledo-Arnuncio JJ, González-Martínez SC, Smouse PE, Alía R. **2010**. Climatic niche and neutral genetic diversity of the six Iberian pine species: a retrospective and prospective view. *Molecular Ecology* **19**: 1396–409.

Stearns SC. **1976**. Life-history tactics: a review of the ideas. *Quarterly Review of Biology* **51**: 3–47.

Stearns SC. **1989**. Trade-offs in life-history evolution. *Functional Ecology* **3**: 259–268.

Stearns SC. **1992**. *The evolution of life histories*. Oxford University Press.

Sultan SE. **2000**. Phenotypic plasticity for plant development, function and life history. *Trends in Plant Science* **5**: 537–42.

Sultan SE, Horgan-Kobelski T, Nichols LM, Riggs CE, Waples RK. **2013**. A resurrection study reveals rapid adaptive evolution within populations of an invasive plant. *Evolutionary Applications* **6**: 266–278.

Tapias R, Climent J, Pardos JA, Gil L. **2004**. Life histories of Mediterranean pines. *Plant Ecology* **171**: 53–68.

Tapias R, Gil L, Fuentes-Utrilla P, Pardos JA. **2001**. Canopy seed banks in Mediterranean pines of south eastern Spain: a comparison between *Pinus halepensis* Mill., *P. pinaster* Ait., *P. nigra* Arn. and *P. pinea* L. *Journal of Ecology* **89**: 629–638.

Thomas SC. **2011**. Age-related changes in tree growth and functional biology: the role of reproduction. In: Meinzer FC, Lachenbruch B, Dawson TE, eds. Size-and age-related changes in tree structure and function. Springer Netherlands, 33–64.

Varghese M, Kamalakannan R, Harwood CE, Lindgren D, McDonald MW. **2009**. Changes in growth performance and fecundity of *Eucalyptus camaldulensis* and *E. tereticornis* during domestication in southern India. *Tree Genetics & Genomes* **5**: 629–640.

Verdú M. **2002**. Age at maturity and diversification in woody angiosperms. *Evolution* **56**: 1352–1361.

Verdú M, Spanos K, Canová I, Slobodník B, Paule L. **2007**. Similar gender dimorphism in the costs of reproduction across the geographic range of *Fraxinus ornus*. *Annals of Botany* **99**: 183–191.

Verkaik I, Espelta JM. **2006**. Post-fire regeneration thinning, cone production, serotiny and regeneration age in *Pinus halepensis*. *Forest Ecology and Management* **231**: 155–163.

Voltas J, Chambel MR, Prada MA, Ferrio JP. **2008**. Climate-related variability in carbon and oxygen stable isotopes among populations of Aleppo pine grown in common-garden tests. *Trees-Structure and Function* **22**: 759–769.

Walsh B, Blows MW. **2009**. Abundant genetic variation+ strong selection= multivariate genetic constraints: a geometric view of adaptation. *Annual Review of Ecology, Evolution, and Systematics* **40**: 41–59.

Weiner J, Campbell LG, Pino J, Echarte L. **2009**. The allometry of reproduction within plant populations. *Journal of Ecology* **97**: 1220–1233.

Wesselingh RA, de Jong TJ. **1995**. Bidirectional selection on threshold size for flowering in *Cynoglossum officinale* (hound's-tongue). *Heredity* **74**: 415–424.

Wesselingh RA, Klinkhamer PGL. **1996**. Threshold size for vernalization in *Senecio jacobaea*: genetic variation and response to artificial selection. *Functional Ecology* **10**: 281–288.

Wesselingh RA, Klinkhamer PGL, de Jong TJ, Boorman LA. **1997**. Threshold size for flowering in different habitats: effects of size-dependent growth and survival. *Ecology* **78**: 2118–2132.

West S. **2009**. *Sex Allocation*. Princeton University Press.

White TL, Adams WT, Neale DB. **2007**. *Forest Genetics*. CABI Publishing.

Willis CG, Ruhfel B, Primack RB, Miller-Rushing AJ, Davis CC. **2008**. Phylogenetic patterns of species loss in Thoreau's woods are driven by climate change. *Proceedings of the National Academy of Sciences of the United States of America* **105**: 17029–17033.

Wilson AJ, Réale D, Clements MN, Morrissey MM, Postma E, Walling C a, Kruuk LEB, Nussey DH. **2010**. An ecologist's guide to the animal model. *The Journal of Animal Ecology* **79**: 13–26.

Wright SJ, Joseph Jaramillo MA, Alejandra Pavon JJ, Condit RR, Hubbell SPSP, P. Foster RBRB. **2005**. Reproductive size thresholds in tropical trees: variation among individuals, species and forests. *Journal of Tropical Ecology* **21**: 307–315.

Wu HX, Ying CC. **2004**. Geographic pattern of local optimality in natural populations of lodgepole pine. *Forest Ecology and Management* **194**: 177–198.

Zas R. **2006**. Iterative kriging for removing spatial autocorrelation in analysis of forest genetic trials. *Tree Genetics & Genomes* **2**: 177–185.

Zhang DY. **2006**. Evolutionary stable reproductive investment and sex allocation in plants. In: Harder LD, Barrett SCH, eds. The ecology and evolution of flowers. Oxford University Press, 41–59.

ACKNOWLEDGEMENTS / AGRADECIMIENTOS

8. ACKNOWLEDGEMENTS / AGRADECIMIENTOS

The present study has been developed within the framework of several research projects: VAMPIRO (CGL2008-05289-C02-02), AdapCon (CGL2011-30182-C02-01), FENOPIN (AGL2012-40151-C03-02), Proyecto Singular Estratégico RESTAURA (PSS-310000-2008-4), TREESNIPS (QLK3-CT2002-01973), EVOLTREE excellence network (16322-GOCE), NOVELTREE (FP7/ 2007-2013 n.211868), MITIGENFOR (RTA 2011-00016-00-00), Project INIA - AT07-002, Project INIA – RTA -07-100, Project INIA-AT10-007, and Project CC03-048 (agreement between Dirección General de Biodiversidad and INIA). My work was also supported by the Spanish Ministry of Education via a doctoral grant (FPU AP-2007-03302). The data used in this research are part of the Spanish Network of Genetic Trials (GENFORED). We thank all persons and institutions linked to the establishment and measurement of the field trials and to the maintenance of the network. Data are publicly available upon request through www.genfored.es.

Mi trabajo no hubiera sido posible sin el esfuerzo de las personas que promovieron la creación de la red de ensayos genéticos, entre los que me gustaría destacar a Ricardo Alía y Dolores Agúndez. Extiendo el agradecimiento a las numerosas personas que durante años han participado en la instalación, mantenimiento y toma de datos en los ensayos, entre ellos Fernando del Caño, Diana Barba, Eduardo Ballesteros, Elena Alamillo, Regina Chambel, Isabel Rodríguez, Abel Blanco, David Lafuente y los estudiantes del programa FCT en los IIEESS de El Escorial y de Villaviciosa de Odón (Madrid). También quiero destacar el empeño de Eduardo Notivol y Regina Chambel para poner en marcha la plataforma online de Genfored.

La idea original de esta tesis, así como el planteamiento de buena parte de los artículos corresponde enteramente a mi director, José Climent. Suyo es el mérito de entender la importancia de estudiar los caracteres reproductivos en la red de ensayos genéticos forestales, labor que comenzó antes de esta tesis y con la que he tenido suerte de colaborar. Ciertamente, sus ganas e interés en este proyecto han superado los míos propios y por ello, esta tesis es tanto o más suya que mía.

I am also indebted to everyone who contributed to my scientific training. Empezando con Baudilio Herrero y Pilar Zaldívar, quienes me proporcionaron mi primer trabajo relacionado con la investigación. Later on came that wonderful year in Finland, back in 2005/06, where I realised how much I liked science and the (self-described) extrange Finns. Thanks and greetings to Tino Warinoski, Teemu Teeri, Marja, Suvi, Sanna, Miia, Mika and Roosa plus Elisa, Wilmer, Agnes, Tuuli, Marjo and Anssi. Luego de vuelta en España, tuve una brevísima etapa en el departamento de Economía donde lo mejor fue coincidir con amigos como Jordi y Chabi. Después pasé por el laboratorio de diagnóstico genético de Elena Hidalgo en Palencia, donde especialmente gracias a Ana I de Lucas empecé a conocer los pormenores de la investigación forestal. Al mismo tiempo, trabajaba en otra de mis pasiones, los hongos, en ADRI Cerrato Palentino (un abrazo a Miguel, Fernando y Ana). Llegado el momento tuve que elegir entre hongos y desarrollo rural o doctorado, y opté por la segunda opción, eso sí, sin perder la esperanza de seguir en contacto con el mundo de la micología. Ese hubiera podido ser otro camino alternativo, a buen seguro igual de satisfactorio que el presente, con maestros y amigos como Juan Andrés Oria, Raúl, Jaime, Bea, Carlos, Pablo y Amparo. De vuelta al mundo académico, guardo en mi memoria excelentes momentos de Palencia junto con todos mis compañeros y profesores del Master de doctorado, ¡demasiado numerosos para nombrarlos! La etapa de Palencia llegó a su fin y el destino quiso que mi camino hacia el doctorado continuara en el CIFOR-INIA en Madrid.

A pesar de ser un nuevo comienzo, éste no fue difícil gracias al apoyo y arrope de Jose y toda la gente del departamento de Ecología y Genética Forestal, y muy especialmente también gracias a Rafael Zas y Eduardo Notivol ayudándome con el primer paper, casi puesto en bandeja. Agradezco también la colaboración científica a todas las personas que de una forma u otra han contribuido a mis publicaciones, bien como coautores o también de forma importante aportando y discutiendo ideas. Thanks to John Pannell, Stephen Bonser, Rafael Zas, Luis Sampedro, Eduardo Notivol, Regina Chambel, Fernando Valladares, Ricardo Alía, Santiago González-Martínez, Juanjo Robledo, Ana I de Lucas, Rosario Sierra, Elena Hidalgo, Juan Majada y una vez más, José Climent.

Ya asentado en el almacén de becarios, me tocó el turno de partir de estancia. En un primer momento hacia el Department of Plant Sciences de Oxford, junto con John Pannell quien me acogió sin reparos ni condiciones durante algo más de medio año y me brindó la oportunidad de ampliar mis horizontes científicos. Allí tuve la oportunidad de conocer mucha gente interesante y explorar mis habilidades de jugador de cricket, casi confirmando mi vida pasada como un gentleman inglés. Greetings to Julia, Jamie, John Russell, Suvi (again here!), Lizzie, Nadine and all the Department of Plant Sciences cricket team. Mi segunda estancia me llevó hasta las antípodas, en la University of New South Wales, Australia junto a Stephen Bonser durante dos meses. Un lugar donde pude respirar de nuevo el ambiente académico y donde compartí interesantes journal club sessions con Begoña Peco, Angela Moles, Fatih, Justin, Josh, Ali and Samiya.

La vida en el almacén de becarios se sucedía entre conferencias, seminarios, cursos y salidas de campo mientras que los antiguos becarios completaban su migración hacia la luz y los nuevos llegaban a la zona de tinieblas. Un saludo especial para mis coetáneos Marina, Katha y Javi, que también están peleando con sus tesis y para Paloma Ruiz y Rubén Manso, que ya ganaron la batalla. Mucho ánimo también para los que están en la cola de salida: Quique, Gregor, Natalia, Isabel (gracias por aguantarme aún estando “tésico”), Maje, Paloma Torroba y Rose, y para también Laura y Javi, del departamento enemigo, ¡disfrutad el camino!

Finalmente, unas palabras de agradecimiento para mi familia y amigos por haberme acompañado y apoyado durante toda esta aventura que me ha llevado alrededor del mundo, ¡y que espero que también algún día me traiga de vuelta!

Lausana, Suiza, 18 de septiembre de 2013

APPENDIX

9. APPENDIX

Articles published in Science Citation Index journals:

(I) Variation of early reproductive allocation in multi-site genetic trials of Maritime pine and Aleppo pine. **Santos-del-Blanco L**, Notivol E, Zas R, Chambel MR, Majada J, Climent J. 2010. Forest Systems 19: 381–392.

(II) Genetic differentiation for size at first reproduction through male versus female functions in the widespread Mediterranean tree *Pinus pinaster*. **Santos-del-Blanco L**, Climent J, González-Martínez SC, Pannell JR. 2012. Annals of Botany 110: 1449–1460.

(III) Plasticity in reproduction and growth among 52 range-wide populations of a Mediterranean conifer: adaptive responses to environmental stress. **Santos-del-Blanco L**, Bonser SP, Valladares F, Chambel MR, Climent J. 2013. Journal of Evolutionary Biology. 26: 1912-1924.

Complementary manuscripts and conference proceedings:

(IV) Correlated effects on reproductive traits derived from domestication of a forest tree are relevant for future forest resilience. **Santos-del-Blanco L**, Alía R, González-Martínez S, Sampedro L, Lario F, Climent, J. 2013. Manuscript.

(V) Somatic and genetic costs of reproduction in a conifer tree. **Santos-del-Blanco L**, Climent J. Manuscript.

(VI) Guidelines for estimation of quantitative genetic parameters of reproductive traits. **Santos-del-Blanco L**, Climent J. Manuscript.

(VII) Ecología evolutiva de la reproducción en dos pinos mediterráneos: *Pinus pinaster* y *Pinus halepensis*. **Santos-del-Blanco L**, Chambel R, Notivol E. Alía R, Climent J. Proceedings 6º Congreso Forestal Español. Sociedad Española de Ciencias Forestales, Vitoria, 2013.

APPENDIX I

Variation of early reproductive allocation in multi-site genetic trials of Maritime pine and Aleppo pine. **Santos-del-Blanco L**, Notivol E, Zas R, Chambel MR, Majada J, Climent J. 2010. Forest Systems 19: 381–392.

Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA)
Available online at www.inia.es/forestsystems

Forest Systems 2010 19(3), 381-392
ISSN: 1131-7965
eISSN: 2171-9845

Variation of early reproductive allocation in multi-site genetic trials of Maritime pine and Aleppo pine

L. Santos-del-Blanco[1,5]*, R. Zas[2], E. Notivol[3], M. R. Chambel[1,5],
J. Majada[4] and J. Climent[1,5]

[1] *CIFOR-INIA. Madrid. Spain*
[2] *CITA. Zaragoza. Spain*
[3] *MBF-CSIC. Pontevedra. Spain*
[4] *SERIDA. Asturias. Spain*
[5] *Sustainable Forest Management Research Institute UVa-INIA. Palencia. Spain*

Abstract

Life histories in Mediterranean pines are well known to be closely related to different fire and disturbance regimes. Variation in these factors is also reflected in reproductive strategies at the specific and intraspecific level. Specifically, the onset of reproduction is a crucial stage for any organism because it has profound implications on fitness. In this paper we focus on the intraspecific variation and plasticity in the threshold size for reproduction and reproductive allocation in two Mediterranean pines, assessed at the onset of reproduction, when trade-offs between reproduction and growth are expected to be greater. Replicated common garden provenance and progeny trials of Aleppo pine and Maritime pine were used to estimate genetic parameters for reproduction and vegetative growth, as well as variation in plasticity in reproductive strategies at the intraspecific level. In both species, high variation among populations was found for both threshold size for reproduction and for reproductive allocation. Reproductive allocation was also highly variable within populations and showed moderate to high values of heritability and high coefficients of additive genetic variation. These results indicate a high genetic control of these reproductive traits, while high additive genetic variation is maintained, allowing to face selective pressures. Moreover, reproductive strategies although plastic, showed low genotype × environment interaction, and intraspecific variation was highly consistent across trial sites both at the population and the family levels. The former data confirm the strong genetic control of reproductive traits in these species. Finally, the fact that Aleppo pine starts its reproductive phase as female while Maritime pine can start reproducing either as male or female deserves further attention.

Key words: *Pinus halepensis*; *Pinus pinaster*; genetic variability; plasticity; trade-offs; early reproductive strategies; threshold size for reproduction.

Resumen

Variación en asignación reproductiva temprana en ensayos multi-localidad de pino carrasco y pino negral

Las estrategias de historia vital en pinos mediterráneos están estrechamente ligadas a diversos regímenes de incendios y perturbaciones. A su vez, la variabilidad de esos factores también se ve reflejada en las estrategias reproductivas a niveles inter e intraespecíficos. Concretamente, el comienzo de la reproducción es una etapa crucial para cualquier organismo debido a su profunda influencia en su adaptación al medio. Este trabajo se enfoca en el estudio de la variabilidad intraespecífica y la plasticidad en el tamaño umbral de reproducción y la asignación reproductiva en dos pinos mediterráneos. El estudio se realizó durante el comienzo de la fase reproductiva, momento en que se estima que la compensación entre reproducción y crecimiento es de mayor importancia. Se utilizaron ensayos multi-sitio de procedencias y progenies de pino carrasco y pino negral para estimar los parámetros genéticos de caracteres reproductivos y de crecimiento vegetativo, así como para conocer la variabilidad en la plasticidad de las estrategias reproductivas a nivel intraespecífico. En ambas especies se halló una alta variabilidad entre poblaciones para el tamaño umbral de reproducción y para la asignación reproductiva. La asignación reproductiva también fue altamente variable dentro de poblaciones y mostró valores de heredabilidad de moderados a altos y altos coeficientes de varianza genética aditiva. Estos resultados indican un alto control genético de los rasgos reproductivos, mientras se mantiene una alta varianza genética, permitiendo afrontar futuras presiones selectivas. Además, a pesar de la existencia de plasti-

---

* Corresponding author: santos.luis@inia.es

Received: 15-04-10; Accepted: 21-07-10.

cidad en las estrategias reproductivas, éstas mostraron una baja interacción genotipo × ambiente, y la variación intraespecífica fue acorde en los diferentes lugares de ensayo tanto a nivel poblacional como familiar. Los datos anteriores confirman el alto control genético de los rasgos reproductivos en estas especies. Finalmente, el hecho de que el pino carrasco comience su fase reproductora como hembra mientras que el pino negral pueda comenzar su reproducción bien como macho o bien como hembra, merece una atención más detallada.

Palabras clave: *Pinus halepensis; Pinus pinaster;* variabilidad genética; plasticidad; trade-offs; estrategias de reproducción temprana; tamaño umbral de reproducción.

Introduction

Due to its direct link to fitness, information on the intraespecific variation in the time at which organisms reach maturity and the amount of resources allocated to reproduction is central to understand how evolution has shaped these traits in the past and to infer how can they be affected by future evolutionary forces. In long-living plant species, optimal size or age at maturity reflects the balance between the benefits of an early reproduction and its costs reflected in disminished future reproduction and survival (Wesselingh *et al.*, 1997). Theory predicts that mortality risk and its predictability drive the time at first reproduction within and among species (Kozlowski, 1992).

Pines are known to have developed astonishing adaptations to fire such as the grass stage or serotiny, but also a group of other life history traits, being reproductive traits like precocity and intensity of early reproduction among the most important (Keeley and Zedler, 1998). Species adapted to frequent crown fires and unable to resprout, are expected to show an early intense reproduction while species not adapted to fire or adapted to ground fires typically show a delayed reproduction (Agee, 1998). Mediterranean pine ecosystems are frequently affected by forest fires and droughts (Richarson *et al.*, 2007), and thus Mediterranean pines can provide a good example for studying the relationship between early reproductive strategies and disturbance regimes.

General reproductive strategies are known for many pine species. However, there is a lack of information at the intraspecific level. The vast majority of the studies at the intraspecific level are biased towards economically important species, related to breeding programs interested in reducing the time between cycles, increasing seed crops and avoiding unequal contributions from some genotypes in seed orchards (Koenig and Knops, 2000; Kang *et al.,* 2003). Consequently, an evolutionary and ecological discussion on this topic is not common.

In widely distributed Mediterranean pines like Aleppo pine (*Pinus halepensis* Mill.) and Maritime pine (*Pinus pinaster* Ait.), information on reproductive trait variation within species holds great interest for understanding adaptation to contrasting local conditions as seen, for example, in the model genus *Arabidopsis* (Bonser and Aarssen, 2001; Rutter and Fenster, 2007). This information can also prove useful for an optimum deployment of genetic materials as a mean to increase forest resilience facing frequent disturbances and to facilitate adaptation to climate change (Thompson *et al.,* 2009). Common garden trials of forest trees, usually planted for genetic breeding, can offer valuable information on these subjects, provided the necessary cooperation between foresters and evolutionists.

Extensive research on the ecology and population genetics regarding *P. pinaster* and *P. halepensis* is available, but only few references focus on reproduction (Richardson, 1998), despite its close relation to fitness. Although both species have distribution areas that overlap partially at several points within the Iberian Peninsula, some differences are remarkable. Maritime pine spreads across the western Mediterranean basin from North Morocco with Mediterranean climate to South Western Atlantic coast of France, with a humid Atlantic climate. Three different main gene pools have been differentiated (Bucci *et al.*, 2007). On the other hand, Aleppo pine has a circunmediterranean distribution with genetically diverse populations in Greece and Turkey but more genetically uniform populations towards the west of the basin following a proposed colonization route (Grivet *et al.*, 2009).

According to its genetic diversity, common garden trials have shown a high variability in reproductive strategies for Maritime pine (Tapias *et al.*, 2004) and variable differentiation in Aleppo pine populations depending on the trait and experimental site (Climent *et al.*, 2008). Genetic differentiation among populations for reproductive traits is proposed to reflect local selective pressures, consistent with empirical examples about how fire can act to shape early reproductive allocation (González-Ochoa *et al.*, 2004; Gil *et al.*, 2009).

Genetic parameters such as narrow sense heritability (h^2), additive genetic coefficient of variation (CV_a), the quantitative differentiation between populations (Q_{st}), and genetic correlations among traits are relevant to describe the genetic control of reproductive traits, to assess quantitative variation within and among populations and to describe the existence of trade-offs between traits (Roff, 2000). The comparison of the genetic architecture of traits closely linked to fitness with that of other traits is scientifically challenging, since it deals with the interplay between past directional selection (reducing within population variation and promoting differentiation between populations) and the availability of enough additive genetic variation to enable future evolutionary processes (Merilä and Sheldon, 1999). Furthermore, there is growing evidence showing the importance of plasticity in trees affecting the estimation of genetic parameters and the necessity to conduct experiments with genetic entries replicated in a range of environmental conditions (Sgrò and Hoffmann, 2004).

In this work, we focus on describing the intraspecific variability in multi-site genetic trials of *P. halepensis* and *P. pinaster* for reproductive traits, namely threshold size for reproduction and reproductive allocation from an evolutionary quantitative genetic approach. We aim also to determine whether early reproduction in these species entail vegetative fitness costs, which will be reflected as negative genetic correlations between reproductive and vegetative traits.

Material and methods

Field trials and Plant material

For **Aleppo pine**, we used a provenance-progeny trial replicated in two sites in inland Spain (Megeces —AMEG—, and Montañana —AMON—) comprising 148 open-pollinated families of 32 populations covering the species' natural range in the Iberian Peninsula and Balearic Islands plus three additional sources from planted stands of unknown origin (Table A2). One year old seedlings were planted in 1995 in a randomized complete block design with seven blocks, and two contiguous plants per plot. Spacing was 2.5 × 2 m at AMEG and 5.2 × 1 m at AMEG and AMON. Trial sites were ecologically contrasting (Table A1): AMEG is situated on a dry shallow calcic soil with < 15% slope in the Castilian Plateau; as a result of the harsher conditions, mortality in this site was high (33%). Despite being outside the species natural range, AMEG lies within an area with extensive Aleppo pine plantations. AMON is sited on a deep fertile alluvial soil, well irrigated during summer, and more favorable for pine growth, although mortality after plantation affected many seedlings.

Maritime pine trials are represented by a progeny trial replicated in two sites (Rebordelo —PREB—, and Rianxo —PRIA—) and a provenance-progeny trial, also replicated in two sites (A Merca —PMER—, and Cavada —PCAV—), all located at Northwestern Spain, under temperate Atlantic climate (Table A1). Soil and climate in PREB and PCAV are rather similar, with high annual and summer precipitation, while PMER represents a transition towards Mediterranean conditions, with much lower summer rainfall and PRIA is representative of mild winter coastal conditions.

In PREB and PRIA, 28 open-pollinated (o.p.) families of superior trees selected within the Atlantic coast of Galicia were planted in 2003 under different establishment fertilization treatments. The original experimental design included three unimproved seed lots that were not considered in this study. The experimental layout in both sites was a split-plot design in ten blocks, with nine fertilization treatments acting as the main factor and the genetic entries as the split factor (see details in Martíns *et al.*, 2009). Althoug fertilized plants attained bigger size and more cones, fertilization did not affect the ratio between cones and tree size *i.e.* reproductive allocation (data not shown), therefore fertilization was not further considered in this work. Both progeny trials were thus considered to follow a randomized complete block design with 90 blocks and single-tree plots. Spacing was 3 × 2 m.

In PMER and PCAV, 250 open-pollinated families pertaining to 26 natural populations covering most of the natural range were planted in 2005 (Table A3). Experimental layout was a complete randomized block design with 4 plants per family and block and 4 blocks. Spacing was 3 × 2 m.

Assessments

Reproductive and growth-related variables were measured in all sites at young ages, after a significant proportion of the trees started to produce cones. Nevertheless, it was not possible to measure the same variables coding for reproduction or growth in all sites due to differences in development.

In the Aleppo pine trials, basal diameter and total tree height were measured and cones belonging to different cohorts were counted in 2005 and 2009 when trees were 11 and 15 years old, respectively. The small tree size and good visibility in the AMEG site allowed for distinguishing up to three cone cohorts, representing yearly reproduction. In winter 2005 and 2009, first and second year developing conelets were counted. Male reproduction was also recorded through a binary variable, present or absent. Regarding its reproductive status, each individual tree was classified as male (protandrious, with only male cones), female (protogynous, with only female cones), synchronous (with both male and female cones) or juvenile (no cones).

In AMON, only one measurement was carried out in winter 2009, recording basal diameter and tree height. The better growing conditions in this site, compared with AMEG, were reflected in a more vigorous growth and more advanced ontogenic development. Trees were too high to allow a clear visibility of all developing cones and to distinguish them from older ones. Since Aleppo pine cones remain attached to the branches, either opened or as serotinous cones, we used an alternative method to estimate accumulated cone production trough the tree life. Cones counted in 15 seconds were taken as a surrogate of reproduction (Koenig *et al.*, 1994). According to a preliminary sub-sample, counting cones during 15 seconds was considered to provide a reliable estimation of the total cone number per tree, comparing estimations in trees with different cone loads.

Reproductive allocation, RA, (following Karlsson and Méndez, 2005), was calculated for each tree as a ratio between the number of cones (sum of developing first and second year conelets (Cone count, Cc) for AMEG and the total number of cones counted in 15 seconds for AMON) and stem volume over bark (Vob), a surrogate for biomass, and hence, resource availability (Climent *et al.*, 2008). Vob was calculated according to the formula:

$$Vob = \frac{\pi}{12}\,\mathrm{Db}^2\mathrm{H}$$

where Db is basal diameter and H is total tree height.

In the PRIA and PREB maritime pine progeny trials, total tree height and basal diameter were measured in December 2007, when trees were 5 years old. First and second year conelets of the two coexisting cohorts within the crown were counted in all trees, and RA was estimated as described before.

In PMER and PCAV height was measured, first and second year conelets were counted and male reproductive status was recorded as a binary variable in 2009, when trees were 5 years old. This was the time in which a significant proportion reached maturity since in previous years reproduction was almost absent. As in Aleppo pine, individuals were classified as juvenile, male, female or synchronous. We used data from this trial series exclusively to assess the variation and plasticity of the threshold size for male and female reproduction.

Data analysis and genetic parameters

Threshold size for reproduction (TSR) was studied with a logistic model similar to that used by other authors (Wesselingh *et al.*, 1997; Méndez and Karlsson, 2004). Reproduction probability was analyzed by adjusting a variable termed CATREP, representing the reproductive status of an individual (0, non reproductive, 1, reproductive, bearing female and/or male cones). Since we were interested mostly in the variation between populations for TSR, we applied this analysis to the provenance-progeny trials at the time of maximum differentiation, that is, when close to 50% individuals were reproductive. Sites AMEG of Aleppo pine in 2005 and PMER and PCAV of Maritime pine fulfilled this requisite. Logistic curves were adjusted with size (stem volume over bark in *P. halepensis* or height in *P. pinaster*) as a quantitative factor. A first analysis was made considering all populations per site as a categorical factor in order to test for its significance. Then, a curve was fitted for each provenance (Fig. 1):

$$CATREP = \frac{e^{(a+bx)}}{1+e^{(a+bx)}}$$

being *a* and *b* coefficients adjusted for each regression and *x* was either *Vob* in Aleppo pine or H in Maritime pine. V_{50} or H_{50} were defined as the volume or height at which the probability for a tree to have reached sexual maturity is 50% (Méndez and Karlsson, 2004).

Genetic parameters

Target variables *Vob, Cc* and *RA* were analyzed by Mixed Linear Models testing for variability at provenance and family within provenance levels as follows:

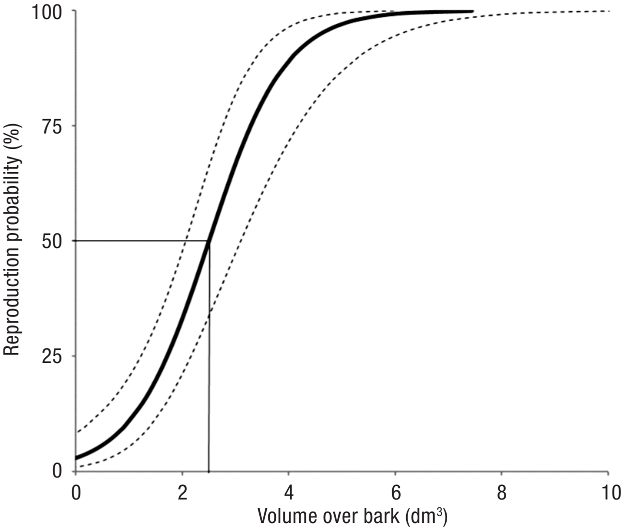


Figure 1. Example of a logistic curve representig probability of reproduction as a function of size (stem volume over bark) in Pinus halepensis from Hijar, NE Spain. Dashed lines indicate the 75% confidence intervals. The vertical line denotes the volume V_{50} at which the probability for a tree to have reached sexual maturity is 50%.

$$Y_{i,j,k} = \mu + P_r + F_j(Pr_i) + B_k + \varepsilon$$

where Y is the dependent variable, μ is the general mean, Pr is the random effect of the provenance, $F(Pr)$ is the random effect of family within provenance, B is the random effect of the block, and ε is the error term. The two progeny trials PREB and PRIA, were analyzed with an equivalent linear model without the provenance effect, and excluding the three unimproved seed sources from the analysis.

Narrow sense heritability (h^2) was calculated assuming the open-pollinated families as true half-sibs:

$$h^2 = \frac{\sigma^2_A}{\sigma^2_P} = 4\,\frac{\sigma^2_f}{\sigma^2_f + \sigma^2_\varepsilon}$$

where σ^2_A is the additive variance, σ^2_P is the phenotypic variance, σ^2_f is the familiar variance and σ^2_ε is the variance due to error.

Quantitative trait variation (Q_{st}) was calculated as

$$Q_{st} = \frac{\sigma^2_f}{\sigma^2_f + 2 \cdot 4\sigma^2_{f(Pr)}}$$

where $\sigma^2_{f(Pr)}$ is the family variance within provenances.

Coefficient of additive genetic variance CV_a was defined as

$$CV_a = \frac{\sigma^2_A}{\mu}$$

being μ the general mean.

Phenotypic r_p and genetic correlations r_A were calculated according to

$$r_A \frac{COV_f\,(x,y)}{\sqrt{\sigma^2_{fx} \cdot \sigma^2_{fy}}} \quad \text{and } r_P = \frac{COV\,(x,y)}{\sqrt{\sigma^2_x \cdot \sigma^2_y}}$$

where COV_{xy} is the covariance between any two variables x and y and σ^2_x and σ^2_y are their corresponding variances. COV_{fxy} is the family variance, obtained as follows:

$$COV_{fxy} = \frac{\sigma^2_{fxy} - \sigma^2_{fx} - \sigma^2_{fy}}{2}$$

where σ^2_{fxy} is the family variance of a composite variable resulting from the sum of any two variables x and y.

Results

Threshold size for reproduction

In *Pinus halepensis* trials, mean height was higher and ontogenic development more advanced in AMON than in AMEG as a result of less limiting ecological conditions. In *P. pinaster* PMER and PCAV trials had a similar development, with slightly bigger trees but less sexually developed in PCAV. In PREB and PRIA trials, height was also very similar but in PRIA the number of reproductive trees was lower. Overall, survival in *P halepensis* trials was lower than in *P. pinaster* ones (Table1).

In Aleppo pine (AMEG trial assessed in 2005), both Vob ($\chi^2 = 561.9$, $p < 0.0001$) and population ($\chi^2 = 70.3$, $p < 0.0001$) contributed significatively to the fact of being adult or juvenile (CATREP). There was a 3.6-fold difference among populations for V50 and a 8.5-fold difference for RA. V50, despite large errors, showed a significant correlation with mean RA at the population level (Fig. 2). Trees reached maturity at a mean height of 243 cm, and this was virtually always as females since just 2 out of 1,493 trees bore male cones only (Fig. 3).

Similarly, in the two Maritime pine trials (PMER and PCAV sites) both tree height ($\chi^2 = 512.9$ for PMER, and $\chi^2 = 93.5$ for PCAV, $p < 0.0001$) and population ($\chi^2 = 114.2$ for PMER and $\chi^2 = 164.5$ for PCAV, $p < 0.0001$) had a significant effect on CATREP. There was a 2.4-fold (152 cm) difference between the most and lest precocious populations in PMER, and a 3.3-fold (226 cm) difference in PCAV, with strong among provenance correspondence between the two test sites (Fig. 4). Unlike Aleppo pine, Maritime pine trees reached maturity either as males or females (Fig. 3). In both sites, 95% confidence level intervals showed

Table 1. Summary information of Aleppo pine and Maritime pine genetic trials: code, number of plants, number of populations, number of families, assessment age (years), survival (%), average tree height (cm) and reproductive trees (%)

Trial	Plants	Populations	Families	Assessment age (yrs)	Survival (%)	H (cm)	R (%)
Aleppo pine							
AMEG	2,182	32	148	11/15	67.0	217	73.8
AMON	2,037	32	148	15	67.1	784	99.8
Maritime pine							
PMER	3,152	25	217	5	81.0	161	64.2
PCAV	3,456	25	224	5	83.9	182	52.4
PREB	2,007	—	28	5	80.9	359	75.4
PRIA	2,098	—	28	5	83.2	350	64.7

differences between average heights in juvenile, and female trees respect to male and synchronous ones (Fig. 3).

Intraespecific variation in reproductive allocation and tree size

In Aleppo pine, both volume and reproductive allocation differed significantly among populations ($p < 0.001$) and among families within populations ($p < 0.001$) in both years, although in 2005, variation among families within populations was just marginally significant ($p < 0.10$) for RA. Because measures in RA were not comparable between the two sites, variation in plasticity was ilustrated by rank Spearman correlation in 2009 (Fig. 5). A consistent behaviour was found among populations between both sites for RA ($\rho = 0.80$), indicating limited population × site interaction; *i.e.* low differences for plasticity between populations.

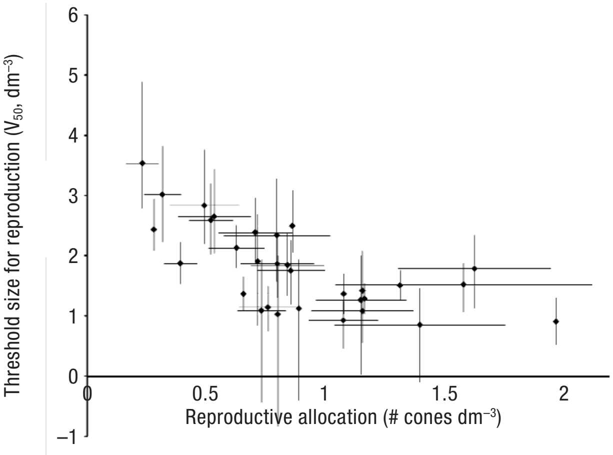


Figure 2. Relationship across populations between reproductive allocation (RA) in 2005 in AMEG and the corresponding Volume over bark at which the reproduction probability was 50% (V_{50}). Each point represents a population. Vertical lines indicate 75% confidence intervals for V_{50} and horizontal lines standard errors for RA in year 2005.

Significant within population variation ($p < 0.001$) in RA and Vob was also observed in the *P. pinaster* trials, with a very high genetic correlation among sites for RA (Fig. 6). The high correspondance between sites in reproductive traits constrast with the strong genotype × environment interaction observed for growth ($r = 0.13$ for Vob *vs* $r = 0.89$ for RA).

Heritabilities for RA ranked from 0.27 to 0.63 and they were higher than those for Vob (0.14-0.22). Heritabilities for RA were also more variable than for Vob. The highest value was attained in the Aleppo pine trial AMEG in 2009. The coefficient of quantitative variation (Qst) did not show a consistent difference for RA and Vob. Hence, Qst for RA was much higher than for volume in AMEG in 2005, similar in 2009 but lower in AMON. However, the greatest difference for genetic parameters between reproductive allocation and tree size was that of the coefficients of additive genetic variance (CVa), which were consistently higher (up to 5.6-fold) for RA across species, sites and years (Table 2).

Phenotypic and genetic correlations

Phenotypic correlations between RA and Vob were negative in all cases except for AMEG in 2005, being between moderate and low ($r_P = -0.18$ to 0.11) except in AMON ($r_P = -0.37$). Genotypic correlations were also negative but much stronger ($r_A = -0.30$ to -0.97) than phenotypic correlations except in AMEG in 2009 ($r_A = -0.05$) (Table 3)

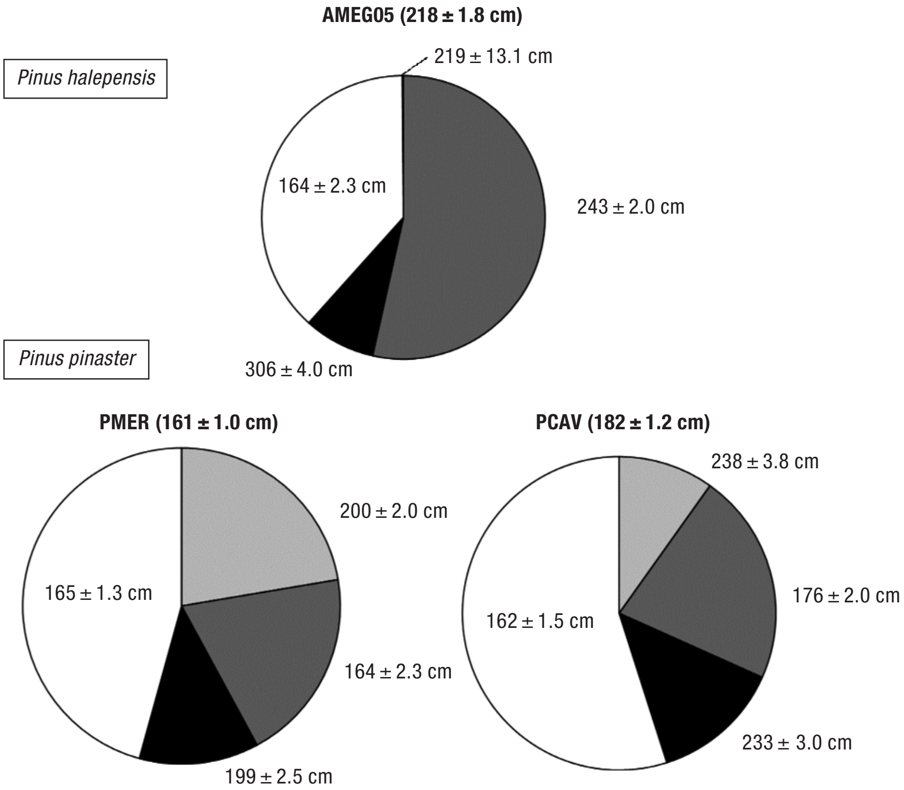


Figure 3. Proportions of juvenile (white), male (pale grey), female (dark grey) or synchronous (black) individuals in AMEG (Aleppo pine) and in PMER and PCAV (Maritime pine) trial sites. Numbers indicate average (± s.e) height for each group or site.

Discussion

Our results show significant intraspecific variation on reproductive traits both in Aleppo pine and in Maritime pine, consistent with previous information. Reproductive allocation was studied here during the onset of reproduction, when it is most relevant in the case of short fire return intervals and when trade-offs with vegetative growth are expected to be greater (Wesselingh *et al.*, 1997).

Intraespecific variation in threshold size for reproduction and reproductive allocation

TSR in plants has been assessed in the frame of developmental biology mainly for herbaceous plants, existing however only few examples (but see Méndez and Karlsson, 2004). Estimation of intraspecific TSR in trees is inherently challenging due to their late maturity (up to many years), and their relatively larger size. There is however some information regarding age

Table 2. Genetic parameter estimates for early reproductive allocation (*RA*) and Volume over bark (*Vob*) in different Aleppo pine and maritime pine sites and ages.

Species	Site	Age	h^2		Q_{st}		*CVa*	
			RA	*Vob*	*RA*	*Vob*	*RA*	*Vob*
Aleppo pine	AMEG	11	0.29	0.22	0.48	0.18	82.29	43.31
	AMEG	15	0.63	0.22	0.12	0.12	100.56	33.02
	AMON	15	0.27	0.14	0.21	0.29	40.23	21.91
Maritime pine	PREB	5	0.47	0.14			82.34	11.65
	PRIA	5	0.32	0.18			71.21	17.51

h^2: narrow sense heritability. Q_{st}: coefficient of quantitativ variation. *CVa*: coefficient of additive genetic variance.

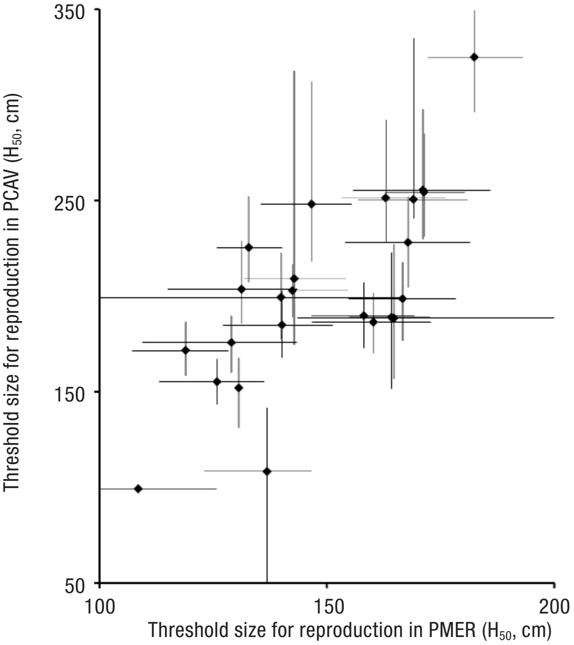


Figure 4. Relationship across populations between threshold size for reproduction (H_{50}, height at which reproduction probability is 50% for a given population) in two Maritime pine trial sites, PMER and PCAV. Vertical and horizontal lines indicate 75% confidence intervals for H_{50}. Lines were not included if logistic curve adjusting for a given population and site was not significant.

or size at maturity for some tree species, including Mediterranean pines (Schmida *et al.*, 2000), but most studies lack any control of environmental conditions or genetic background.

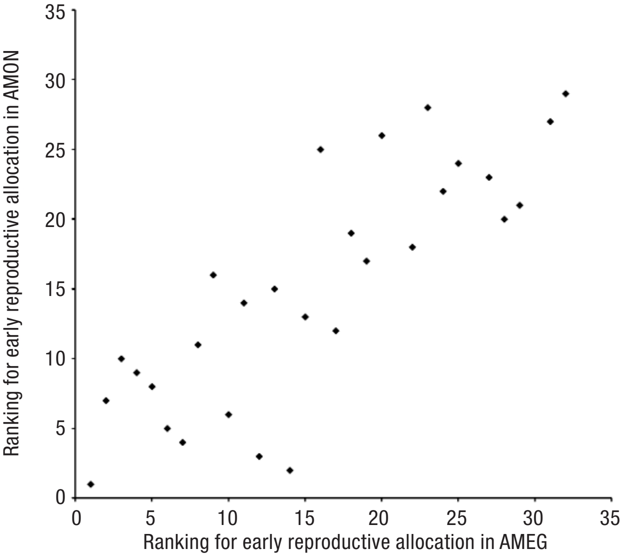


Figure 5. Relationship between the population ranking for early reproductive allocation (RA) estimated in two contrasting *P. halepensis* sites in 2009, AMEG and AMON. Each point represents a population.

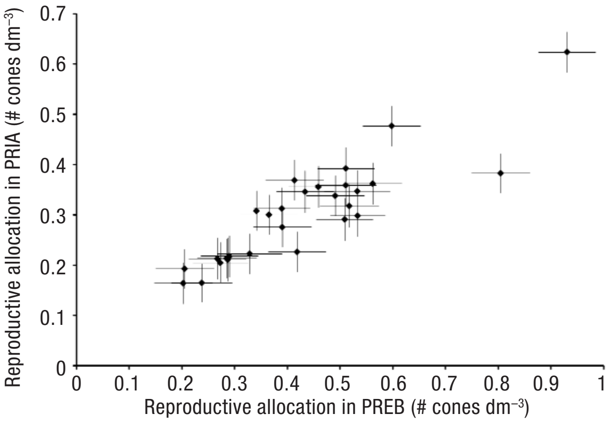


Figure 6. Family relationship in early reproductive allocation between two *P. pinaster* progeny trials, PREB and PRIA. Each point represents an open-pollinated familiy. Vertical and horizontal lines denote standard errors.

In Aleppo pine three main findings stand out. First, we found large differences between populations in both threshold size for reproduction (TSR) and reproductive allocation (RA), consistent with previous results in this species (Climent *et al.*, 2008) despite Spanish populations were reported to be more genetically uniform (Grivet *et al.,* 2009). Second, a close inverse relationship between reproductive allocation (RA) and TSR was found (Fig. 2) confirming that precocious populations tend also to invest more in reproduction. Third, reproductive allocation at the population level showed a consistent pattern across sites (AMEG and AMON) despite the widely contrasting experimental environments. This evidence supports the idea that the differential reproductive strategies among populations are not affected by the environmental conditions, and remain largely consistent across environments, even though as it was the case in AMON, growth conditions were almost unlimited. This high genetic control for female reproduction in Aleppo pine is in line with results from breeding programs reporting high heritability for flower and cone production and consistent behaviour along time (Matziris, 1997).

Significant variability between populations for TSR was also found in Maritime pine and, as in Aleppo pine, with a high consistency of behaviours across environments (*i.e.* low genotype × environment interaction). However, contrasting with Aleppo pine, male reproduction was a highly relevant factor to explain those differences. It has been described an enhanced male reproductive allocation in pines as a result of disturbances such as herbibory or shadow (Schmida *et al.*,

Table 3. Phenotypic (r_p) and genetic (r_a) correlations between cone count (*Cc*) and stem volume over bark (*Vob*) and between reproductive allocation (*RA*) and stem volume over bark for Aleppo pine and Maritime pine at different sites and measurement times. All correlations were significant

Species	Site	Age	*Cc-Vob*		*RA-Vob*	
			r_a	r_p	r_a	r_p
Aleppo pine	AMEG	11	0.21	0.56	–0.30	0.11
	AMEG	15	0.42	0.49	–0.05	–0.09
	AMON	15	–0.93	0.29	–0.97	–0.37
Maritime pine	PREB	5	–0.21	0.16	–0.36	–0.12
	PRIA	5	–0.21	0.12	–0.42	–0.18

2000; Cobb *et al.*, 2002) but to our knowledge, little attention has been paid to interspecific variation in early sex allocation (Richardson, 1998). Sexual specialization patterns between genotypes in relation to environmental conditions should be checked in the future, at the light of the diverse life history traits closely related to fire regime (Tapias *et al.*, 2004). An early female but not male reproduction has been suggested as a consequence of a higher reproductive success in post-fire situations where some adult trees have survived in the surroundings and can pollinate precocious protogynous individuals (Ne'eman *et al.*, 2004). Our data suggest differential selection pressures between Maritime pine populations that could be related to a complex combination of population history, local environment and perturbation regimes.

Moreover, a high additive genetic control for female reproductive allocation between families was found in Maritime pine. In PRIA and PREB, the almost perfect genetic correlations between both sites confirm that although plasticity exists, genetic differences of plasticity between families are negligible. This strong genetic correlation emerges in spite of heavy damage caused by pine weevil *Hylobius abietis* L. in the PRIA trial during the two first years after planting. The pine weevil attack, which differentially affected families (Zas *et al.*, 2005), caused deep alterations of resource allocation and pine growth patterns (Sampedro *et al.*, 2009). Although it is known that herbivory may also affect reproduction traits in pine trees (Cobb *et al.*, 2002; Mueller *et al.*, 2005), it seems to be not the case here, as family variation in reproductive allocation remained fairly consistent irrespective of the incidence of the herbivore. This meaningful result links to a wide scientific discussion about the plasticity of fitness traits, that largely overpasses the objectives of this paper (Schlichting, 2002; Sultan, 2003).

Evolutionary implications

The moderate or high differentiation in reproductive allocation among populations (Q_{st}) is in agreement with a high fitness value of female early reproductive allocation, as it had been postulated based on the species' life histores (Ne'man *et al.*, 2004; Tapias *et al.*, 2004; Climent *et al.*, 2008). Moreover, the very high additive genetic variation between families across populations (CV_a) observed both in Aleppo and Maritime pine could be also interpreted as a sign of high fitness value (Merilä and Sheldon, 1999) despite current controversy in this issue (Glazier, 2002). Nontheless, if a high differentiation between populations can be thought as a fingerprint of different directional selection processes in each environment, a high additive genetic variation provides the fuel for future adaptation to fast changes in perturbation regimes associated to global change. A precocious high female fecundity is advantageous in fire-prone habitats, as seen dramatically in Spain in repeteadly-burned forest stands (Gil *et al.*, 2009) but it can also be thought to increase overall fitness under other environmental constraints, as postulated in *Arabidopsis* (Rutter and Fenster, 2007).

But early female fecundity, as a part of a reproductive strategy is not cost free, and seems to have implications in other traits, as reflected by the negative genetic correlations between reproductive allocation and tree size, also reported previously in pines (Schmidtling, 1981). Our data point towards a clear antagonism between reproduction and growth in both pine species, consistent with previous results in Aleppo pine, showing that most abundant cone yields are produced by middle-sized, not bigger individuals (Climent *et al.*, 2008). However, it should be noted that genetic correlation between reproductive allocation and growth were estimated here upon two non-independent variables, Vob and RA = Cc / Vob. The inclusion of Vob in the denominator for RA estimation may imply a mathematical artifact leading to spurious correlation (Brett, 2004). Although the negative correlation between cone count and volume observed in all trials except AMEG (Table 3) is supporting that the trade-off between growth and early reproduction does exist, bootstrap or Montecarlo simulations should be used in the future to confirm the actual magnitude of this trade-off (Brett, 2004). Actually, costs of reproduction measured as trade offs between reproductive allocation and vegetative growth are a classic and prolific research field, although mainly focused in herbaceous species (Karlsson and Méndez, 2005). Evaluation of costs of reproduction in trees is more challenging than in herbaceous plants, and thus examples are scarce and almost absent in Mediterranean pines. Some examples exist in which no costs were found, even for masting species like *Picea abies* (Seifert and Müller-Starck, 2009) and *Fagus crenata* (Yasumura *et al.*, 2006). To explain that, the existence of compensatory mecanisms (resource storage, enhanced resource acquisition), rather than actual absence of costs of reproduction has been proposed (Karlsson and Méndez, 2005).

Results shown here point to a higher than suspected diversification among Aleppo pine and Maritime pine populations in early reproductive allocation, providing an excellent example of evolution as a response to ecological conditions in two widespread species. The fact that Maritime pine can start its reproductive phase either as male or female, while Aleppo pine consistently starts as female (confirmed by authors' unpublished data) deserves further attention, integrating genetic and environmental control and the different costs of male and female reproduction.

Acknowledgements

Many people collaborated in stablishing and measuring the field trials: R. Alía, D. Agúndez, F. del Caño, N. Godoy, D. Barba, E. Ballesteros, A. Villar, L.M. Alquézar, E. Alamillo, E. Álvarez, M. García, R. Ferradás, P. Martíns, X. Moreira and others. We are thankful to all of them. This research was developed as part of projects AT07-002 and INIA-RTA07-100 of INIA and PSS-310000-2008-4 of the Spanish Ministry of Science and Innovation. Genetic trials reported in this paper form part of Genfored network of forest genetic trials in Spain, implemented mainly through project CC03-048 (DGB-INIA agreement). PMER and PCAV trials were installed as part of project TREESNIPS (QLK3-CT2002-01973). PRIA and PREB trials were established under the Genetic Breeding Program of Galicia by the Centro de Investigación Forestal de Lourizán. Luis Santos is supported by the Spanish Ministry of Education via a doctoral grant (FPU AP-2007-03302).

References

AGEE J.K., 1998. Fire and pine ecosystems. In: Ecology and biogeography of *Pinus* (Richardson D.M., ed). Cambridge University Press, Cambridge, UK. pp. 193-218.

BONSER S.P., AARSSEN L.W., 2001. Allometry and plasticity of meristem allocation throughout development in Arabidopsis thaliana. Journal of Ecology 89, 72-79.

BRETT M.T., 2004. When is a correlation between non-independent variables «spurious»? Oikos 105, 647-656.

BUCCI G., GONZÁLEZ-MARTÍNEZ S.C., LE PROVOST G., PLOMION C., RIBEIRO M.M., SEBASTIANI F., ALIA R.,VENDRAMIN G.G., 2007. Range-wide phylogeography and gene zones in *Pinus pinaster* Ait. revealed by chloroplast microsatellite markers. Molecular Ecology 16, 2137-2153.

CLIMENT J., PRADA M.A., CALAMA R., CHAMBEL M. R., DE RON D.S., ALIA R., 2008. To grow or to seed: Ecotypic variation in reproductive allocation and cone production by young female Aleppo pine (*Pinus halepensis*, *Pinaceae*). American Journal of Botany 95, 833-842.

COBB N.S., TROTTER R.T., WHITHAM, T.G., 2002. Long-term sexual allocation in herbivore resistant and susceptible pinyon pine (*Pinus edulis*). Oecologia 130, 78-87.

GIL L., LÓPEZ R., GARCÍA-MATEOS Á., GONZÁLEZ-DONCEL I., 2009. Seed provenance and fire-related reproductive traits of *Pinus pinaster* in central Spain. International Journal of Wildland Fire 18, 1003-1009.

GLAZIER D.S., 2002. Resource-allocation rules and the heritability of traits. Evolution 56, 1696-1700.

GONZÁLEZ-OCHOA A.I., LÓPEZ-SERRANO F.R., DE LAS HERAS J., 2004. Does post-fire forest management increase tree growth and cone production in *Pinus halepensis*? Forest Ecology and Management 188, 235-247.

GRIVET D., SEBASTIANI F., GONZÁLEZ-MARTÍNEZ S.C., VENDRAMIN G.G., 2009. Patterns of polymorphism resulting from long-range colonization in the Mediterranean conifer Aleppo pine. New Phytologist 184, 1016-1028.

KANG K.S.,BILA A.D., HARJU A.M., LINDGREN D., 2003. Estimation of fertility variation in forest tree populations. Forestry 76, 329-344.

KARLSSON P.S., MÉNDEZ M., 2005. The resource economy of plant reproduction. In: Reproductive allocation in plants. Elsevier, Amsterdam, The Netherlands. pp. 1-40.

KEELEY J.E., ZEDLER P.H., 1998. Evolution of life histories in *Pinus*. In: Ecology and biogeography of *Pinus*. (Richardson D.M., ed). Cambridge University Press, Cambridge, UK. pp. 193-218.

KOENIG W.D., KNOPS J.M.H., CARMEN W.J., STANBACK M.T., MUMME R.L., 1994. Estimating acorn crops using visual surveys. Canadian Journal of Forest Research 24, 2105-2112.

KOENIG W.D., KNOPS, J.M.H., 2000. Patterns of annual seed production by northern hemisphere trees: a global perspective. American Naturalist 155, 59-69.

KOZLOWSKI J., 1992. Optimal allocation of resources to growth and reproduction: implications for age and size at maturity. Trends in Ecology & Evolution 7, 15-19.

MARTÍNS P., SAMPEDRO L., MOREIRA X., ZAS R., 2009. Nutritional status and genetic variation in the response to nutrient availability in *Pinus pinaster*. A multisite field study in Northwest Spain. Forest Ecology and Management 258, 1429-1436.

MATZIRIS D., 1997. Variation in growth, flowering and cone production in a clonal seed orchard of Aleppo pine grown in Greece. Silvae Genetica 46, 224-228.

MÉNDEZ M., KARLSSON P.S., 2004. Between-population variation in size-dependent reproduction and reproductive allocation in *Pinguicula vulgaris* (*Lentibulariaceae*) and its environmental correlates. Oikos 104, 59-70.

MERILÄ J., SHELDON B.C., 2000. Genetic architecture of fitness and nonfitness traits: empirical patterns and development of ideas. Heredity 83, 103-109.

MUELLER R., WADE B., GEHRING C., WHITHAM T., 2005. Chronic herbivory negatively impacts cone and seed production, seed quality and seedling growth of susceptible pinyon pines. Oecologia 143, 558-565.

NE'EMAN G., GOUBITZ S., NATHAN R., 2004. Reproductive traits of *Pinus halepensis* in the light of fire - a critical review. Plant Ecology 171, 69-79.

RICHARDSON D.M. (ed), 1998. Ecology and biogeography of *Pinus*. Cambridge University Press, Cambridge, UK.

RICHARDSON D.M., RUNDEL P.W., JACKSON S.T., TESKEY R.O., ARONSON J., BYTNEROWICZ A., WINGFIELD M.J., PROCHES S., 2007. Human impacts in pine forests: past, present, and future. Annual Review of Ecology Evolution and Systematics 38, 275-297.

ROFF D.A., 2000. Trade-offs between growth and reproduction: an analysis of the quantitative genetic evidence. Journal of Evolutionary Biology 13, 434-445.

RUTTER M.T., FENSTER C.B., 2007. Testing for Adaptation to Climate in *Arabidopsis* thaliana: a calibrated common garden approach. Ann Bot 99, 529-536.

SAMPEDRO L., MOREIRA X., MARTÍNS P., ZAS R., 2009. Growth and nutritional response of *Pinus pinaster* after a large pine weevil (*Hylobius abietis*) attack. Trees-Structure and Function 23, 1189-1197.

SCHLICHTING C.D., 2002. Phenotypic plasticity in plants. Plant Species Biology 17, 85-88.

SCHMIDA A., LEV-YADUM S., GOUBITTZ S., NE'EMAN G., 2000. Sexual allocation and gender segregation in *Pinus halepensis*, *P. brutia* and *P. pinea*. In: Ecology, biogeography and management of *Pinus halepensis* and *P. brutia* forest ecosystems in the Mediterranean Basin. (Ne'eman G., Trabaud L., eds). Backhuys Publishers, Leiden, The Netherlands. pp. 91-104.

SCHMIDTLING R.C., 1981. The inheritance of precocity and its relationship with growth in Loblolly pines. Silvae Genetica 30, 188-192.

SEIFERT T., MÜLLER-STARCK G., 2009. Impacts of fructification on biomass production and correlated genetic effects in Norway spruce (*Picea abies* L. Karst.). European Journal of Forest Research 128, 155-169.

SGRO C.M., HOFFMANN A.A., (2004). Genetic correlations, tradeoffs and environmental variation. Heredity 93, 241-248.

SULTAN S.E., 2003. Phenotypic plasticity in plants: a case study in ecological development. Evolution & Development 5, 25-33.

TAPIAS R., CLIMENT J., PARDOS J.A., GIL L., 2004. Life histories of Mediterranean pines. Plant Ecology 171, 53-68.

THOMPSON I., MACKEY B., MCNULTY S., MOSSELER A., 2009. Forest resilience, biodiversity, and climate change. A synthesis of the biodiversity/resilience/stability relationship in forest ecosystems. Secretariat of the Convention on Biological Diversity, Montreal, Canada. Technical Series no. 43. 67 pp.

WESSELINGH R.A., KLINKHAMER P.G.L., DE JONG T.J., BOORMAN L.A., 1997. Threshold size for flowering in different habitats: effects of size-dependent growth and survival. Ecology 78 2118-2132.

YASUMURA Y., HIKOSAKA K., HIROSE T., 2006. Resource allocation to vegetative and reproductive growth in relation to mast seeding in *Fagus crenata*. Forest Ecology and Management 229, 228-233.

ZAS R., SAMPEDRO L., PRADA E., FERNÁNDEZ-LÓPEZ J., 2005. Genetic variation of *Pinus pinaster* Ait. seedlings in susceptibility to the pine weevil *Hylobius abietis* L. Ann For Sci 62, 681-688.

Appendix

Table A1. Summary of trial design and climatic conditions per site

Species	Site (abr.)	Trial	Altitude	M (°C)	M (°C)	P (mm)	Sp (mm)	Longitude	Latitude
Pinus halepensis	Megeces AMEG	P-p	779	12.1	–0.1	413	66	4°33’30” W	41°25’18” N
	Montañana AMON	P-p	216	14.9	1.5	350	70	0°49’31” W	41°41’5” N
Pinus pinaster	A Merca PMER	P-p	454	12.8	2.5	1,018	92	7°56’17” W	42°14’42” N
	Cavada PCAV	P-p	349	12.6	3.1	1,328	183	6°32’36” W	43°25’15” N
	Rianxo PRIA	p	90	14.6	5.6	1,866	165	8°46’49” W	42°41’7” N
	Rebordelo PREB	p	530	12.5	2.8	2,335	235	8°28’35” W	42°27’40” N

Trial: P-p, provenance-progenies, p, progenies. M: mean annual temperature. m: mean temperature of the coldest month. P: mean annual rainfall. Sp: summer rainfall.

Table A2. List and location of the Spanish populations comprised in the AMEG and AMON Aleppo pine provenance progeny trials. Asterisks indicate seed sources from Northern Plateau afforestations

Population	Latitude	Longitude
Altura	39°47’ N	0°36’ W
Benamaurel	37°42’ N	2°44’ W
Benicasim	40°04’ N	0°01’ E
Cabanellas	42°14’ N	2°47’ E
Carratraca	36°50’ N	4°50’ W
Cazorla	38°06’ N	2°47’ W
Escorca	39°49’ N	2°53’ E
Frigiliana	36°49’ N	3°55’ W
Hijar	41°06’ N	0°25’ W
Lentegi	36°49’ N	3°41’ W
Luna	42°13’ N	0°00’ W
Monroyo	40°47’ N	0°01’ E
Palma de Mallorca	39°08’ N	2°56’ E
Paterna	38°37’ N	2°16’ W
Quesada	37°44’ N	3°09’ W
Ricote	38°08’ N	1°25’ W
Sant Salvador de Guardiola	41°40’ N	1°45’ E
Santanyí	39°17’ N	3°02’ E
Santiago de la Espada	38°13’ N	2°28’ W
Tibi	38°31’ N	0°38’ W
Tivissa	41°03’ N	0°00’ E
Tuéjar	39°49’ N	1°09’ W
Valbuena de Duero*	41°39’ N	4°17’ W
Valdeconcha	40°26’ N	2°52’ W
Valtablado del Río	40°44’ N	2°23’ W
Vega de Valdetronco*	41°35’ N	5°04’ W
Velez Blanco	37°47’ N	2°00’ W
Villa de Ves	39°10’ N	1°14’ W
Villajoyosa	38°29’ N	0°18’ W
Villanueva de Huerva	41°21’ N	1°03’ W
Villavieja de Tordesillas*	41°36’ N	4°55’ W
Zuera	41°55’ N	0°55’ W

Table A3. List and location of the populations comprised in PMER and PCAV Maritime pine provenance progeny trials

Population	Latitude	Longitude	Country
Alto de la Llama	43°17’ N	6°29’ W	Spain
Arenas de San Pedro	40°11’ N	5°06’ W	Spain
Armayán	43°18’ N	6°27’ W	Spain
Bayubas de Abajo	41°31’ N	2°57’ W	Spain
Cadavedo	43°32’ N	6°25’ W	Spain
Carbonero	41°10’ N	4°16’ W	Spain
Castropol	43°30’ N	6°58’ W	Spain
Cenicientos	40°16’ N	4°29’ W	Spain
Coca	41°15’ N	4°29’ W	Spain
Cuellar	41°22’ N	4°29’ W	Spain
Lamuño	43°33’ N	6°13’ W	Spain
Leiria	39°47’ N	8°57’ W	Portugal
Mimizan	44°08’ N	1°18’ E	France
Oria	37°31’ N	2°21’ E	Spain
Pineta	41°57’ N	9°02’ W	France
Pinia	42°01’ N	9°27’ W	France
Pleucadec	47°46’ N	2°20’ W	France
Puerto de vega	43°32’ N	6°37’ W	Spain
Rodoiros	43°25’ N	6°32’ W	Spain
San Cipriano de Ribarteme	42°07’ N	8°21’ W	Spain
San Leonardo	41°50’ N	3°3’ W	Spain
Sergude	42°49’ N	8°27’ W	Spain
Sierra de Barcia	43°31’ N	6°29’ W	Spain
Tamrabta	33°36’ N	5°01’ W	Morocco
Valdemaqueda	40°30’ N	4°05’ W	Spain

APPENDIX II

Genetic differentiation for size at first reproduction through male versus female functions in the widespread Mediterranean tree *Pinus pinaster*. **Santos-del-Blanco L**, Climent J, González-Martínez SC, Pannell JR. 2012. Annals of Botany 110: 1449–1460.

Annals of Botany Page 1 of 12
doi:10.1093/aob/mcs210, available online at www.aob.oxfordjournals.org

ANNALS OF BOTANY
Founded 1887

PART OF A SPECIAL ISSUE ON POPULATION BIOLOGY

Genetic differentiation for size at first reproduction through male versus female functions in the widespread Mediterranean tree *Pinus pinaster*

L. Santos-del-Blanco[1,2,*], J. Climent[1,2], S. C. González-Martínez[1,2] and J. R. Pannell[3]

[1]*INIA-CIFOR, Ctra A Coruña km 7·5, E-28040, Madrid, Spain,* [2]*Sustainable Forest Management Research Institute, University of Valladolid, Avda. Madrid 44, E-34071, Palencia, Spain and* [3]*Department of Ecology and Evolution, Biophore Building, University of Lausanne, CH-1015, Lausanne, Switzerland*
** For correspondence. E-mail* santos.luis@inia.es

Received: 5 March 2012 Returned for revision: 24 April 2012 Accepted: 17 July 2012

- *Background and Aims* The study of local adaptation in plant reproductive traits has received substantial attention in short-lived species, but studies conducted on forest trees are scarce. This lack of research on long-lived species represents an important gap in our knowledge, because inferences about selection on the reproduction and life history of short-lived species cannot necessarily be extrapolated to trees. This study considers whether the size for first reproduction is locally adapted across a broad geographical range of the Mediterranean conifer species *Pinus pinaster*. In particular, the study investigates whether this monoecious species varies genetically among populations in terms of whether individuals start to reproduce through their male function, their female function or both sexual functions simultaneously. Whether differences among populations could be attributed to local adaptation across a climatic gradient is then considered.
- *Methods* Male and female reproduction and growth were measured during early stages of sexual maturity of a *P. pinaster* common garden comprising 23 populations sampled across the species range. Generalized linear mixed models were used to assess genetic variability of early reproductive life-history traits. Environmental correlations with reproductive life-history traits were tested after controlling for neutral genetic structure provided by 12 nuclear simple sequence repeat markers.
- *Key Results* Trees tended to reproduce first through their male function, at a size (height) that varied little among source populations. The transition to female reproduction was slower, showed higher levels of variability and was negatively correlated with vegetative growth traits. Several female reproductive traits were correlated with a gradient of growth conditions, even after accounting for neutral genetic structure, with populations from more unfavourable sites tending to commence female reproduction at a lower individual size.
- *Conclusions* The study represents the first report of genetic variability among populations for differences in the threshold size for first reproduction between male and female sexual functions in a tree species. The relatively uniform size at which individuals begin reproducing through their male function probably represents the fact that pollen dispersal is also relatively invariant among sites. However, the genetic variability in the timing of female reproduction probably reflects environment-dependent costs of cone production. The results also suggest that early sex allocation in this species might evolve under constraints that do not apply to other conifers.

Key words: *Pinus pinaster*, conifers, sex-dependent threshold size for first reproduction, size-dependent sex allocation, clinal variation, neutral genetic structure.

INTRODUCTION

It is widely appreciated that plants are enormously variable in key life-history traits such as seed number, germination rate, growth rates and time to flowering. To the extent that life-history variation has an additive genetic component, these observations are puzzling, because natural selection is expected to deplete genetic variation for traits that affect individual fitness (reviewed by Barton and Keightley, 2002). Much of the variation in life-history traits within populations is attributable to phenotypic plasticity (Sultan, 2000), but it is also well established that plant populations contain large amounts of additive genetic variation for life-history traits, including seed size and number (Mazer, 1987), plant relative growth rate (Sánchez-Gómez *et al.*, 2010), time to flowering (Montague *et al.*, 2008), and flower size and number (Worley and Barrett, 2000). Understanding the nature and maintenance of this variation remains an important challenge for plant evolutionary biologists. One idea is that life-history variation is rendered effectively neutral as a result of antagonistic pleiotropy, i.e. fitness trade-offs between different life-history traits (Walsh and Blows, 2009), or by fluctuating selection from among generations (Bonser and Aarssen, 2009; Childs *et al.*, 2010). Another idea is that much of the variation we observe is maintained in a balance between purifying selection, which depletes it, and its replenishment by mutation or immigration (Barton and Keightley, 2002).

The importance of immigration as a process that introduces new genetic variation for life-history traits into a population will ultimately depend on the maintenance of this variation among populations by adaptive responses to natural selection locally, i.e. on the adaptive divergence among populations for the life-history traits concerned. Measuring population genetic divergence for life-history traits is thus an important task, yet discriminating between variation due to phenotypic plasticity and that due to genetic divergence requires the measurement of traits expressed by individuals from different populations under comparable environmental conditions (i.e. tested in common gardens or reciprocal transplant experiments). In standard common-garden experiments, genotypes sampled from a range of different populations are grown together in a common environment. Unlike reciprocal transplant experiments, common gardens are unable to test the hypothesis of local adaptation for all populations, but they allow an assessment of genetic divergence among more populations that could be accommodated in fully reciprocal transplant experiments. Common garden experiments thus offer a promising avenue towards understanding the maintenance of life-history variation in geographically widespread species.

The age and/or size of first reproduction, i.e. the onset of reproduction, and the number of offspring produced represent particularly important life-history traits. Slight changes in size at maturity and fecundity can have dramatic effects on lifetime reproductive output in perennial species, because early reproduction can be costly in terms of future reproductive potential (Kozłowski, 1992; Roff, 2000). Theory predicts that selection will cause plants with a large life expectancy and a strongly positive relationship between size and fecundity to delay their onset of flowering (Roff, 1992). It is widely accepted that plants tend to use size rather than age as a developmental cue for reproduction (de Jong and Klinkhamer, 2005; but see Metcalf *et al.*, 2003). However, size and age are clearly linked, not least because the age schedule of survival and the disturbance regime, which affects life expectancy, can have an important impact on size at reproduction (Klinkhamer and de Jong, 1987; Burd *et al.*, 2006; Weiner *et al.*, 2009).

Plant size at first reproduction has received relatively little attention in long-lived polycarpic trees (Thomas, 1996; Dodd and Silvertown, 2000; Niklas and Enquist, 2003; Wright *et al.*, 2005), although it is well studied in monocarps (Wesselingh *et al.*, 1997; Rees *et al.*, 1999; Callahan and Pigliucci, 2002; Kuss *et al.*, 2008; Kagaya *et al.*, 2009) and perennial herbs (Méndez and Karlsson, 2004; Brys *et al.*, 2011). These studies have not only documented substantial variability in size at first reproduction among populations, but they have also shown high within-population variability. Moreover, they confirmed theoretical predictions on the role of environmental effects that act through changes in disturbance regimes and growth conditions (see Méndez and Karlsson, 2004).

Although it may sometimes be useful to refer to size at first reproduction as a single trait, individuals in sexual populations transmit their genes through both male and female functions, and the size threshold for each may differ. Thus, in the case of dioecious species, males often begin flowering earlier than females (Delph, 1999). Similarly, in monoecious or hermaphroditic species, male function often precedes female function (Freeman *et al.*, 1981). In some species, this decoupling of male and female functions in relation to size, i.e. a sex-dependent threshold size for first reproduction (TSFR), can result in the presence of individuals functioning as pure males, as females or as hermaphrodites expressing both sexes simultaneously during their early stages of sexual maturity (de Jong and Klinkhamer, 2005; Zhang, 2006). In the extreme, populations can express sequential hermaphroditism, as in the classical example of the jack-in-the-pulpit, where plants are male when small and either female or hermaphroditic one to several reproductive seasons later (Kinoshita, 1987). Such transitions in the functional gender of individuals as they grow have been interpreted in terms of adaptive responses to natural selection when the fitness gains through male and female functions depend on size (Cadet *et al.*, 2004; de Jong and Klinkhamer, 2005; Zhang, 2006).

Size effects on fitness expectations through male vs. female functions can be direct or indirect (Klinkhamer *et al.*, 1997). In wind-pollinated species, pollen dispersal may be enhanced directly by growth in height, so that tall individuals preferentially express their male function (Pickup and Barrett, 2012). In animal-pollinated species, this ‘direct effect’ of height on male fitness is less likely. Here, larger plants are more likely to enhance their female function if the female fitness gain curve flattens off less quickly with investment than the male curve, as seems likely; these are the so-called ‘budget effects’ of size on sex allocation (Klinkhamer *et al.*, 1997). Because a plant’s resource base, and therefore its growth rate and ultimate size, will inevitably depend on habitat quality, we might then expect selection through these indirect budget effects to vary among populations of a species exposed to environments that differ across the species’ geographical range. For example, the relative costs of male versus female functions may be different under different environments, such that female function may be borne optimally by large individuals in some, but not all, populations (see Klinkhamer *et al.*, 1997). If so, differences in sex-specific TSFR may evolve in accordance with the differing trade-offs between reproduction through each sexual function and growth. Such geographical variation in this important life-history trait would allow a more subtle understanding of the action of natural selection on sex allocation that is possible through broader comparisons among species, for example with different pollination modes. There has, however, been very little research on among-population variation in TSFR through male versus female functions (hereafter male or female TSFR) despite the abundant recent literature dealing with sex allocation in plants.

Forest trees offer a valuable opportunity to test hypotheses concerning variation in size-dependent sex expression and sex allocation (SDS). Not only do they often have large distribution ranges (Petit and Hampe, 2006), but the several-fold differences in size between small and large plants within populations has probably favoured the selection of SDS patterns (Burd and Allen, 1988). For instance, variability in the gender of monoecious individuals in conifers does seem to be associated with variability in size related to environmental stress (Cobb *et al.*, 2002; Kang, 2007). Interestingly, this is in some ways similar to the well-established observation of an increased tendency towards dioecy with environmental stress in species that vary in the degree to which their sexes are separate (Ashman, 2006; and see Discussion).

In this study, we use a large common garden to assess patterns of genetic variation in early reproductive life-history traits among populations of the widespread Mediterranean species *Pinus pinaster* (maritime pine). *P. pinaster* is a monoecious, wind-pollinated, wind-dispersed conifer with a remarkably wide ecological niche (Tapias *et al.*, 2004). Previous work, based on data from common-garden experiments, has described among-population variation in size at reproduction for this species (Santos-del-Blanco *et al.*, 2010). Here, we investigate whether *P. pinaster* shows differences in the threshold size at first reproduction through male versus female function among populations from a broad geographical range across a strong climatic gradient, from Atlantic (i.e. mild winters, high annual precipitation and low altitude) to dry continental environments. We sought evidence for a genetic trade-off between allocation to growth and early reproduction, and we tested the prediction that the median threshold size at first reproduction through each of the two sexual functions should correlate with the environment of the source populations. Finally, by comparing the among-population genetic structure in terms of life-history traits with that displayed by neutral genetic markers, we tested the hypothesis that observed clinal variation in the former has resulted from responses to natural selection as opposed to drift or historical effects (Alleaume-Benharira *et al.*, 2006; Grivet *et al.*, 2011).

MATERIALS AND METHODS

Common garden

We established our common garden at Parderrubias, Ourense Province, north-west Spain (Fig. 1A). This common garden belongs to a broader series of experiments aimed at describing variability in adaptive traits in *Pinus pinaster*, and was chosen for the present study as it was placed in a transitional area from Atlantic to Mediterranean climate, as indicated by Gower's distance (Fig. 1B). The common garden comprised 194 open-pollinated families from a total of 23 natural populations distributed over the greater part of the species' natural range, including Atlantic Iberian Peninsula and France, Mediterranean Spain, Corsica and Morocco (Fig. 1, Supplementary Data Table S1). Two additional non-natural populations present in the common garden were excluded from the present study.

Half-sib families in natural populations were sampled from trees that were spaced at least 100 m apart. In total, 2767 2-year-old seedlings were established from seed following standard nursery procedures, and were planted in the common garden in 2005 in a resolvable alpha design, with four replicates of 71 incomplete blocks and four contiguous plants from the same family per block. This design was originally chosen because it allows efficient control of spatial heterogeneity, which is a common problem in large common gardens of forest trees. Additional seedlings from a local provenance were used as a border as well as to fill in the gaps of missing plants. Individuals were planted at a spacing of 2 m × 3 m. The soil in the common garden was a humic cambisol. Despite generally uniform climatic conditions across the common garden, there was inevitably small-scale environmental heterogeneity due to a soil depth gradient, and this was probably responsible for some of the (random) variation in plant size. Of the 2767 trees initially planted, 2240 were still alive when we last measured them in 2010.

Measurement of traits and environmental variables

We measured reproductive and growth-related traits in 2007, 2009 and 2010, a period that covered the transition from a point where most individuals were still vegetative to one when most had become reproductive. We also calculated a number of derived size-dependent fitness components (Méndez and Karlsson, 2004), as set out in Table 1. Correlation among size-dependent fitness components was tested using Pearson's correlation coefficient. Population means of size-dependent fitness components were also included in association tests with environmental data (see below). Following previous research (Climent *et al.*, 2008; Grivet *et al.*, 2011), three spatial and six climatic variables were considered (Table 1). Climatic data for Iberian populations were obtained from a functional phytoclimatic model based on raw data from meteorological stations (Gonzalo-Jiménez, 2010). Climatic data for non-Iberian populations were obtained from WorldClim-Global Climate Data at 5′ resolution (Hijmans *et al.*, 2005).

Molecular analysis

Needles from between six and 30 individuals per population (mean = 16) were collected and dried in silica gel for subsequent DNA extraction. Twelve nuclear microsatellites (simple sequence repeats, SSRs) were genotyped: ITPH4516 (Mariette *et al.*, 2001); RPtest1, ctg275, ctg4363, NZPR1078 and NZPR544 (Chagné *et al.*, 2004); A6F03 (Guevara *et al.*, 2005); pEST2669 (Steinitz *et al.*, 2011); and gPp14, epi3, epi5 and epi6 (F. Sebastiani and G. G. Vendramin, Istituto di Genetica Vegetale, CNR, Florence, pers. comm., June 2011; Supplementary Data Table S2). Forward primers were 5′ end-labelled with fluorochromes (HEX, FAM, VIC or PET) and amplified using the Qiagen Multiplex PCR Kit (Qiagen, Venlo, the Netherlands) following the manufacturer's instructions. Amplified allele fragments were separated using an ABI 3730 genetic analyser (Applied Biosystems, Carlsbad, CA, USA) and fragment sizes were determined with reference to the GeneScan^{TM} −500 LIZ$^{®}$ Size Standard (Applied Biosystems) using GeneMapper software version 4·0 (Applied Biosystems).

Data analysis

Genetic variation among populations for flowering probability. Based on the common-garden data, individual male and female reproductive status was coded as a binary variable at each sampling time for each individual (4, 6 and 7 years old). Given the 2-year developmental cycle of female strobili, female reproduction was recorded as present for any tree bearing 1st- or 2nd-year female cones.

The probability of reproduction was modelled by a generalized linear mixed model (GLMM) with a binomial family distribution and logit link function fitted by Laplace approximation, as implemented by the lme4 package (Bates *et al.*, 2011) on the R platform (R Development Core Team, 2012). Total height (i.e. size), provenance and their interaction were used as fixed terms, and family was included as a random factor. Block structure was not included in these models, as preliminary analyses showed no influence of spatial heterogeneity

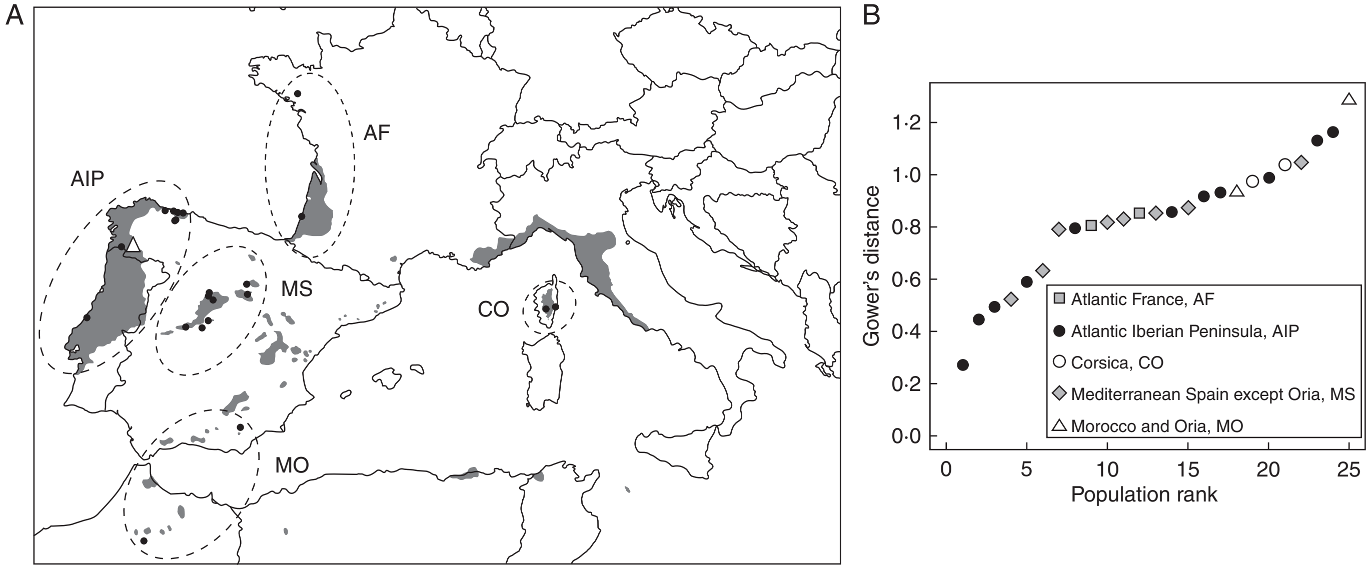


FIG. 1. Distribution map of *Pinus pinaster* source populations (circles) and common garden (triangle). Shaded areas indicate the species' natural distribution range; see key in (B) for population abbreviations. (B) Gower's (absolute) environmental distance between each population to the common garden location. Values closer to 0 indicate greater similarity between the population of origin and the common garden site with respect to the environmental variables used for the calculation of the distance. Symbols represent genetic groups, as indicated in the key.

on the allometry of reproduction (i.e. the block effect or spatial auto-correlation disappeared when tree size variation was included in the models). This first model was used to test the existence of genetic variation among populations for the relationship between size (estimated by height) and reproduction, both by using a likelihood ratio test (LRT) for models with and without the interaction term, and in terms of overall Akaike information criterion.

Ontogenetic and climatic effects on reproduction were necessarily confounded in our experiment, as all trees were the same age. Because we did not include age/year information in the model, our results should thus be interpreted as reflecting the overall behaviour of trees during their transition to maturity, but also to some extent influenced by among-year climatic variation.

We fitted a GLMM for both male and female reproduction using Markov chain Monte Carlo (MCMC) methods, with uninformative priors and fixing residual variance at 1 (MCMCglmm R package; Hadfield, 2011). This approach was preferred over restricted maximum-likelihood methods (Hadfield, 2010), which did not achieve satisfactory convergence for some populations when random effects were included, and also because MCMC methods allowed a more accurate estimation of errors in estimates of the median threshold size for reproduction. The Markov chain was run for 5500 000 iterations, sampling each 5000 after a burn-in period of 500 000 iterations. These settings proved suitable, as successive values of the chain were uncorrelated. Convergence of the chain was also checked graphically. From these models, posterior mode values for the parameters μ (intercept) and α (slope associated with height) were extracted as size-dependent fitness components commonly used in these studies (Méndez and Karlsson, 2004; Brys *et al.*, 2011).

Age-dependent size distribution of reproductive stages. We classified each individual tree as female, male, cosexual or juvenile depending on the sex expressed for its first reproductive event; juveniles were those individuals that had still not started reproducing in 2010. We also recorded the height of individuals at which the first reproductive event took place. We tested whether plant size for each class of reproductive status differed significantly overall and among populations by using the mixed model $H_{ijk} = \mu + R_i \ * \ P_j + F_k + e_{ijk}$, where H was plant height, μ was the general mean, R was the reproductive class (female, male, cosexual or juvenile), P was the population (fixed factor), F was the family (random factor) and e was the random error term. The GLMM was implemented in R. Finally, we recorded the changes in sex choice for all individuals across the last two years (Fig. 2).

Genetic population structure and environmental correlations. Maritime pine is characterized by strong population genetic structure at the range-wide scale (e.g. Bucci *et al.*, 2007). When testing for environmental correlations at wide spatial scales, geographical genetic structure in maritime pine results in large numbers of false positives (estimated at approx. 84 % by Grivet *et al.*, 2011). Thus, here we used linear models to test for genetic associations between early reproductive life-history traits and environmental data that controlled for neutral population structure (i.e. associations between plant traits and environmental data that could be due to demographical history) using molecular markers (nuclear SSRs) as covariates. Neutral population structure was assessed using the Bayesian clustering method implemented in STRUCTURE 2·2 (Pritchard *et al.*, 2000). We ran an admixture model with correlated allele frequencies between clusters. Ten runs were performed for each number of clusters from $K = 1$ to $K = 10$, with a burn-in length of 50 000 and a

TABLE 1. *Common garden measured traits, derived size-dependent fitness components and native location environmental data of* Pinus pinaster *trees*

Variable		Description
Measured traits		
H	Height (cm)	Total height of each tree in 2007, 2009 and 2010
F. Rep	Female reproduction	Presence or absence of seed cones in each tree, yearly from 2007
M.Rep	Male reproduction	Presence or absence of pollen cones in each tree in 2009 and 2010
Derived size-dependent fitness components		
μ		Intercept of reproduction-size logistic regression at the population level
α		Slope of reproduction-size logistic regression at the population level
TSFR	Median threshold size for first reproduction (cm)	Size at which the probability for a tree within a population to have reached sexual maturity was 50 %, equal to μ/α
SRI	Smallest reproductive individual (cm)	Size of the smallest reproductive individual in the population
LVI	Largest vegetative individual (cm)	Size of the largest vegetative individual in the population
RAN		Range between LVI and SRI
%VEG	Percentage of vegetative trees (cm)	Percentage of non-reproductive trees larger than the population TSFR
%REP	Percentage of reproductive trees (cm)	Percentage of reproductive trees in the population
Environmental data		
Alt	Altitude (m)	
Long	Longitude (m)	
Lat	Latitude (m)	
AMT	Annual mean temperature (°C)	
MTWM	Mean temperature of the warmest month (°C)	
MTCM	Mean temperature of the coldest month (°C)	
CI	Continentality index (°C)	Difference between mean temperature of the warmest month and mean temperature of the coldest month
AP	Annual precipitation (mm)	
PDM	Precipitation during the warmest quarter (mm)	Precipitation in June, July and August

run length of 500 000 iterations. The optimal number of population clusters, *K*, was calculated following Evanno *et al.* (2005) and the guidelines provided by STRUCTURE developers in the manual. Population means for individual assignment probability (Fig. 3) to each of the *K* clusters (i.e. the Q matrix, Yu *et al.*, 2006) were used to control for neutral population structure in environmental associations (see similar appoaches in Eckert *et al.*, 2010; Grivet *et al.*, 2011). Additionally, to allow a more synthetic interpretation, we carried out a principal component analysis (PCA) with *varimax* rotation to reduce the number of environmental variables. To decide the number of principal components to retain, we ran a parallel analysis with 1000 iterations (Hayton *et al.*, 2004) and selected only those principal components with eigenvalues for observed data larger than those obtained in simulations. The analysis was implemented by the psych package (Revelle, 2011) on the R platform (R Development Core Team, 2012). PCA loadings of retained components were used in correlation analysis with plant traits. Finally, we used a likelihood ratio test to compare a model including only neutral population genetic structure with one including both neutral population genetic structure and PCA loadings or single environmental variables to explain phenotypic differences among populations.

RESULTS

Phenotypic variation in early sex expression

Trees began to reproduce sexually from 5 years of age, i.e. in 2008, and most were reproductive by year 7 (in 2010). The majority of these early individuals expressed only their male function, with similar lower numbers expressing either only female or both sexual functions (Table 2). In 2009, about half of the plants were juvenile, some remained juvenile in 2010, many began to express their male function and a minority expressed their female function, either purely or together with their male function. Individuals bearing only female or male cones in 2009 tended to produce only male cones or both male and female cones in 2010, so that individuals producing only female cones over two consecutive years were rare. Finally, all trees with female and male cones in 2009 continued producing male cones, but some abandoned their female function (Fig. 2).

Overall, juveniles were the smallest class of individual (206·0 ± 23·9 cm), followed by individuals expressing their female function (233·2 ± 23·6 cm), then those expressing their male function (321·1 ± 23·3 cm) and finally those expressing both functions (372·9 ± 25·2 cm). However, this association between gender expression and plant size also depended significantly on the population of origin (population × gender interaction, $P < 0{\cdot}0001$).

Associations between reproduction and size

Median TSFR varied significantly among populations for both male ($P < 0{\cdot}0001$) and female functions ($P < 0{\cdot}0001$). At the population level, the size effect was highly significant for all populations except the Pineta population (Corsica), which was dropped in subsequent analyses. Across all other populations, intercepts were similar for male and female logistic regressions, while slopes where approximately double for male compared with female reproduction, reflecting the faster transition from juvenile to male reproductive stage.

Median male TSFR was relatively uniform among populations and lower than female TSFR, which varied substantially among populations (Fig. 4).

Average height of the largest vegetative individual (LVI) was sensibly larger for plants considering female function, whereas heights were only slightly smaller for female function compared with male function regarding the smallest reproductive individual (SRI). Accordingly, size ranges were larger for female function (Table 3). Size-dependent fitness components at the population level are given in Supplementary Data Tables S3 and S4.

Correlations and trade-offs between size-dependent fitness components

There was low variability for male function, and correlations between early male reproductive traits with other traits were non-significant (data not shown). In contrast, significant correlations were found between female fitness components, height and the percentage of individuals reaching sexual maturity through female function (Table 4). Height was positively correlated with female TSFR (i.e. delayed female reproduction) and negatively correlated with the number of individuals that became reproductive through female function, thus indicating an overall negative correlation between growth in height and female function at the population level.

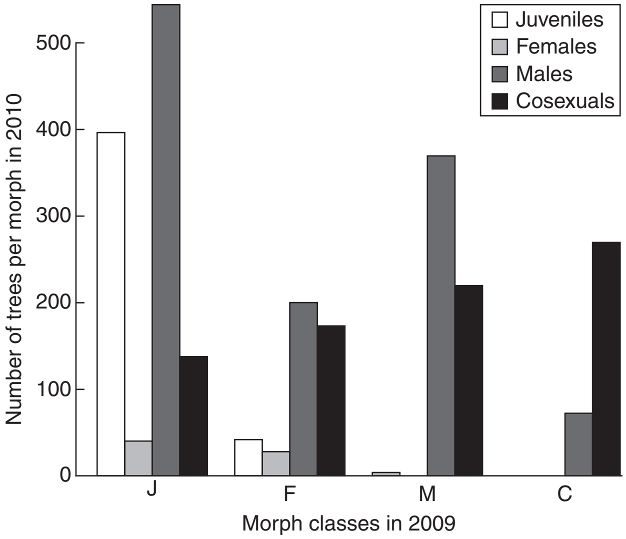


FIG. 2. Transitions between reproductive developmental stages across *Pinus pinaster* populations grown in a common garden in north-west Spain. The *x*-axis represents morph classes at age 6, and within each class bars represent the number of individuals behaving as juveniles (J), females (F), males (M) or cosexuals (C) at age 7.

Environmental correlations

Parallel analysis associated with PCA for environmental traits revealed that only one principal component (PC1),

TABLE 2. *Percentages of* Pinus pinaster *trees remaining non-reproductive (J), or having reached sexual maturity as females (F), males (M) or cosexuals (C) until year 7*

Population	GG	% J	% F	% M	% C	*n*
Mimi	AF	15	13	43	30	96
Pleu	AF	12	36	20	31	108
Alto	AIP	9	12	68	11	65
Arma	AIP	14	8	58	20	71
Cada	AIP	14	3	76	7	87
Cast	AIP	15	15	50	20	74
Lamu	AIP	15	12	55	18	67
Leir	AIP	15	12	61	12	132
Puer	AIP	16	3	66	15	61
Sanc	AIP	10	31	33	26	39
Segu	AIP	7	13	55	24	165
Sier	AIP	11	7	72	11	46
Pine	CO	59	11	30	0	27
Pini	CO	11	6	70	13	54
Oria	MO	20	27	40	13	143
Tamr	MO	43	32	20	5	125
Aren	MS	12	16	48	24	126
Bayu	MS	20	35	31	13	143
Carb	MS	15	35	27	23	48
Ceni	MS	5	16	47	31	55
Coca	MS	32	25	41	1	68
Cuel	MS	14	44	30	12	153
Rodo	MS	17	16	46	21	76
SanL	MS	18	22	40	20	137
Vald	MS	14	18	54	15	74
Average or total		16	19	48	17	2240

N, number of trees per population. GG, genetic group; see Fig. 1 for GG codes and Supplementary Data Table S1 for population codes.

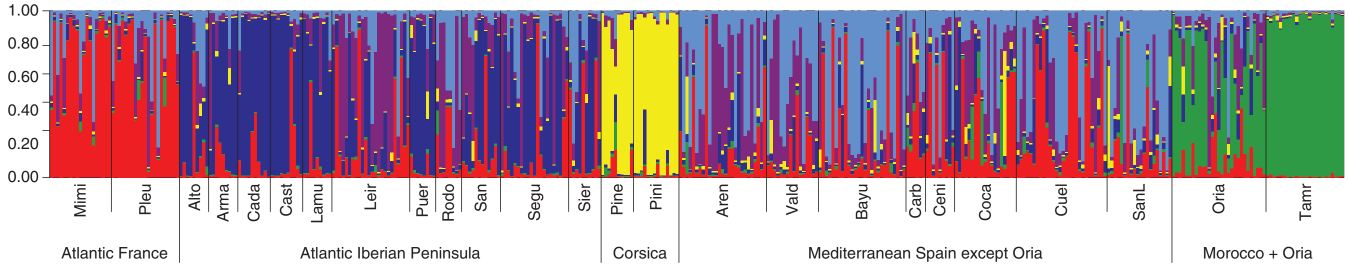


FIG. 3. Barplot of individual assignment probability to each of the optimal $K = 6$ clusters representing five geographical genetic groups in *Pinus pinaster* as produced by STRUCTURE 2·2 software. Each individual is represented as a line segment which is vertically partitioned into *K*-coloured components, representing the individual's estimated proportions of ancestry in the *K* clusters. Population abbreviations are given in Supplementary Data Table S1.

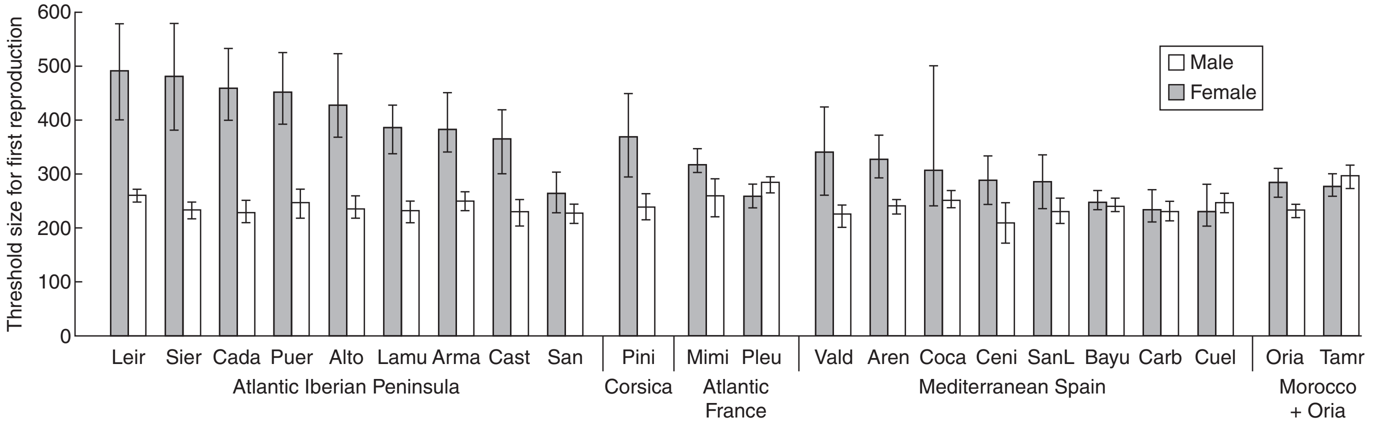


FIG. 4. Median threshold size for first reproduction through male and female functions (as indicated) in *Pinus pinaster* populations grown in the common garden in north-west Spain. Bars represent the posterior mode of Bayesian estimates, with lower and upper 95 % credible intervals. Population abbreviations are given in Supplementary Data Table S1.

explaining 64 % of the variance, should be retained. Overall, this principal component reflected favourable growth conditions. This factor was positively related to longitude, mean temperature of the coldest month (MTCM), precipitation during the warmest quarter (PDM) and annual precipitation (AP), and negatively to altitude, mean temperature of the warmest month (MTWM) and continentality index (CI). Latitude and annual mean temperature (AMT) had loadings below 0·7.

Male TSFR was not correlated with PC1 or any single spatial or climatic variable (Table 5 and Supplementary Data Table S5), but we found significant positive associations between PC1 and female TSFR, height and the percentage of individuals expressing their male function, and negative associations between PC1 and the percentage of individuals expressing their female function. Among single climatic parameters, models for MTCM and CI showed the highest significance levels (see Supplementary Data Table S5). Trees with larger female TFSR tended to come from sites with more favourable growing conditions, as those with milder winters and a reduced degree of continentality (Fig. 5).

Nuclear SSR summary information is provided in Supplementary Data Table S2. A STRUCTURE analysis using Bayesian clustering based on SSRs showed the existence of five clearly differentiated genetic groups in *P. pinaster* corresponding roughly to Atlantic France, Atlantic Iberian Peninsula, Corsica, Mediterranean Spain (except the Oria population in southern Spain) and Morocco plus Oria (Fig. 3 and Supplementary Data Table S6). Atlantic and Mediterranean Iberian populations displayed varying degrees of admixture, which has been suggested to be the result of historical gene flow among Mediterranean and Atlantic maritime pine glacial refugia (de Lucas *et al.*, 2009). Once neutral population genetic structure was integrated in the models, the overall correlations of early reproductive life-history traits with favourable growth conditions (as resumed in PC1) remained significant. However, several correlations for single environmental parameters became non-significant, including one for CI (see Supplementary Data Table S5). This indicated that either some true environmental associations were confounded with neutral genetic structure imposed by demographic history (and thus were not reliable), or that they were false-positives.

TABLE 3. *Mean values for size-dependent fitness components for male and female reproduction, averaged across 22 natural* Pinus pinaster *populations; abbreviations are as defined in Table 1*

	Male	Female
μ	−8·07 ± 1·79	−5·75 ± 0·90
α	0·033 ± 0·007	0·018 ± 0·005
TSFR	241·7 ± 19·7	339·1 ± 81·9
LVI	322 ± 50	393 ± 39
SRI	113 ± 17	105 ± 22
RAN	209 ± 52	254 ± 100
%VEG	4·0 ± 2·4	1·7 ± 2·3

DISCUSSION

Our results provide evidence for clear genetic differences in key life-history traits among populations of *P. pinaster* sampled across a wide environmental range. Because much of this genetic variation was associated with a strong environmental gradient among the sites we sampled, some of which remained significant after neutral genetic structure was accounted for, the observed life-history trait clines are likely to be the result of local adaptation rather than simply the outcome of divergence through drift or historical accident. This is among the first reports of reproductive life-history trait variation in a widespread forest species (Climent *et al.*, 2008; Santos-del-Blanco *et al.*, 2010) but also, as far as we know, the first report of adaptive genetic differentiation for both male and female threshold sizes for reproduction in a (monoecious) plant species.

The *P. pinaster* individuals in our study tended to start reproducing preferentially as males, and they tended to maintain their male function fairly constantly through time. Accordingly, male median TSFR was lower than female TSFR. This finding is somewhat surprising, given that most

TABLE 4. *Among-population correlations for early female reproductive traits in* Pinus pinaster*; abbreviations are as defined in Table 1.*

	fμ	fα	fTSFR	fLVI	fSRI	fRAN	%fVEG	%J	%F
H	−0·305	−0·360	0·691***	0·654***	0·744***	0·269	−0·363	−0·634**	−0·747***
fμ		−0·523*	−0·148	−0·505*	−0·553**	−0·222	0·117	−0·036	0·315
fα			−0·730***	−0·161	−0·094	−0·128	0·521*	0·152	0·517*
fTSFR				0·624**	0·544**	0·372	−0·694***	−0·231	−0·865***
fLVI					0·557**	0·815	−0·337	−0·149	−0·579**
fSRI						−0·026	−0·324	−0·420	−0·689***
fRAN							−0·180	0·113	−0·217
%fVEG								0·284	0·768***
%J									0·322

Significant at: ***$P < 0·001$, **$P < 0·01$, *$P < 0·05$.

conifer species tend to start reproducing as females (Williams, 2009). In perennial plants and, most notably, in trees, the existence of threshold sizes for reproduction arises from the need to invest all available resources in a large vegetative body during early developmental stages to maximize further reproduction (reviewed by Thomas, 2011). The existence of greater female costs of reproduction has been related to a generally lower threshold size for male reproduction in dioecious and monoecious plants and to failures in setting fruit in species with perfect flowers (Iwasa, 1991; de Jong and Klinkhamer, 2005). This pattern, largely observed in woody angiosperms, differs from that typically observed in conifers, which display earlier expression of female function. *P. pinaster* would thus seem to have evolved a pattern of early reproduction and sex allocation more similar to woody angiosperms than other conifers.

Although the trend for early male reproduction in *P. pinaster* is interesting and unusual, the more striking pattern we observed is that male TSFR is much more canalized, both within and among populations, than female TSFR: individuals in our common garden started producing male cones at around 2·5 m in height, whereas female function commenced in individuals over a very wide range of heights (Fig. 4). Why should male TSFR be so uniform, and why should the female TSFR vary so widely?

An increase in maleness with size in wind-pollinated plants is a well-established prediction from SDS theory (de Jong and Klinkhamer, 2005), and forest trees have been identified as suitable organisms to test it (Cruden and Lyon, 1985). The prediction rests on the presumed advantage to plants that release their pollen from greater heights, regardless of the seed dispersal mode (Friedman and Barrett, 2009). However, in conical-shaped conifers, like many pine species, male cones are not located in the treetop but rather on lower secondary branches, and it is the female cones that are borne by upper vigorous branches. This segregation is more evident in adult trees (Shmida *et al.*, 2000), but we have observed it also in our young maritime pine specimens. This architectural pattern has been proposed either as a strategy to avoid selfing, as male and female flowering are synchronous in this species (Miguel-Pérez *et al.*, 2002), and/or as a consequence of the larger size of female cones which could only be borne on stronger upper vertical branches (Ne'eman *et al.*, 2011). Either way, the hypothesis that increased height favours male

TABLE 5. *Environmental associations of* Pinus pinaster *reproductive life-history traits (first column) with environmental variables represented by population scorings on first component of PCA analysis*

	Corrected		Uncorrected	
Trait	Slope	*P*	Slope	*P*
fTSFR	109·10 ± 26·97	0·001	56·34 ± 13·30	0·000
mTSFR	8·36 ± 8·90	n.s.	−1·89 ± 4·39	n.s.
H	32·21 ± 11·80	0·016	24·30 ± 4·16	0·000
%M	15·28 ± 6·15	0·025	10·43 ± 2·97	0·002
%F	−12·34 ± 4·85	0·023	−7·74 ± 2·04	0·001

Corrected values indicate slopes, standard errors and *P*-values for the association after including neutral genetic structure corrections (i.e. likelihood ratio test between a full model and a reduced model with just neutral genetic structure); uncorrected values indicate association parameters without correcting for neutral genetic structure (i.e. just environmental data and traits data in the model). n.s., not significant.

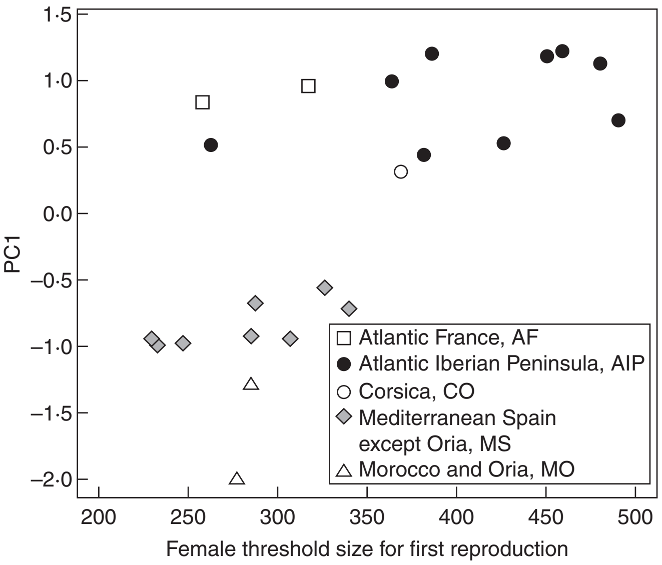


FIG. 5. Variation of *Pinus pinaster* median female threshold size for first reproduction along an environmental harshness gradient, as estimated by population scorings on PC1. Symbols represent genetic groups, as indicated in the key.

function would not offer a convincing explanation for size-dependent sex allocation in conifers (Fox, 1993). Moreover, the size advantage hypothesis only applies in the presence of clear differences in size among individuals within a population (Friedman and Barrett, 2009), which is not common in Mediterranean conifer forests. The established theory for size-dependent sex allocation thus would not seem to apply to conifers in a straightforward manner.

Given that the position of male and female cones is conserved across conical-shaped conifers and thus appears to be phylogenetically constrained, for whatever reason, a more plausible explanation for canalization of male TSFR is simply that pollen needs to be released above a threshold height to be lifted by horizontal winds and updrafts; if so, it would not be surprising if the same physical constraint applied to all individuals, irrespective of their provenance. The high value for the slope of the male flowering probability curve with size ($\alpha = 0{\cdot}033$) indicates low genetic variation for this trait and a common sharp threshold for male TSFR within and across populations. We therefore hypothesize that the low among-population variation in the threshold sizes for male reproduction found in our study points to the existence of similar male fitness gain curves and might be the result of rather uniform conditions for pollen release and transport across populations. To our knowledge, this possibility has not yet been considered in the SDS literature.

Female TSFR not only showed high variability within populations, but also clear evidence for high among-population differentiation; this variation, in turn, was significantly correlated with most early reproductive life-history traits (Table 4). For example, we found a positive correlation among populations between the average height of individuals and female TSFR, and a negative correlation between the average height in the population and the number of individuals first reproducing as females (as opposed to early males). These patterns emerge as negative correlations of population means for reproductive and growth traits measured in a common garden environment, so our results should be considered at the genetic, not phenotypic or physiological, level (Reznick, 1985; de Jong and Klinkhamer, 2005). Thus, the observed patterns probably reflect genetic trade-offs between growth and female reproduction that have been selected under different environments.

A particularly interesting finding is that much of the observed among-population variation was associated with climatic differences among sites. At least some of this variation might underlie possible differences in the intensity of within-stand competition (e.g. competition might be more important at more mesic sites; Grime, 1977; Grivet *et al.*, 2011). Thus, in favourable (yet strongly competitive) environments, early investment in the more costly female function would increase the risk to individuals of being suppressed by their neighbours, so a delay of reproduction might be advantageous (Thomas, 2011). By contrast, in unfavourable (but less competitive) environments, selection would tend to favour stress tolerance at the expense of growth or competitive ability, so that a low female TSFR would be expected (Roff, 1992).

In accordance with this reasoning, we found lower female TSFR and a higher proportion of individuals first expressing their female function in populations from sites offering less favourable conditions for growth, a pattern that remained significant after accounting for neutral genetic structure. It would thus seem plausible that, on the one hand, populations have become differentiated for female TSFR in response to selection under possible differences in the competitive environment and disturbance regime among sites, and, on the other hand, a canalized male TSFR has evolved under the possible site-independent physical constraints facing pollen dispersal in conifers.

The idea that selection will have caused populations of *P. pinaster* to diverge for reproductive traits across its range is largely in keeping with observed clinal variation in morphological traits for a number of widespread species (Davis *et al.*, 2005); in some cases, this clinal variation has evolved over short time periods, for example flowering-time variation in *Verbascum thapsus* (Ansari and Daehler, 2010) and *Lythrum salicaria* (Montague *et al.*, 2008; Colautti *et al.*, 2010). Similarly, artificial selection experiments on size at reproduction in *Cynoglossum officinale* also elicited a fast and direct response (Wesselingh and de Jong, 1995), and the cultivation of *Eucalyptus* trees in India resulted in the rapid evolution of fecundity traits (Varghese *et al.*, 2009). These studies argue for the existence of large genetic variation for size at reproduction, allowing fast among-population differentiation due to strong selection.

If the above explanation is able to account for among-population patterns in TSFR, how might we explain the differences in within-population variability in this important trait between male and female functions? A differential plastic response in female TSFR of populations grown out of their native habitat could account for variability registered in the common garden. This variability might not be present in native habitats, where a more uniform female TSFR could exist. However, we found no evidence of correlation between environmental distance and range of median threshold sizes in each population ($t_{21} = 0{\cdot}761$, $P > 0{\cdot}45$). Another possibility is that the greater variance in TSFR in female function within populations is attributable to the evolution of a bet-hedging strategy, either through stochastic or plastic expression of the same underlying genotypes, or through the frequency-dependent maintenance of genetic variation for TSFR under environmental stochasticity (Rees *et al.*, 2004; Metcalf *et al.*, 2008; Weiner *et al.*, 2009; Childs *et al.*, 2010). The role of environmental stochasticity on reproductive strategies has been described mainly for monocarps, which can suffer high rates of mortality before reaching maturity. Woody species usually have more stable demographic patterns than monocarps, probably buffered by ongoing trade-offs between investment in reproduction and growth or maintenance. Nevertheless, Mediterranean pine species suffer unpredictable fire return intervals as well as episodic droughts, both of which are likely to play a major role in reproductive strategies (Pausas *et al.*, 2008). A bet-hedging strategy in a long-lived woody species prone to environmental stochasticity might be expected to evolve for the more expensive reproduction function, i.e. for cone production, as observed. Interestingly, previous studies on *Pinus* spp. report lower heritabilities for male compared with female cone production, for example in *P. pinaster* (Merlo and Fernández López, 2004) and *P. sylvestris* (Savolainen *et al.*, 1993), suggesting that a similar explanation might apply to several species evolving under a rather broad range of environmental challenges.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Table S1. List and location of the *Pinus pinaster* populations comprising the present study. Table S2. Summary information of nuclear SSR markers used to estimate range-wide population genetic structure in *Pinus pinaster*. Table S3. Size-dependent fitness components for male reproduction in 23 natural *Pinus pinaster* populations. Table S4. Size-dependent fitness components for female reproduction in 23 natural *Pinus pinaster* populations. Table S5. Associations of environmental variables with reproductive life-history traits in *Pinus pinaster*. Table S6. Number of trees per population included in the molecular marker analysis and average individual assignment probability for each of the optimal $K = 6$ clusters representing five geographical genetic groups in *Pinus pinaster*.

ACKNOWLEDGEMENTS

R. Alía and J. Majada established and maintained the common garden (TREESNIPS QLK3-CT2002-01973). The data used in this research are part of the Spanish Network of Genetic Trials (GENFORED). We thank E. Ballesteros, E. Alamillo, F. del Caño, D. Barba and N. Godoy (†) for fieldwork. Thanks are extended to A. I. de-Lucas, J. P. Jaramillo-Correa and G. G. Vendramin for assistance in nuclear microsatellite genotyping, to D. Grivet for advice on environmental correlations and to J. J. Robledo-Arnuncio for valuable discussions. The manuscript also benefited from comments by R. Wesselingh and S. P. Bonser. This study was funded by the Spanish Ministry of Economy and Competitiveness through projects Mitigenfor (RTA 2011-00016-00-00), Restaura (PSS-310000-2008-4), AdapCon (CGL2011-30182-C02-01) and LinkTree (ERAnet-BiodivERsA; EUI2008-03713). Additional funding was provided by the European Community's Seventh Framework Program (FP7/2007-2013) under grant agreement no. 211868 (Project Noveltree) KBBE-2007-1-2-05. Finally, this work was also supported by the Spanish Ministry of Education through a PhD grant to L.S.B. (FPU-AP-03302).

LITERATURE CITED

Alleaume-Benharira M, Pen I, Ronce O. 2006. Geographical patterns of adaptation within a species' range: interactions between drift and gene flow. *Journal of Evolutionary Biology* **19**: 203–215.

Ansari S, Daehler CC. 2010. Life history variation in a temperate plant invader, *Verbascum thapsus along a tropical elevational gradient in Hawaii. Biological Invasions* **12**: 4033–4047.

Ashman TL. 2006. The evolution of separate sexes: a focus on the ecological context. In: Harder LD, Barrett SCH. eds. *Ecology and evolution of flowers*. New York: Oxford University Press, 204–222.

Barton N, Keightley P. 2002. Understanding quantitative genetic variation. *Nature Reviews Genetics* **3**: 11–21.

Bates D, Maechler M, Bolker B. 2011. *Package lme4: linear mixed-effects models using S4 classes*. R package version 0·999375-42. http://CRAN.R-project.org/package=lme4.

Bonser SP, Aarssen LW. 2009. Interpreting reproductive allometry: individual strategies of allocation explain size-dependent reproduction in plant populations. *Perspectives in Plant Ecology, Evolution and Systematics* **11**: 31–40.

Brys R, Shefferson RP, Jacquemyn H. 2011. Impact of herbivory on flowering behaviour and life history trade-offs in a polycarpic herb: a 10-year experiment. *Oecologia* **166**: 293–309.

Bucci G, González-Martínez SC, Le-Provost G, *et al.* 2007. Range-wide phylogeography and gene zones in *Pinus pinaster* Ait. revealed by chloroplast microsatellite markers. *Molecular Ecolology* **16**: 2137–2153.

Burd M, Allen TFH. 1988. Sexual allocation strategy in wind-pollinated plants. *Evolution* **42**: 403–407.

Burd M, Read J, Sanson GD, Jaffré T. 2006. Age-size plasticity for reproduction in monocarpic plants. *Ecology* **87**: 2755–2764.

Cadet C, Metz JAJ, Klinkhamer PGL. 2004. Size and the not so single sex: disentangling the effects of size and budget on sex allocation in hermaphrodites. *The American Naturalist* **164**: 779–792.

Callahan HS, Pigliucci M. 2002. Shade-induced plasticity and its ecological significance in wild populations of *Arabidopsis thaliana*. *Ecology* **83**: 1965–1980.

Chagné D, Chaumeil P, Ramboer A, *et al.* 2004. Cross-species transferability and mapping of genomic and cDNA SSRs in pines. *Theoretical and Applied Genetics* **109**: 1204–1214.

Childs DZ, Metcalf CJE, Rees M. 2010. Evolutionary bet-hedging in the real world: empirical evidence and challenges revealed by plants. *Proceedings of the Royal Society B: Biological Sciences* **277**: 3055–3064.

Climent J, Prada MA, Calama R, Chambel MR, Sánchez de Ron D, Alia R. 2008. To grow or to seed: ecotypic variation in reproductive allocation and cone production by young female Aleppo pine (*Pinus halepensis*, Pinaceae). *American Journal of Botany* **95**: 833–842.

Cobb NS, Trotter RTIII, Whitham TG. 2002. Long-term sexual allocation in herbivore resistant and susceptible Pinyon pine (*Pinus edulis*). *Oecologia* **130**: 78–87.

Colautti RI, Eckert CG, Barrett SCH. 2010. Evolutionary constraints on adaptive evolution during range expansion in an invasive plant. *Proceedings of the Royal Society B: Biological Sciences* **277**: 1799–806.

Cruden RW, Lyon DL. 1985. Patterns of biomass allocation to male and female functions in plants with different mating systems. *Oecologia* **66**: 299–306.

Davis MB, Shaw RG, Etterson JR. 2005. Evolutionary responses to changing climate. *Ecology* **86**: 1704–1714.

Delph L. 1999. Sexual dimorphism in life history. In: Geber MA, Dawson TE, Delph LF. eds. *Gender and sexual dimorphism in flowering plants*. Berlin: Springer, 149–173.

Dodd ME, Silvertown J. 2000. Size-specific fecundity and the influence of lifetime size variation upon effective population size in *Abies balsamea*. *Heredity* **85**: 604–609.

Eckert AJ, van Heerwaarden J, Wegrzyn JL, *et al.* 2010. Patterns of population structure and environmental associations to aridity across the range of loblolly pine (*Pinus taeda* L., Pinaceae). *Genetics* **185**: 969–982.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* **14**: 2611–2620.

Fox JF. 1993. Size and sex allocation in monoecious woody plants. *Oecologia* **94**: 110–113.

Freeman DC, McArthur ED, Harper KT, Blauer AC. 1981. Influence of environment on the floral sex ratio of monoecious plants. *Evolution* **35**: 194–197.

Friedman J, Barrett SCH. 2009. Wind of change: new insights on the ecology and evolution of pollination and mating in wind-pollinated plants. *Annals of Botany* **103**: 1515.

Gonzalo-Jiménez J. 2010. *Diagnosis fitoclimática de la españa peninsular: hacia un modelo de clasificación funcional de la vegetación y de los ecosistemas peninsulares españoles*. Organismo Autónomo de Parques Nacionales.

Grime J. 1977. Evidence for the existence of three primary strategies in plants and Its relevance to ecological and evolutionary theory. *American Naturalist* **111**: 1169–1194.

Grivet D, Sebastiani F, Alía R, *et al.* 2011. Molecular footprints of local adaptation in two Mediterranean conifers. *Molecular Biology and Evolution* **28**: 101–16.

Guevara M, Chagné D, Almeida M, *et al*. 2005. Isolation and characterization of nuclear microsatellite loci in *Pinus pinaster* Ait. *Molecular Ecology Notes* **5**: 57–59.

Hadfield J. 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *Journal of Statistical Software* **33**: 1–22.

Hadfield J. 2011. *Package "MCMCglmm" Reference manual*: http://cran.r-project.org/web/packages/MCMCglmm/MC.

Hayton JC, Allen DG, Scarpello V. 2004. Factor retention decisions in Exploratory Factor Analysis: a tutorial on Parallel Analysis. *Organizational Research Methods* **7**: 191–205.

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

Iwasa Y. 1991. Sex change evolution and cost of reproduction. *Behavioral Ecology* **2**: 56–68.

de Jong TJ, Klinkhamer PGL. 2005. *Evolutionary ecology of plant reproductive strategies*. Cambridge: Cambridge University Press.

Kagaya M, Tani T, Kachi N. 2009. Variation in flowering size and age of a facultative biennial, *Aster kantoensis* (Compositae), in response to nutrient availability. *American Journal of Botany* **96**: 1808–1813.

Kang H. 2007. Changes in gender expression in korean populations of *Pinus densiflora* over a five-year period. *Journal of Plant Biology* **50**: 181–189.

Kinoshita E. 1987. Sex change and population dynamics in *Arisaema* (Araceae) I. *Arisaema serratum* (Thunb.) Schott. *Plant Species Biology* **2**: 15–28.

Klinkhamer PGL, de Jong TJ. 1987. Plant size and seed production in the monocarpic perennial *Cynoglossum Officinale* L. *New Phytologist* **106**: 773–783.

Klinkhamer PGL, de Jong TJ, Metz H. 1997. Sex and size in cosexual plants. *Trends in Ecology & Evolution* **12**: 260–265.

Kozłowski J. 1992. Optimal allocation of resources to growth and reproduction: Implications for age and size at maturity. *Trends in Ecology & Evolution* **7**: 15–9.

Kuss P, Rees M, Ægisdóttir HH, Ellner SP, Stöcklin J. 2008. Evolutionary demography of long lived monocarpic perennials: a time lagged integral projection model. *Journal of Ecology* **96**: 821–832.

de Lucas AI, González-Martínez S, Hidalgo E, Bravo F, Heuertz M. 2009. Admixture, one-source colonization or long-term persistence of maritime pine in the Castilian Plateau?: insights from nuclear microsatellite markers. *Investigación agraria. Sistemas y recursos forestales* **18**: 3–12.

Mariette S, Chagne D, Decroocq S, *et al*. 2001. Microsatellite markers for *Pinus pinaster* Ait. *Annals of Forest Science* **58**: 203–206.

Mazer S. 1987. Quantitative genetics of life history and fitness components in *Raphanus raphanistrum* L. (Brassicaceae): ecological and evolutionary consequences of seed-weight. *American Naturalist* **130**: 891–914.

Merlo E, Fernández López J. 2004. Análisis del balance parental reproductivo en un huerto semillero de Pinus pinaster Ait. *Investigación Agraria. Sistemas y Recursos Forestales* **13**: 387–398.

Metcalf CJE, Rose KE, Rees M. 2003. Evolutionary demography of monocarpic perennials. *Trends in Ecology & Evolution* **18**: 471–480.

Metcalf CJE, Rose KE, Childs DZ, Sheppard AW, Grubb PJ, Rees M. 2008. Evolution of flowering decisions in a stochastic, density-dependent environment. *Proceedings of the National Academy of Sciences* **105**: 10466–10470.

Miguel-Pérez I, González-Martínez SC, Alía R, Gil L. 2002. Growth phenology and mating system of Maritime pine (*Pinus pinaster* Aiton) in central Spain. *Investigación Agraria. Sistemas y Recursos Forestales* **11**: 193–204.

Montague JL, Barrett SCH, Eckert CG. 2008. Re-establishment of clinal variation in flowering time among introduced populations of purple loosestrife (*Lythrum salicaria*, Lythraceae). *Journal of Evolutionary Biology* **21**: 234–245.

Méndez M, Karlsson PS. 2004. Between-population variation in size-dependent reproduction and reproductive allocation in *Pinguicula vulgaris* (Lentibulariaceae) and its environmental correlates. *Oikos* **104**: 59–70.

Ne'eman G, Goubitz S, Werger MJ, Shmida A. 2011. Relationships between tree size, crown shape, gender segregation and sex allocation in *Pinus halepensis*, a Mediterranean pine tree. *Annals of Botany* **108**: 197–206.

Niklas KJ, Enquist BJ. 2003. An allometric model for seed plant reproduction. *Evolutionary Ecology Research* **5**: 79–88.

Pausas JG, Llovet J, Rodrigo A, Vallejo R. 2008. Are wildfires a disaster in the Mediterranean basin?–A review. *International Journal of Wildland Fire* **17**: 713–723.

Petit RJ, Hampe A. 2006. Some evolutionary consequences of being a tree. *Annual Review of Ecology, Evolution and Systematics* **37**: 187–214.

Pickup M, Barrett SCH. 2012. Reversal of height dimorphism promotes pollen and seed dispersal in a wind-pollinated dioecious plant. *Biology Letters* **8**: 245–8.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959.

Rees M, Childs DZ, Rose KE, Grubb PJ. 2004. Evolution of size-dependent flowering in a variable environment: partitioning the effects of fluctuating selection. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **271**: 471–475.

Rees M, Sheppard AW, Briese D, Mangel M. 1999. Evolution of size-dependent flowering in *Onopordum illyricum*: a quantitative assessment of the role of stochastic selection pressures. *American Naturalist* **154**: 628–651.

Reznick D. 1985. Costs of reproduction: an evaluation of the empirical evidence. *Oikos* **44**: 257–267.

Roff DA. 1992. Age and size at maturity. In: Roff DA. ed. *The evolution of life histories: theory and analysis*. New York: Chapman and Hall, 179–241.

Roff DA. 2000. Trade-offs between growth and reproduction: an analysis of the quantitative genetic evidence. *Journal of Evolutionary Biology* **13**: 434–445.

Sánchez-Gómez D, Majada J, Alía R, Feito I, Aranda I. 2010. Intraspecific variation in growth and allocation patterns in seedlings of *Pinus pinaster* Ait. submitted to contrasting watering regimes: can water availability explain regional variation? *Annals of Forest Science* **67**: 505–504.

Santos-del-Blanco L, Notivol E, Zas R, Chambel MR, Majada J, Climent J. 2010. Variation of early reproductive allocation in multi-site genetic trials of Maritime pine and Aleppo pine. *Forest Systems* **19**: 381–392.

Savolainen O, Kärkkäinen K, Harju A, Nikkanen T, Rusanen M. 1993. Fertility variation in *Pinus sylvestris*: a test of sexual allocation theory. *American Journal of Botany* **80**: 1016–1020.

Shmida A, Lev-Yadun S, Goubitz S, Ne'Eman G. 2000. Sexual allocation and gender segregation in *Pinus halepensis*, *P. brutia* and *P. pinea*. In: Ne'Eman G, Trabaud L. eds. *Ecology, biogeography and management of Pinus halepensis and P. brutia Forest Ecosystems in the Mediterranean Basin*. Leiden: Backhuys Publisher, 91–104.

Steinitz O, Troupin D, Vendramin G G, Nathan R. 2011. Genetic evidence for a Janzen-Connell recruitment pattern in reproductive offspring of *Pinus halepensis* trees. *Molecular Ecology* **20**: 5152–4164.

Sultan SE. 2000. Phenotypic plasticity for plant development, function and life history. *Trends in Plant Science* **5**: 537–42.

R Development Core Team. 2012. *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. www.r-project.org.

Revelle W. 2011. *psych: Procedures for personality and psychological research*. Northwestern University, Evanston (R package, version 1·1.12).

Tapias R, Climent J, Pardos JA, Gil L. 2004. Life histories of Mediterranean pines. *Plant Ecology* **171**: 53–68.

Thomas SC. 1996. Relative size at onset of maturity in rain forest trees: a comparative analysis of 37 Malaysian species. *Oikos* **76**: 145–154.

Thomas SC. 2011. Age-related changes in tree growth and functional biology: the role of reproduction. In: Meinzer FC, Lachenbruch B, Dawson TE. eds. *Size- and age-related changes in tree structure and function*. Dordrecht: Springer, 33–64.

Varghese M, Kamalakannan R, Harwood CE, Lindgren D, McDonald MW. 2009. Changes in growth performance and fecundity of *Eucalyptus camaldulensis* and *E. tereticornis* during domestication in southern India. *Tree Genetics and Genomes* **5**: 629–640.

Walsh B, Blows MW. 2009. Abundant genetic variation+ strong selection= multivariate genetic constraints: a geometric view of adaptation. *Annual Review of Ecology, Evolution, and Systematics* **40**: 41–59.

Weiner J, Campbell LG, Pino J, Echarte L. 2009. The allometry of reproduction within plant populations. *Journal of Ecology* **97**: 1220–1233.

Wesselingh RA, de Jong TJ. 1995. Bidirectional selection on threshold size for flowering in *Cynoglossum officinale* (hound's-tongue). *Heredity* **74**: 415–424.

Wesselingh RA, Klinkhamer PGL, de Jong TJ, Boorman LA. 1997. Threshold size for flowering in different habitats: effects of size-dependent growth and survival. *Ecology* **78**: 2118–2132.

Williams CG. 2009. *Conifer reproductive biology*. Berlin: Springer.

Worley AC, Barrett SCH. 2000. Evolution of floral display in *Eichhornia paniculata* (Pontederiaceae): direct and correlated responses to selection on flower size and number. *Evolution* **54**: 1533–1545.

Wright SJ, Jaramillo MA, Pavon J, Condit R, Hubbell SP, Foster RB. 2005. Reproductive size thresholds in tropical trees: variation among individuals, species and forests. *Journal of Tropical Ecology* **21**: 307–315.

Yu J, Pressoir G, Briggs WH, *et al*. 2006. A unified mixed-model method for association mapping that accounts for multiple levels of relatedness. *Nature Genetics* **38**: 203–208.

Zhang DY. 2006. Evolutionary stable reproductive investment and sex allocation in plants. In: Harder LD, Barrett SCH. eds. *The ecology and evolution of flowers*. Oxford: Oxford University Press, 41–59.

SUPPLEMENTARY DATA

TABLE S1. List and location of the *Pinus pinaster* populations comprised in the present study. GG= genetic group (as identified by STRUCTURE; see main text); MTCM = mean temperature of the coldest month (ºC); MTWM = mean temperature of the warmest month (ºC); AMT= annual mean temperature (ºC); CI = continentality index (ºC); PWM = precipitation during the warmest quarter (mm); AP = annual mean precipitation (mm).

Population and abbreviation		Latitude	Longitude	GG	Altitude	MTCM	MTWM	AMT	CI	PWM	AP
Mimizan	Mimi	44°80'N	1°18'W	AF	37	3.2	24.8	13.3	21.6	232	1235
Pleucadec	Pleu	47°46'N	2°20'W	AF	80	2.5	21.9	11.2	19.4	154	804
Alto de la Llama	Alto	43°17'N	6°29'W	AIP	503	2.6	23.4	11.7	20.8	149	1137
Armayán	Arma	43°18'N	6°27'W	AIP	498	2.0	24.0	11.8	22.0	152	1112
Cadavedo	Cada	43°32'N	6°25'W	AIP	210	5.0	22.0	13.2	17.0	204	1316
Castropol	Cast	43°30'N	6°58'W	AIP	391	4.5	22.0	12.6	17.5	184	1179
Lamuño	Lamu	43°33'N	6°13'W	AIP	125	5.3	22.7	13.4	17.4	192	1282
Leiria	Leir	39°47'N	8°57'W	AIP	20	7.4	24.4	15.4	17.0	44	811
Puerto de Vega	Puer	43°32'N	6°37'W	AIP	121	4.9	22.6	13.4	17.7	194	1283
San Cipriano de Ribarteme	San	42°70'N	8°21'W	AIP	300	2.7	26.0	12.3	23.3	121	1600
Sierra de Barcia	Sier	43°31'N	6°29'W	AIP	240	4.7	22.4	13.0	17.7	192	1339
Pineta	Pine	41°58'N	9°20'E	CO	750	6.7	26.0	15.5	19.3	42	583
Pinia	Pini	42°10'N	9°27'E	CO	10	6.3	26.9	15.6	20.6	49	580
Oria	Oria	37°31'N	2°21'W	MO	1223	0.4	30.7	13.1	30.3	29	357
Tamrabta	Tamr	33°36'N	5°10'W	MO	1758	-4.6	30.4	10.7	35.0.	49	745
Arenas de San Pedro	Aren	40°11'N	5°60'W	MS	733	1.2	33.4	14.2	32.2	73	1318
Bayubas de Abajo	Bayu	41°31'N	2°52'W	MS	998	-1.4	29.6	10.6	30.9	96	553
Carbonero	Carb	41°10'N	4°16'W	MS	845	-0.7	31.3	12.3	32.0	72	435
Cenicientos	Ceni	40°16'N	4°29'W	MS	1100	1.3	28.8	12.4	27.5	60	794
Coca	Coca	41°15'N	4°29'W	MS	800	-0.6	31.2	12.3	31.8	77	454
Cuellar	Cuel	41°22'N	4°29'W	MS	830	-0.6	30.9	12.2	31.6	72	468
San Leonardo	SanL	41°50'N	3°30'W	MS	1096	-2.7	27.9	9.3	30.6	120	753
Valdemaqueda	Vald	40°30'N	4°18'W	MS	890	0.5	29.2	12.1	28.7	70	681

TABLE S2. Summary information of nuSSR markers used to estimate range-wide population genetic structure in *Pinus pinaster*. Name = locus name; Motif = SSR repeated motif; Label = fluorescent label; Min, Max = minimun and maximun allele sizes per locus; *N* = number of alleles per locus.

Name	Motif	Label	Forward sequence	Reverse sequence	Min	Max	*N*
A6F03	(AC)17	VIC	CCTGAAAATCGACGGATCG	ATGGTATTTTGCGGGTTGC	258	272	8
rptest1	(ATC)7	NED	AGGATGCCTATGATATGCGC	AACCATAACAAAAGCGGTCG	131	171	18
Ctg4363	(AT)10	VIC	TAATAATTCAAGCCACCCCG	AGCAGGCTAATAACAACACGC	108	124	8
NZPR1078	(AC)10	PET	TGGTGATCAAGCCTTTTTCC	GTTGATGAGTGATGGCATGG	239	259	10
epi3	(TC)15	NED	AGCAACATTTCCCTGGACAC	GGAATAATTGCAGTTGCAGTAGC	202	216	7
gPp14	(ATT)9	VIC	TATTGACGGTGTCTCTTCCT	GACTTTGACCTAAAGCATGG	215	237	8
pEST2669	(TA)19	NED	ATTGCTTCTGAAAGGGCATC	TCCCTTGGCACCATGTTAAT	202	229	6
epi5	(TA)9	PET	GGCGCGAACTACTTCATCTG	CAATGCTGACAAACCCAGAA	166	202	16
NZPR544	(CA)5(AC)12(TA)5	FAM	GCGATGTGCAACCCTTGATA	TGCTATTCCGTCAAAAACCC	340	359	8
ctg 275	(AT)16	FAM	ACGGAGATATATTGCTGGCG	AAAGAATAACGTGAAACAAACCC	259	269	6
epi6	(AT)9	FAM	CCCACCATGACAAGGTTGAT	CGCTGGGCTTGAACATCTA	161	185	11
ITPH4516	(CT)27	PET	TGATGCAAACAAGTTCCATG	AGCACTCGCTAAACTATGAAGG	223	238	6

TABLE S3. Size- dependent fitness components for male reproduction in 23 natural *Pinus pinaster* populations. GG = genetic Group; mμ = intercept of logistic regressions; mα = slope of logistic regressions; mTSFR = median threshold size for male reproduction; loCI,upCI = lower and upper 95% limits of Bayesian credible intervals for mTSFR; LVI = largest vegetative individual respect to male reproduction; SRI = smallest individual bearing male cones; mRAN = transition range (mLVI–mSRI); mVEG = number of vegetative individuals. na = not available.

Pop	GG	mμ ± s.e.	mα ± s.e.	mTSFR	loCI	upCI	mLVI	mSRI	mRAN	%mVEG
Mimi	AF	$-10.72 \pm 5.91 \times 10^{-2}$	$4.15 \times 10^{-2} \pm 2 \times 10^{-4}$	258.2	220.1	291.1	378	146	232	4.2
Pleu	AF	$-8.40 \pm 3.43 \times 10^{-2}$	$3.00 \times 10^{-2} \pm 1 \times 10^{-4}$	283.6	264.4	294.8	327	120	207	2.8
Alto	AIP	$-7.19 \pm 4.39 \times 10^{-2}$	$2.77 \times 10^{-2} \pm 2 \times 10^{-4}$	234.2	217.0	258.7	300	134	166	1.5
Arma	AIP	$-9.78 \pm 2.71 \times 10^{-2}$	$4.15 \times 10^{-2} \pm 2 \times 10^{-4}$	248.4	232.6	267.9	313	118	195	7.0
Cada	AIP	$-5.86 \pm 3.22 \times 10^{-2}$	$2.36 \times 10^{-2} \pm 1 \times 10^{-4}$	226.7	209.3	250.4	231	78	153	1.1
Cast	AIP	$-4.81 \pm 2.95 \times 10^{-2}$	$2.22 \times 10^{-2} \pm 1 \times 10^{-4}$	230.0	203.5	252.5	410	94	316	4.1
Lamu	AIP	$-8.88 \pm 5.13 \times 10^{-2}$	$3.56 \times 10^{-2} \pm 2 \times 10^{-4}$	232.1	209.4	248.3	257	113	144	1.5
Leir	AIP	$-8.68 \pm 3.72 \times 10^{-2}$	$3.61 \times 10^{-2} \pm 1 \times 10^{-4}$	260.4	248.0	271.8	376	100	276	0.8
Puer	AIP	$-9.30 \pm 6.27 \times 10^{-2}$	$4.18 \times 10^{-2} \pm 2 \times 10^{-4}$	247.2	217.5	271.4	280	126	154	4.9
San	AIP	$-11.61 \pm 7.56 \times 10^{-2}$	$5.21 \times 10^{-2} \pm 3 \times 10^{-4}$	227.1	209.2	244.0	267	137	130	2.6
Sier	AIP	$-4.96 \pm 4.14 \times 10^{-2}$	$2.16 \times 10^{-2} \pm 2 \times 10^{-4}$	233.4	217.1	248.8	282	119	163	4.3
Pine	CO	na	na	na			467	127	340	na
Pini	CO	$-6.23 \pm 4.67 \times 10^{-2}$	$2.51 \times 10^{-2} \pm 2 \times 10^{-4}$	238.2	215.4	263.9	347	111	236	1.9
Oria	MO	$-8.59 \pm 3.06 \times 10^{-2}$	$3.56 \times 10^{-2} \pm 1 \times 10^{-4}$	231.9	219.8	243.8	338	101	237	4.9
Tamr	MO	$-9.54 \pm 4.84 \times 10^{-2}$	$3.47 \times 10^{-2} \pm 2 \times 10^{-4}$	296.6	273.1	316.7	383	105	278	3.2
Aren	MS	$-7.49 \pm 3.55 \times 10^{-2}$	$3.14 \times 10^{-2} \pm 1 \times 10^{-4}$	240.2	226.0	252.7	392	112	280	6.3
Bayu	MS	$-8.12 \pm 3.04 \times 10^{-2}$	$3.21 \times 10^{-2} \pm 1 \times 10^{-4}$	239.1	229.7	255.3	361	108	253	4.2
Carb	MS	$-8.38 \pm 6.01 \times 10^{-2}$	$3.64 \times 10^{-2} \pm 3 \times 10^{-4}$	228.8	213.0	249.8	290	117	173	8.3
Ceni	MS	$-6.03 \pm 4.93 \times 10^{-2}$	$2.86 \times 10^{-}2 \pm 2 \times 10^{-4}$	208.6	172.1	246.4	353	132	221	1.8
Coca	MS	$-9.13 \pm 4.27 \times 10^{-2}$	$3.35 \times 10^{-2} \pm 2 \times 10^{-4}$	250.8	237.2	269.7	296	118	178	10.3
Cuel	MS	$-7.07 \pm 3.17 \times 10^{-2}$	$3.01 \times 10^{-2} \pm 1 \times 10^{-4}$	246.8	228.6	264.4	345	106	239	3.3
SanL	MS	$-9.77 \pm 4.18 \times 10^{-2}$	$4.00 \times 10^{-2} \pm 2 \times 10^{-4}$	229.7	207.7	255.7	283	80	203	4.4
Vald	MS	$-6.92 \pm 3.93 \times 10^{-2}$	$2.93 \times 10^{-2} \pm 2 \times 10^{-4}$	225.6	200.9	242.3	272	111	161	4.1

TABLE S4. Size-dependent fitness components for female reproduction in 23 natural *Pinus pinaster* populations. GG = genetic Group; fμ = intercept of logistic regressions; fα = slope of logistic regressions; fTSFR = median threshold size for female reproduction; loCI,upCI = lower and upper 95% limits of Bayesian credible intervals for fTSFR; fLVI = largest vegetative individual respect to female reproduction; fSRI = smallest individual bearing female cones; fRAN = transition range (fLVI–fSRI), fVEG = number of vegetative individuals. na not available.

Pop	GG	fμ ± s.e.	fα ± s.e.	fTSFR	loCI	upCI	fLVI	fSRI	fRAN	%fVEG
Mimi	AF	–6.79 ± 2.23×10^{-2}	2.00×10^{-2} ± 1×10^{-4}	316.8	301.2	346.9	404	129	249	2.1
Pleu	AF	–5.75 ± 1.71×10^{-2}	2.15×10^{-2} ± 1×10^{-4}	258.0	236.3	280.9	422	115	212	7.4
Alto	AIP	–5.47 ± 2.22×10^{-2}	1.16×10^{-2} ± 1×10^{-4}	426.6	368.0	522.8	403	126	174	0
Arma	AIP	–5.32 ± 2.32×10^{-2}	1.51×10^{-2} ± 1×10^{-4}	381.7	341.0	451.0	391	92	221	0
Cada	AIP	–6.42 ± 3.05×10^{-2}	1.43×10^{-2} ± 1×10^{-4}	458.4	399.0	531.9	470	138	93	0
Cast	AIP	–5.53 ± 2.59×10^{-2}	1.55×10^{-2} ± 1×10^{-4}	363.6	299.4	418.7	410	84	326	1.4
Lamu	AIP	–4.97 ± 1.76×10^{-2}	1.33×10^{-2} ± 1×10^{-4}	385.9	336.2	426.8	430	100	157	0
Leir	AIP	–5.26 ± 1.96×10^{-2}	1.06×10^{-2} ± 1×10^{-4}	490.4	399.4	576.8	448	100	276	0
Puer	AIP	–6.62 ± 3.02×10^{-2}	1.39×10^{-2} ± 1×10^{-4}	450.6	391.5	524.0	429	149	131	0
San	AIP	–4.31 ± 2.27×10^{-2}	1.66×10^{-2} ± 1×10^{-4}	262.7	228.1	303.4	353	78	189	2.6
Sier	AIP	–5.16 ± 3.42×10^{-2}	0.96×10^{-2} ± 1×10^{-4}	480.2	381.3	578.9	364	118	164	0
Pine	CO	na	na	na			467	263	204	na
Pini	CO	–8.44 ± 6.33×10^{-2}	2.77×10^{-2} ± 2×10^{-4}	368.8	294.0	448.9	458	147	200	0
Oria	MO	–5.08 ± 1.48×10^{-2}	1.84×10^{-2} ± 1×10^{-4}	284.7	256.3	310.2	366	85	253	2.1
Tamr	MO	–6.40 ± 1.73×10^{-2}	2.22×10^{-2} ± 1×10^{-4}	276.8	259.0	300.3	383	78	305	6.4
Aren	MS	–5.77 ± 1.99×10^{-2}	1.85×10^{-2} ± 1×10^{-4}	325.9	293.1	371.9	400	112	280	1.6
Bayu	MS	–4.46 ± 1.25×10^{-2}	1.80×10^{-2} ± 1×10^{-4}	246.9	233.0	269.6	366	94	267	3.5
Carb	MS	–5.26 ± 2.49×10^{-2}	2.20×10^{-2} ± 1×10^{-4}	233.2	211.2	271.3	314	99	191	6.3
Ceni	MS	–5.43 ± 2.53×10^{-2}	1.95×10^{-2} ± 1×10^{-4}	287.2	242.7	333.5	364	111	242	1.8
Coca	MS	–5.41 ± 3.47×10^{-2}	1.65×10^{-2} ± 1×10^{-4}	306.4	241.0	501.0	367	82	214	0
Cuel	MS	–6.37 ± 2.08×10^{-2}	2.49×10^{-2} ± 1×10^{-4}	229.5	203.3	281.8	383	72	273	3.3
SanL	MS	–5.71 ± 2.07×10^{-2}	2.00×10^{-2} ± 1×10^{-4}	285.2	236.7	335.7	349	103	180	0
Vald	MS	–6.54 ± 3.04×10^{-2}	1.84×10^{-2} ± 1×10^{-4}	339.7	260.6	423.3	377	99	173	0

TABLE S5. Associations of environmental variables (first column) with reproductive life-history traits (second column) in *Pinus piaster*. Corrected values indicate slopes, standard errors and *P*-values for the association after including neutral genetic structure corrections (i.e. likelihood ratio test between a full model and a reduced model with just neutral genetic structure); uncorrected values indicate association parameters without correcting for neutral genetic structure (i.e. just environmental data and traits data in the model).

		Corrected		Uncorrected	
	Trait	Slope	*P*-value	Slope	*P*-value
Alt	fTSFR	$-2.02\times10^{-1} \pm 7.02\times10^{-2}$	0.011	$-9.87\times10^{-2} \pm 3.13\times10^{-2}$	0.005
	mTSFR	$-3.37\times10^{-2} \pm 1.86\times10^{-2}$	0.090	$1.39\times10^{-3} \pm 9.22\times10^{-3}$	0.881
	H	$-4.06\times10^{-2} \pm 3.06\times10^{-2}$	0.205	$-4.68\times10^{-2} \pm 9.77\times10^{-3}$	0.000
	%M	$-2.21\times10^{-2} \pm 1.54\times10^{-2}$	0.171	$-1.90\times10^{-2} \pm 6.65\times10^{-3}$	0.010
	% F	$1.57\times10^{-2} \pm 1.24\times10^{-2}$	0.223	$1.33\times10^{-2} \pm 4.75\times10^{-3}$	0.011
Lat	fTSFR	$-8.22\times10^{-5} \pm 1.97\times10^{-4}$	0.682	$-7.02\times10^{-5} \pm 5.81\times10^{-5}$	0.241
	mTSFR	$-5.23\times10^{-5} \pm 4.45\times10^{-5}$	0.258	$2.09\times10^{-6} \pm 1.45\times10^{-5}$	0.887
	H	$1.84\times10^{-5} \pm 7.31\times10^{-5}$	0.805	$-1.14\times10^{-6} \pm 2.25\times10^{-5}$	0.960
	%M	$2.01\times10^{-5} \pm 3.67\times10^{-5}$	0.593	$-6.81\times10^{-7} \pm 1.24\times10^{-5}$	0.957
	% F	$-1.95\times10^{-5} \pm 2.90\times10^{-5}$	0.512	$1.96\times10^{-7} \pm 8.80\times10^{-6}$	0.982
Long	fTSFR	$2.08\times10^{-5} \pm 1.41\times10^{-4}$	0.885	$6.58\times10^{-5} \pm 5.81\times10^{-5}$	0.271
	mTSFR	$5.50\times10^{-5} \pm 3.01\times10^{-5}$	0.087	$-8.97\times10^{-5} \pm 1.43\times10^{-5}$	0.537
	H	$1.84\times10^{-5} \pm 5.22\times10^{-5}$	0.729	$6.02\times10^{-5} \pm 1.79\times10^{-5}$	0.003
	%M	$-1.24\times10^{-5} \pm 2.63\times10^{-5}$	0.645	$1.61\times10^{-5} \pm 1.18\times10^{-5}$	0.189
	% F	$1.16\times10^{-5} \pm 2.09\times10^{-5}$	0.585	$-1.08\times10^{-5} \pm 8.42\times10^{-6}$	0.214
AMT	fTSFR	30.58 ± 16.22	0.079	29.46 ± 10.76	0.013
	mTSFR	–4.59 ± 4.07	0.277	–1.63 ± 3.01	0.595
	H	9.24 ± 6.24	0.159	10.26 ± 4.12	0.022
	%M	6.86 ± 2.88	0.031	6.46 ± 2.16	0.007
	% F	–4.87 ± 2.37	0.058	–4.31 ± 1.57	0.012
MTCM	fTSFR	29.43 ± 7.48	0.001	20.01 ± 3.99	0.000
	mTSFR	–0.91 ± 2.49	0.719	–1.20 ± 1.42	0.407
	H	8.36 ± 3.31	0.023	7.67 ± 1.44	0.000
	%M	4.83 ± 1.56	0.007	3.99 ± 0.85	0.000
	% F	–3.35 ± 1.33	0.024	–2.72 ± 0.63	0.000
MTWM	fTSFR	–18.34 ± 6.06	0.009	–14.18 ± 3.66	0.001
	mTSFR	–2.19 ± 1.72	0.221	–0.19 ± 1.16	0.872
	H	–5.14 ± 2.52	0.059	–5.61 ± 1.30	0.000
	%M	–2.12 ± 1.33	0.132	–2.32 ± 0.85	0.013
	% F	1.52 ± 1.08	0.177	1.78 ± 0.59	0.006
CI	fTSFR	–14.08 ± 3.39	0.001	–9.71 ± 1.91	0.000
	mTSFR	–0.72 ± 1.15	0.542	0.21 ± 0.69	0.762
	H	–3.97 ± 1.53	0.020	–3.78 ± 0.67	0.000
	%M	–1.94 ± 0.79	0.026	–1.75 ± 0.45	0.001
	% F	1.37 ± 0.65	0.052	1.27 ± 0.31	0.001
AP	fTSFR	$4.22\times10^{-2} \pm 5.85\times10^{-2}$	0.482	$1.13\times10^{-1} \pm 4.28\times10^{-2}$	0.015
	mTSFR	$2.47\times10^{-3} \pm 1.40\times10^{-2}$	0.862	$-6.57\times10^{-3} \pm 1.19\times10^{-2}$	0.586
	H	$3.09\times10^{-2} \pm 2.06\times10^{-2}$	0.153	$4.69\times10^{-2} \pm 1.53\times10^{-2}$	0.006
	%M	$7.67\times10^{-3} \pm 1.10\times10^{-2}$	0.495	$2.00\times10^{-2} \pm 9.22\times10^{-3}$	0.043
	% F	$-1.23\times10^{-2} \pm 8.28\times10^{-3}$	0.157	$-1.77\times10^{-2} \pm 6.09\times10^{-3}$	0.009
PDM	fTSFR	$1.14 \pm 5.19\times10^{-1}$	0.044	$5.54\times10^{-1} \pm 2.66\times10^{-1}$	0.050
	mTSFR	$1.47\times10^{-1} \pm 1.35\times10^{-1}$	0.294	$-1.11\times10^{-2} \pm 7.05\times10^{-2}$	0.877
	H	$2.39\times10^{-1} \pm 2.12\times10^{-1}$	0.277	$2.61\times10^{-1} \pm 9.27\times10^{-2}$	0.011
	%M	$1.81\times10^{-1} \pm 1.02\times10^{-1}$	0.096	$9.66\times10^{-2} \pm 5.64\times10^{-2}$	0.102
	% F	$-1.85\times10^{-1} \pm 7.49\times10^{-2}$	0.026	$-8.79\times10^{-2} \pm 3.81\times10^{-2}$	0.032

TABLE S6. Number of trees per population included in the molecular marker analysis and average of individual assignment probability for each of the optimal K=6 clusters (C1 through C6) representing five geographical genetic groups (GG) in *Pinus pinaster*.

Population	*N*	C1	C2	C3	C4	C5	C6	GG
Mimi	19	0.74	0.01	0.02	0.01	0.06	0.15	AF
Pleu	21	0.67	0.02	0.03	0.01	0.12	0.16	AF
Alto	9	0.14	0.01	0.59	0.02	0.09	0.14	AIP
Arma	9	0.08	0.01	0.70	0.02	0.14	0.05	AIP
Cada	10	0.09	0.00	0.82	0.01	0.04	0.04	AIP
Cast	10	0.12	0.01	0.80	0.01	0.04	0.03	AIP
Lamu	9	0.10	0.01	0.73	0.01	0.10	0.06	AIP
Leir	24	0.19	0.01	0.24	0.01	0.42	0.13	AIP
Puer	8	0.05	0.01	0.76	0.04	0.10	0.05	AIP
San	12	0.15	0.01	0.51	0.02	0.25	0.06	AIP
Segu	21	0.12	0.01	0.50	0.01	0.23	0.13	AIP
Sier	10	0.21	0.01	0.41	0.02	0.13	0.21	AIP
Pine	10	0.05	0.03	0.03	0.80	0.05	0.04	CO
Pini	14	0.01	0.02	0.02	0.92	0.01	0.01	CO
Oria	29	0.10	0.54	0.07	0.03	0.16	0.11	MO
Tamr	24	0.01	0.95	0.01	0.01	0.01	0.01	MO
Aren	27	0.14	0.02	0.09	0.03	0.34	0.38	MS
Bayu	27	0.22	0.01	0.07	0.03	0.14	0.53	MS
Carb	6	0.28	0.04	0.11	0.06	0.20	0.31	MS
Ceni	9	0.30	0.01	0.13	0.02	0.19	0.35	MS
Coca	19	0.26	0.08	0.13	0.06	0.26	0.22	MS
Cuel	28	0.34	0.02	0.07	0.01	0.20	0.36	MS
Rodo	8	0.16	0.02	0.03	0.02	0.42	0.35	MS
SanL	20	0.14	0.06	0.07	0.06	0.11	0.56	MS
Vald	16	0.11	0.01	0.05	0.03	0.36	0.44	MS
Total	399							

APPENDIX III

Plasticity in reproduction and growth among 52 range-wide populations of a Mediterranean conifer: adaptive responses to environmental stress. **Santos-del-Blanco L**, Bonser SP, Valladares F, Chambel MR, Climent J. 2013. Journal of Evolutionary Biology. 26: 1912-1924.

JOURNAL OF Evolutionary Biology eseb European Society for Evolutionary Biology

doi: 10.1111/jeb.12187

Plasticity in reproduction and growth among 52 range-wide populations of a Mediterranean conifer: adaptive responses to environmental stress

L. SANTOS-DEL-BLANCO*†, S. P. BONSER‡, F. VALLADARES§, M. R. CHAMBEL* & J. CLIMENT*†

*Department of Forest Ecology and Genetics, INIA-CIFOR, Madrid, Spain
†Sustainable Forest Management Research Institute, INIA-University of Valladolid, Palencia, Spain
‡Evolution & Ecology Research Centre, School of Biological, Earth and Environmental Sciences, The University of New South Wales, Sydney, NSW, Australia
§Laboratorio Internacional de Cambio Global LINC-Global, Museo Nacional de Ciencias Naturales, MNCN-CSIC, Madrid, Spain

Keywords:

ecotypic trends;
genotype-by-environment interaction;
reproductive allometry;
threshold size for reproduction;
trade-offs.

Abstract

A plastic response towards enhanced reproduction is expected in stressful environments, but it is assumed to trade off against vegetative growth and efficiency in the use of available resources deployed in reproduction [reproductive efficiency (RE)]. Evidence supporting this expectation is scarce for plants, particularly for long-lived species. Forest trees such as Mediterranean pines provide ideal models to study the adaptive value of allocation to reproduction vs. vegetative growth given their among-population differentiation for adaptive traits and their remarkable capacity to cope with dry and low-fertility environments. We studied 52 range-wide *Pinus halepensis* populations planted into two environmentally contrasting sites during their initial reproductive stage. We investigated the effect of site, population and their interaction on vegetative growth, threshold size for female reproduction, reproductive–vegetative size relationships and RE. We quantified correlations among traits and environmental variables to identify allocation trade-offs and ecotypic trends. Genetic variation for plasticity was high for vegetative growth, whereas it was nonsignificant for reproduction. Size-corrected reproduction was enhanced in the more stressful site supporting the expectation for adverse conditions to elicit plastic responses in reproductive allometry. However, RE was unrelated with early reproductive investment. Our results followed theoretical predictions and support that phenotypic plasticity for reproduction is adaptive under stressful environments. Considering expectations of increased drought in the Mediterranean, we hypothesize that phenotypic plasticity together with natural selection on reproductive traits will play a relevant role in the future adaptation of forest tree species.

Introduction

The timing of the onset of reproduction and the number of offspring produced by an individual are two fundamental life-history traits closely linked to fitness in an environment (Stearns, 1992; Braendle *et al.*, 2011). According to life-history theory, individuals that start reproducing earlier in life tend to be favoured under harsh environments, due to reduced life expectancy (Roff, 1992). The initiation of reproduction in plants is often related to size rather than age (De Jong & Klinkhamer, 2005). For example, individuals should build a large vegetative body and invest all available resources in reproduction just before death, that is, a bang-bang strategy (King & Roughgarden, 1982). But uncertainty about the moment of death, for example, due to disturbances will tend to favour reproduction at smaller sizes (and younger ages), a graded reproductive investment and bet-hedging strategies (Childs *et al.*, 2010). Thus, it is

Correspondence: Luis Santos-del-Blanco, Department of Ecology and Evolution, University of Lausanne, Biophore Building, CH1015 Lausanne, Switzerland. Tel.: +41 21 622 42 47; fax: +41 21 692 41 65; e-mail: luis.santosdelblanco@unil.ch

expected that plants, particularly long-lived perennials, will delay reproduction in favourable environments until they reach an optimal size for reproduction both by means of genetic change and phenotypic plasticity provided that selective forces act at local and broad scales (Kozłowski, 1992; Roff, 1992).

Experiments on herbaceous plants demonstrate that varying environmental factors – namely resource availability and competition – induce plasticity in reproductive strategies (Sultan, 2000; Weiner *et al.*, 2009b; Anderson *et al.*, 2011; Nicholls, 2011). In addition, plant populations are often genetically differentiated along environmental clines for size at reproduction and reproductive allometry, that is, the relationship between reproductive output and vegetative size (Lacey, 1988; Alexander *et al.*, 2009; Guo *et al.*, 2012). However, phenotypic plasticity of reproduction is driven to an important extent by size effects, as a strong positive relationship between vegetative and reproductive size is typically found and vegetative traits commonly respond plastically to environmental conditions. In comparison, phenotypic plasticity of the relationship between vegetative and reproductive size has been claimed to have a minor contribution to reproductive output, but this is still debated (Weiner *et al.*, 2009a,b).

Long generation time in long-lived perennials implies that the same genotypes cope with year-to-year changing environmental conditions. On the other hand, populations of annuals or short-lived perennials can undergo genetic changes in shorter periods (Franks & Weis, 2008). Therefore, plasticity might be of greater importance as an adaptive strategy in trees and woody plants compared with short-lived plant species (Willson, 1983) such that long-lived species might exhibit plasticity in both vegetative (Chambel *et al.*, 2005) and reproductive traits like size at reproduction and reproductive investment. The few studies published on long-lived species highlight strong selection on the threshold size at first reproduction and the allometry of reproduction, leading to genetic differentiation at large spatial scales (Thomas, 1996; Matziris, 1997; Niklas & Enquist, 2003; Climent *et al.*, 2008; Santos-del-Blanco *et al.*, 2010) and promoting phenotypic plasticity in life histories at local scales (Fang *et al.*, 2006). Despite consistent predictions of plasticity in the threshold size of reproduction, little is known about the costs of plasticity in terms of final reproductive output relative to vegetative size (Roff, 2000). Reproductive efficiency (RE) can be defined as the slope of the reproductive–vegetative size developmental trajectory that connects threshold size for reproduction with reproduction at a given developmental stage or at the onset of senescence (Bonser & Aarssen, 2009). It is expected that early reproduction will imply lower RE, modifying reproductive allometries and, in turn, reproductive variability within and among populations. However, this has rarely been tested even in short-lived semelparous species (but see Bonser *et al.*, 2010).

The Mediterranean pine *Pinus halepensis* Mill. (Aleppo pine) is a suitable model species for testing hypotheses on the evolution of reproductive strategies in long-lived perennials. It is precocious, bearing female cones from as early as 3 to 6 years of age, and commits heavily and regularly to reproduction, most notably female reproduction (Ne'eman *et al.*, 2004). *Pinus halepensis* is widespread over a large circum-Mediterranean distribution area, and low population differentiation in neutral markers has been reported in the Iberian Peninsula due to recent range expansion (Soto *et al.*, 2010). *Pinus halepensis* shows a wide ecological breadth among populations and is putatively adapted to a large range of abiotic stressors and perturbations, particularly fire and drought (Ne'eman *et al.*, 2004), although intense drought episodes might be detrimental to reproduction (Girard *et al.*, 2011). However, information regarding among-population variation in phenotypic traits in this species remains scarce.

Previous works described significant ecotypic differentiation for size at maturity in *P. halepensis* (Climent *et al.*, 2008; Santos-del-Blanco *et al.*, 2010). In this study, we focus on phenotypic plasticity and among-populations genetic variation in plasticity for reproductive allometry in range-wide *P. halepensis* populations assessed in a common garden experiment replicated in two contrasted sites (low and high environmental stress). Our objectives are to (i) assess the existence of phenotypic plasticity for size at maturity and the reproductive–vegetative size (R–V) relationship in range-wide populations subject to contrasting field conditions; and (ii) to compare genotype × environment patterns for vegetative and reproductive traits and correlations between both sets of traits representing trade-offs that might describe adaptive strategies. First, we expect that similar environmental cues defining favourable or unfavourable growth conditions will act in the same direction considering genetic differentiation and plasticity (Anderson *et al.*, 2012; Chevin *et al.*, 2012). Based on life-history theory, this would imply that the more stressful the environment (both at the origin of populations and at the trial site), the greater amount of resources would be devoted to reproduction. Specifically, we expect that environmental stress will induce reproduction at smaller sizes, associated with higher slopes of the R–V relationship. Finally, we also expect reproductive strategies to be governed by trade-offs between precocity and lifetime fitness, so that individuals that reproduce late benefit from a higher lifetime reproductive investment relative to their size.

Materials and methods

Study species and common gardens

A multisite *P. halepensis* common garden experiment was set up in 1997 replicated at six different sites in eastern and central Spain. The trial includes 52 native populations from continental Spain, Balearic Islands (Spain), France, Italy, Greece and Tunisia, as well as four non-native populations (see Climent *et al.*, 2008 for details) (Fig. 1, Table S1), thus covering most of the species' range. Only data relative to native populations were used in the present study. The minimum requirements for assessing plasticity in our experiment were, first, data measured at same age and identical protocols between sites and, second, contrasted enough environments. Only two of the six sites fulfilled both requirements.

Summary data of environmental conditions at both trial sites obtained from a functional model (Gonzalo-Jiménez, 2010) are shown in Table 1. Valdeolmos trial site (hereafter 'low-stress site') has sandy loam deep soil, whereas Rincón de Ademuz trial site (hereafter 'high-stress site') has shallow and rocky soil. In addition, mean annual rainfall is ca. 25% higher in the low-stress site, and winters are slightly warmer compared with the high-stress site. As a result of combined effects of poorer soil, lower rainfall and slightly colder winters, the high-stress site is much more limiting for *P. halepensis* vegetative growth compared with the low-stress site. This constant environmental difference between sites should not be confounded with within-site year-to-year meteorological variation that has been previously described in this species (Girard *et al.*, 2011).

Population seedlots were obtained by bulking open-pollinated seeds from a subsample of 20 to 30 trees spaced at least 100 m apart in each population. At both sites, 832 one-year-old seedlings from native populations were planted in 1997 in a row–column design on the intersections of a 2.5 × 2.5 m grid, with four replicates and four contiguous plants per population and replicate (16 trees per population). One replicate in the low-stress site was lost due to rabbit herbivory and was not included in this study (624 trees remaining). Due to other causes of mortality, final sample size for this study was 589 in the low-stress site and 633 in the high-stress site.

Measurement of traits and environmental variables

We measured height for each tree at ages 7, 11 and 13 years for both sites (2003, 2007 and 2009, respectively). Diameter at breast height was measured at both sites at ages 11 and 13 years and used to infer biomass from allometric models (Montero *et al.*, 2005) (Table 2).

The onset of female and male reproductive functions in *P. halepensis* is decoupled, with trees generally starting reproduction as females (protogyny) and male reproduction being delayed for up to several years (Shmida *et al.*, 2000). Thus, we focused on the study of *P. halepensis* early investment in female function to estimate threshold sizes for first reproduction and reproductive investment.

Female cones in *P. halepensis* remain attached to the branches even after dehiscence (normally also delayed several years, Ne'eman *et al.*, 2004). Differences in size and colour allow discrimination of several cohorts within tree crowns (Ne'eman *et al.*, 2011) and therefore enable retrospective record of female reproduction. Up to three successive cohorts of female cones were counted at ages 7 and 13 years (2003 and 2009) therefore dating back to the very first reproductive events up to the generalization of reproduction at both sites.

We defined the cumulative reproductive investment (CRI) as the sum of all counted female cones produced

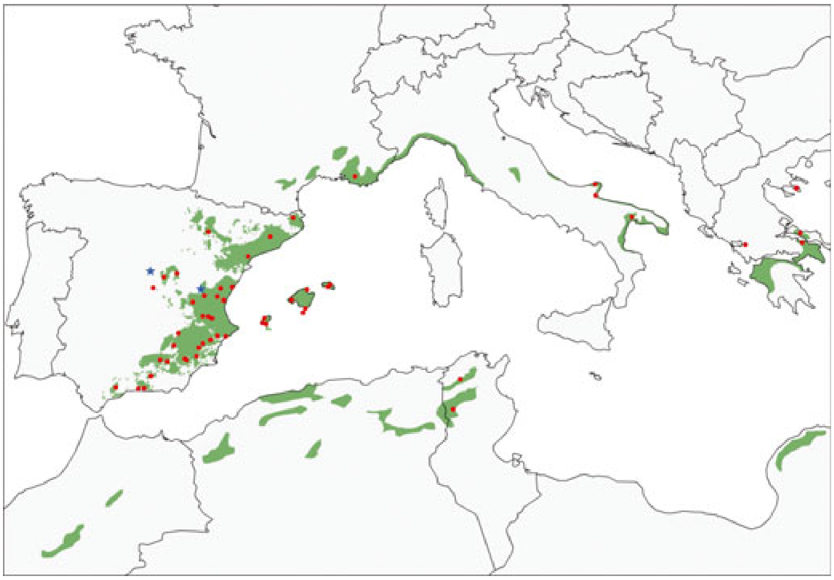


Fig. 1 Distribution map of *Pinus halepensis* source populations (circles) and common garden (stars). Green areas indicate the species' natural distribution range.

Table 1 Climatic descriptors for two *Pinus halepensis* common garden study sites, derived from functional climatic models (Gonzalo-Jiménez, 2010).

Abr.	Site	Valdeolmos Low-stress site	Rincón de Ademuz High-stress site
Long	Longitude	3°26′44″W	1°14′14″W
Lat	Latitude	40°38′42″N	40°06′38″N
Alt	Altitude (m)	731	844
SP	Spring precipitation (mm)	129	99
PDQ	Precipitation of the warmest quarter (mm)	62	94
PDM	Precipitation of the driest month (mm)	13	23
P	Annual precipitation (mm)	475	364
AMT	Annual mean temperature (°C)	12.9	12.3
MTWM	Mean temperature of the warmest month (°C)	29.9	27.6
MTCM	Mean temperature of the coldest month (°C)	0.7	0.2

by an individual until last measurement at age 13 (Table 2).

We collected data for six meteorological and three spatial variables describing the environmental conditions found in the natural populations (Table 1, Table S1). Meteorological data for Iberian populations were obtained from a functional model (Gonzalo-Jiménez, 2010), and data for other populations (i.e. Balearic Islands, France, Tunisia, Italy and Greece) were obtained from WorldClim-Global Climate Data at 5′ resolution (Hijmans *et al.*, 2005). To test hypotheses of local adaptation, we also calculated the Gower's ecological distance for each population at both trial sites (Rutter & Fenster, 2007). This adimensional index informs about the environmental distance between the native environment of each population and the environment where they were grown in the common garden. The analysis was limited to Iberian and Balearic populations due to the unbalanced number of eastern Mediterranean populations in the experiment.

Data analysis

All reported models and tests were implemented in *R* (R Development Core Team., 2012) using packages lme4 (Bates *et al.*, 2011) and MCMCglmm (Hadfield, 2010).

Survival and vegetative size

Mixed linear models for size (height and biomass) at age 13 and generalized linear mixed models (logit link, binomial error) for survival and proportion of reproductive individuals at age 13 were fitted. In all models, site effect was treated as fixed. Population, site-by-population interaction and replicate within site were treated as random. A common interpretation of model parameters is as follows: significant differences among populations indicate intraspecific genetic variability; significant differences between sites reflect phenotypic plasticity, and significant site × population interaction indicates genetic variation for plasticity among populations (Schlichting, 1986). However, deviations from that framework need also to be considered. For example, environmental factors can significantly affect seeds

Table 2 Common garden measured and derived vegetative and reproductive traits of *Pinus halepensis* trees. Measurement age in parenthesis.

Variable		Description
Measured traits		
H	Height (cm)	Total height of each tree (7, 11, 13)
DBH	Diameter at breast height (cm)	Diameter of the tree trunk at 1.30 m (11,13)
CC	Cone number (*n*)	Number of seed cones in each tree per cohort (7, 13)*
Surv	Survival	Status of each tree: dead (0) or alive (1) (7, 11, 13)
Derived traits		
Vegetative		
Biomass	Biomass (kg)	Biomass inferred from DBH (Montero *et al.*, 2005)
Reproductive		
TSFR	Median threshold size for first reproduction (cm)	Size at which the probability for a tree within a population to have reached sexual maturity was 50%
CRI	Cumulative reproductive investment (*n*)	Sum of all seed cones produced by a tree
RA	Reproductive allocation (*n* per kg)	Number of seed cones divided into total tree biomass
R–V intercept		Intercept of R–V GLMM Poisson model
R–V slope		Slope of R–V GLMM Poisson model
RE	Reproductive efficiency	CRI/(height at last measurement − TSFR)

*7 corresponds to ages 5 and below, 6 and 7 years; 13 corresponds to 11, 12 and 13 years.

during development, causing epigenetic changes in gene expression (Johnsen *et al.*, 2005). Also, a significant site × population interaction can indicate local adaptation if populations have a better performance in the site most similar to the conditions of their site of origin (Vergeer & Kunin, 2013). To test the significance of site, population and site × population terms, we performed likelihood ratio tests (LRTs) comparing full models containing all terms with those lacking the relevant term to be tested. Variance components and adjusted means for size at final measurement of each population were derived from analogous models fitted for each trial site.

Size at first reproduction

A generalized linear mixed model (logit link, binomial error) was fitted for cumulative female reproduction (either present or absent) data at ages 7 and 13. We included height as covariate and height × site interaction as fixed term. Then, independent models were fitted for each population and site by Markov Chain Monte Carlo (MCMC) methods (see Santos-del-Blanco *et al.*, 2012 for further details), and median threshold size for first reproduction (TSFR) was defined as the size at which the probability for a tree to have reached sexual maturity was 50% (Wesselingh *et al.*, 1997) and computed by dividing slope by intercept estimates. We also calculated the size of the smallest reproductive individual (SRI) at each population and used this information to classify nonreproducing trees into juvenile (smaller than SRI) or vegetative (larger than SRI) (Mendez & Karlsson, 2004).

Fecundity and reproductive–vegetative size relationships

Generalized linear mixed models (log link, Poisson error) were fitted to the CRI. Juveniles were removed from the data set prior to analysis. The models also included an individual-level random effect to model additive overdispersion (Elston *et al.*, 2001).

Reproduction in plants is typically size dependent (Niklas & Enquist, 2003; Weiner, 2004). We accounted for size-dependent differences in reproductive allocation by calculating reproductive allocation per population first as the mean value across individuals (CRI/biomass) and second as the expected reproductive value based on fitted reproductive–vegetative size (R–V) models and then divided into average size. Thus, each approach represents the mean reproductive allocation per population and reproductive allocation of an average-sized individual in a population, respectively. Similar values for both indexes would indicate that the estimation of reproductive allocation is robust, although issues remain about spurious correlations with size.

Reproductive–vegetative size models describe the relationship between reproductive and vegetative allocation using two parameters – an intercept and a slope (Weiner *et al.*, 2009a; Guo *et al.*, 2012). However, depending on the range of sizes used to fit the models, those two parameters might not be independent in a set of populations due to collinearity (Pinheiro & Bates, 2000). Thus, to summarize reproductive output and compare R–V relationships while accounting for tree vegetative size, we fitted generalized linear mixed models (log link, Poisson error) with independent intercept and slopes to CRI data according to:

$$\eta_i = \ln(\mu_i) = \mathrm{x}'\beta + \mathrm{z}'\mathrm{b} + \mathrm{e}; \mathrm{b} \sim N(0, \sigma^2); \mathrm{e} \sim \mathrm{Pois}(\lambda)$$

where η_i is the linear predictor linked to the expected value of natural logarithm of CRI [ln (μ_i)]. x′ represents the design matrix containing the values for the fixed size effects. β is a vector containing the fixed intercept and slope associated with size, to be estimated. z′ is the design matrix for the random populations effects. b is the vector of random coefficients that follow a normal distribution. e is the vector containing the errors that follow a Poisson distribution. Two models were fitted per site, the first one with b containing random effects for intercepts and the second containing random effects for the slopes. AIC values from both models at each site were very close, indicating that either random intercept or random slope models had similar explanatory power. Population-adjusted intercepts and slopes were derived from MCMC models fitted at each site and used as fecundity indicators; this allowed us to compare general estimates from both sites. Random intercepts associated with population reflect constant deviations across sizes from the general model, that is, a constant higher or lower commitment to reproduction across sizes. Random slopes associated with population represent deviations on reproductive output proportional to vegetative size, that is, enhanced or decreased commitment to reproduction along vegetative size. Our analysis of size at first reproduction and reproductive output divided in two steps (binomial and Poisson submodels) was thus similar to a hurdle model (Brophy *et al.*, 2007; Haymes & Fox, 2012).

Reproductive efficiency

We estimated RE as the slope of the size–reproduction developmental trajectory, linking vegetative size at first reproduction and vegetative and reproductive output at final development (age 13) (Bonser & Aarssen, 2009). RE was estimated at the population level for both sites. We tested whether there were significant correlations between the threshold size for reproduction and RE at each site and whether RE was affected by the environment, comparing RE values between both sites with a paired *t*-test.

Local adaptation patterns

Pearson's correlation tests at each trial site were used to test the relationship between Gower's distance and fitness. We used CRI and female TSFR, as the variables most closely related to fitness but also explored the correlation between Gower's distance and vegetative growth traits (Leimu & Fischer, 2008). We also tested whether increased environmental distances were correlated with changes in trait means.

Plant trait correlations and ecotypic trends

We calculated Pearson's correlations among plant traits at the population level and among those traits and environmental conditions found in the natural populations. Correlations among plant traits can be interpreted as genetic correlations modified by common environmental effects. Correlations were conducted at each trial site separately to check whether trait–trait correlations and ecotypic trends of variation were site dependent. We also obtained the site-to-site correlations for phenotypic traits, as a double-check of site-by-population interaction (Pigliucci, 2001).

Results

Vegetative traits

We found that plants in the high-stress site had lower biomass and height compared with those in the low-stress site, thus confirming that overall environmental differences between both sites had an effect on vegetative growth (Table 3, Fig. 2). In addition, tree survival was significantly lower in the high-stress site compared with the low-stress site ($\chi^2_1 = 76.2$, $P < 0.001$) (Table 3). All populations attained larger sizes in the low-stress than in the high-stress site. However, there was no evidence for population effect alone, but differences between populations were site specific, and a significant site-by-population interaction was found for all vegetative traits (Table 4, Figs 2 and 3), consistent with genetic variation in plasticity for vegetative traits among populations and, possibly, local adaptation. Among-population variance was larger for biomass in the low-stress site [45.0 (31.6–69.2)] compared with the high-stress site [4.7 (3.3–7.2)], but no significant differences were found for height [829 (582–1275) low-stress site; 797 (560–1225) high-stress site]. Population means for vegetative traits at the low- and high-stress sites can be accessed in Tables S2 and S3, respectively.

Table 3 Mean values (± standard errors or credible intervals in brackets) for *Pinus halepensis* vegetative and reproductive traits at two experimental sites with contrasting environmental conditions. Values at each site are averaged across 52 natural populations; abbreviations and units are as defined in Table 2.

	Low-stress site	High-stress site
H	339.6 ± 3.4	274.9 ± 3.0
Biomass	12.16 ± 0.67	4.10 ± 0.24
Survival	0.65 ± 0.03	0.61 ± 0.01
CRI	17.1 ± 1.1	16.3 ± 0.8
RA	5.61 ± 1.11	20.09 ± 3.99
TSFR	204.5 ± 6.9	147.3 ± 6.1
Intercept	1.60 (1.36–1.77)	2.05 (1.87–2.20)
Slope	0.033 (0.030–0.042)	0.070 (0.056–0.090)

CRI, cumulative reproductive investment; TSFR, threshold size for first reproduction; RA, reproductive allocation.

Reproduction and threshold sizes

Mean size of reproductive individuals was greater than that of nonreproductive ones at both sites and both years (low-stress site: biomass $\chi^2_1 = 21.0$, height $\chi^2_1 = 34.7$; high-stress site: biomass $\chi^2_1 = 85.8$, height $\chi^2_1 = 242.1$, all tests $P < 0.001$). At the early measurement date (age 7 years), the proportion of reproductive individuals was slightly greater in the low-stress site than in the high-stress site ($\chi^2_1 = 58.6$, $P < 0.001$). However, at 13 years of age, 96% of trees were reproductive in the stressed environment, whereas only 84% were in the more favourable environment ($\chi^2_1 = 34.7$, $P < 0.001$). As a result, at final measurement, the number of vegetative individuals was higher in the low-stress than in high-stress site (34 vs. 5).

We found a significant effect of both site and population on the threshold size for reproduction, as shown by the significant site and population terms (Table 4). Thus, threshold size for reproduction is both highly plastic and variable among populations (Figs 2 and 3). By contrast, the site-by-population interaction term was not significant, indicating that there was no significant genetic variation for plasticity in the threshold size for reproduction among populations. The probability of reproducing at a given size was significantly smaller in the low-stress site than in high-stress site, evidenced by a reduced slope of the model (data not shown).

We were able to fit independent threshold models for all but three populations in the low-stress site and all populations but one in the high-stress site. For all but two populations, the point estimate of the threshold size for reproduction was higher in the low-stress site than in the high-stress one (Figs 2 and 3).

For CRI, site and population effects were significant, but not, although marginally, population-by-site interaction (Table 4). When tree biomass was included as a covariate in the R–V model, site (indicating a different R–V relationship in both sites), and population terms were significant, but not site × population interaction (Table 4). When height was used as a covariate, similar results were obtained although site was not significant (Table 4). The subsequent GLMM models fitted by MCMC aimed at estimating fecundity at the population level while controlling for size effects revealed an enhanced reproductive allocation in the high-stress site with respect to the low-stress one, defined by a larger intercept and slope (Table 3). Here, a positive intercept must not be regarded as biologically implausible, as it represents a population, not an individual developmental trajectory. Mean reproductive allocation per

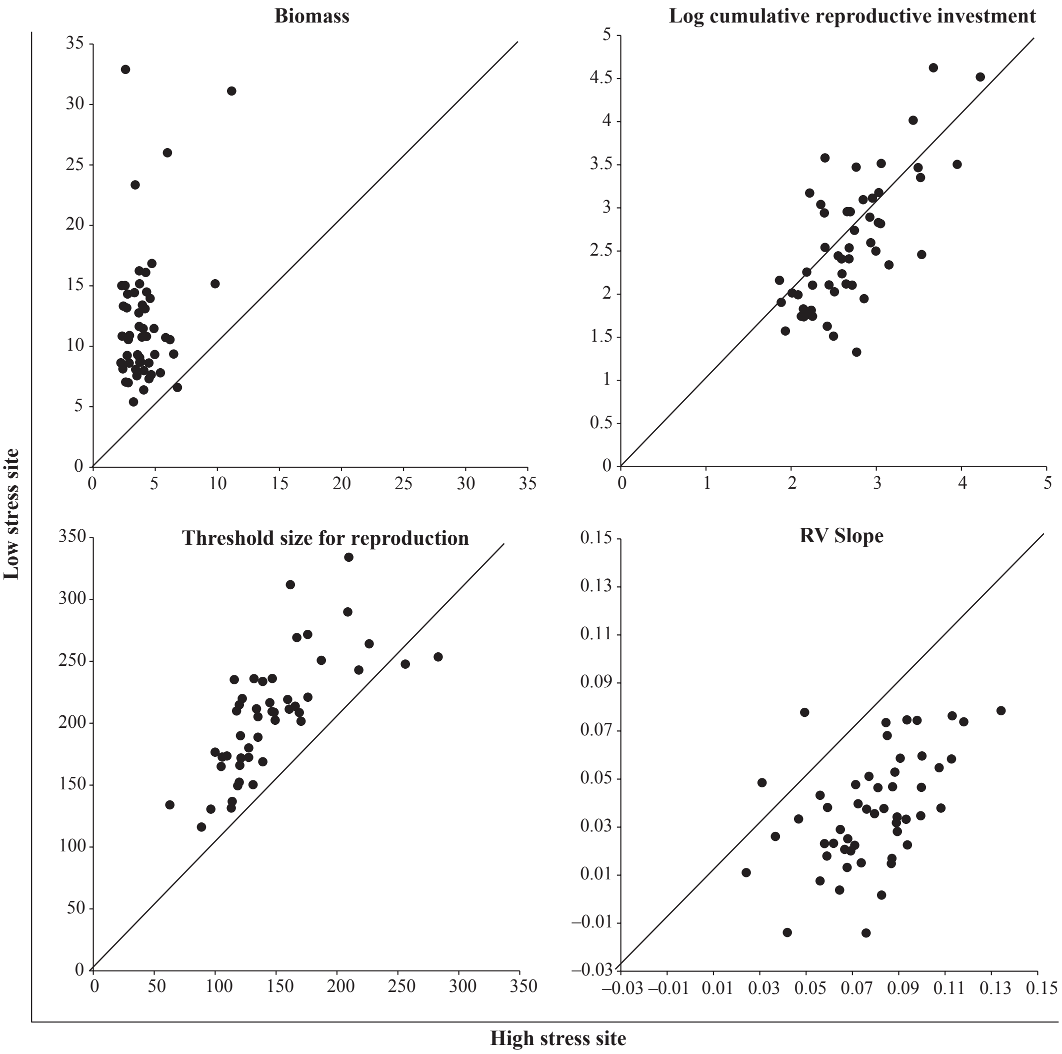


Fig. 2 Site–site graphs for the interpretation of phenotypic plasticity in several vegetative and reproductive traits measured in two *Pinus halepensis* provenance common gardens subject to contrasting environmental conditions (low and high environmental stress). Points represent mean values per source population. Units are as defined in Table 2.

population and expected reproductive allocation of an average-sized individual per population yielded similar results (data not shown), so we used the first index to describe reproductive allocation (RA) as it was derived primarily from the data. Consistently with fecundity descriptors, RA was larger in the high-stress than in the low-stress site. Population means for reproductive traits at the low- and high-stress sites can be accessed in Tables S2 and S3, respectively.

Plant trait correlations and ecotypic trends

Differences in CRI were related to the Gower's environmental distance at both trial sites. That is, there was a significant positive relationship between the environmental similarity between each population with respect to the common garden and the number of cones it produced as revealed by Pearson's correlation tests (Table 5). Female threshold size for reproduction was also significantly negatively related to Gower's distance in the low-stress site but only marginally in the high-stress site. By contrast, for vegetative traits, even when correlations were only marginally significant, they showed opposite patterns at each trial site. Correlations at the high-stress site were negative and at the low-stress site were positive (Table 5). The threshold size for reproduction was the only variable significantly correlated with a change in environmental distance. Closer distances were related to larger thresholds for reproduction. Correlation with CRI was negative but nonsignificant (Table 5).

Table 4 Results of general and generalized linear mixed models for *Pinus halepensis* vegetative and reproductive traits measured in two experimental sites with contrasting environmental conditions. Full models were fitted including all terms. Site, population and population-by-site models were fitted excluding the relevant terms to test plasticity, genetic variation and genetic variation for plasticity. log-likelihood (logLik) is given for each model. Chi-square statistic (Chisq), degrees of freedom (d.f.) and P-value are given for likelihood ratio tests between the full model and reduced models.

	logLik	Chisq	d.f.	*P*-value
Vegetative traits				
Biomass				
Full model	−3449			
Site	−3452	5.6	1	0.018*
Population	−3450	1.7	1	0.190
Site × population	−3466	33.7	1	<0.001***
H				
Full model	−5153			
Site	−5156	6.135	1	0.013*
Population	−5153	0.908	1	0.341
Population × site	−5166	25.6	1	<0.001***
Reproductive traits				
CRI‡				
Full model	−1646			
Site	−1649	5.8	1	0.016*
Population	−1658	23.3	1	<0.001***
Site × population	−1648	3.8	1	0.051†
CRI~h‡				
Full model	−1544			
Site	−1544	0.025	1	0.875
Population	−1564	40.59	2	<0.001***
Population × site	−1545	3.2	2	0.200
CRI~biomass‡				
Full model	−1575			
Site	−1581	13.3	1	<0.001***
Population	−1592	34.97	2	<0.001***
Site × population	−1576	2.7	2	0.257
TSFR§				
Full model	−857			
Site	−876	37.6	1	<0.001***
Population	−871	27.1	2	<0.001***
Site × population	−859	3.0	2	0.222

CRI, cumulative reproductive investment; TSFR, threshold size for first reproduction.

‡Poisson model.

§Binomial model.

*$P < 0.05$, ***$P < 0.01$; †$P < 0.1$.

Site–site correlations of population-adjusted means were not significant for vegetative traits, but strong positive correlations were found for reproductive traits (Table S4). This corroborates the high site-by-population interaction for vegetative traits vs. nonsignificant site-by-population interaction for reproductive traits seen by previous analyses (Table 4).

Within sites, correlations among traits representing potential trade-offs between growth and reproduction (e.g. height and reproductive allocation) showed, in general, stronger correlations in the high-stress site than in the low-stress one (Table S4). We found significant positive correlations between height and TSFR in the high-stress site, but not in the low-stress one. All other correlations between vegetative and reproductive traits were nonsignificant. We also found extensive correlations within vegetative traits and reproductive traits (Table S4).

We found no significant difference in RE between sites ($t_{47} = 1.442$, $P = 0.156$), nor for growth above the site-specific median threshold size for reproduction ($t_{47} = -1.037$, $P = 0.305$). RE was negatively correlated with TSFR in the low-stress site but not in the high-stress site (Table S4).

Correlations between environmental factors and plant traits, reflecting ecotypic trends of variation, were higher in reproductive traits compared with vegetative traits. In turn, they were higher in the low-stress site, but in both sites, the sign of the correlation was the same. Traits indicative of more precocious or abundant reproduction were related to higher altitude, and warmer summers and colder winters (therefore higher continentality index). However, no significant correlations were found between rainfall and any of the phenotypic traits measured at either site (Table S4).

Discussion

Our experiment showed that at the more stressful site, *P. halepensis* trees started reproducing at smaller sizes and completed female reproductive maturity earlier – both in size and in time – than at the least stressful site, therefore following theoretical expectations (Roff, 1992). By definition, threshold size for reproduction accounts for differences in size, so a plastic response in this trait should be considered as a true plastic response and not driven solely by plasticity in size (Sugiyama & Bazzaz, 1998; Weiner, 2004). Hypotheses regarding plasticity of threshold size for reproduction have been addressed in plants only in few cases (Bonser & Aarssen, 2009; Kagaya *et al.*, 2009; Bonser *et al.*, 2010), in contrast with predictions of the high relevance of this type of plasticity (Burd *et al.*, 2006). However, evidences pointing at this phenomenon are common through the literature (Nagy & Proctor, 1997; Fang *et al.*, 2006). We relied on two natural environments to test our hypothesis, which also allowed us to study local adaptation patterns. Nonetheless, a more precise control of environmental stress could be achieved by artificially inducing drought or watering or by setting the experiment at contrasting soil depths and/or nutrient levels, for example, leading to more general conclusions.

The adaptive value of reproduction at larger sizes in favourable conditions relies on a positive relationship between fecundity and size at reproduction, so that

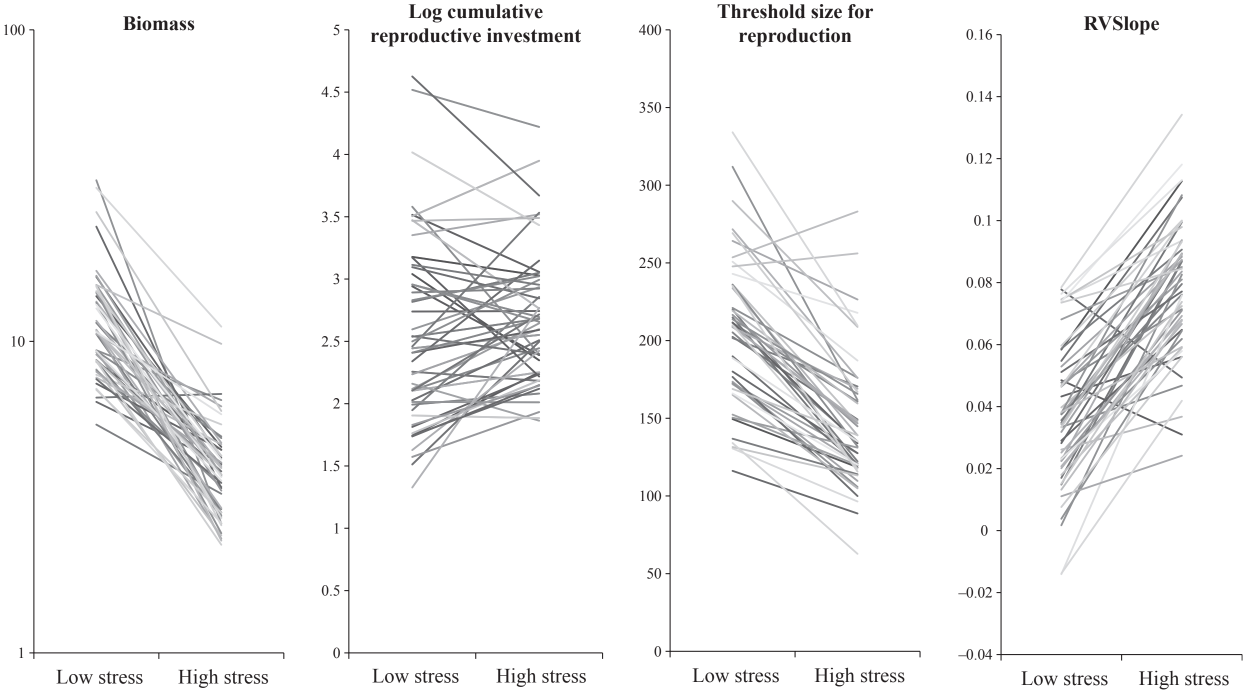


Fig. 3 Reaction norms for vegetative and reproductive traits measured in two *Pinus halepensis* provenance common gardens subject to contrasting environmental conditions (low and high environmental stress). Lines connect mean values per source population at each common garden. Units are as defined in Table 2.

Table 5 Pearson's correlations and *t*-tests between *Pinus halepensis* adjusted population mean values for plant traits and Gower's environmental distance at either high- or low-environmental-stress experimental sites. 'Between sites' refers to the correlation of differences in Gower's distance between sites and differences in mean values for plant traits.

Trait	Site	*r*	*t*	d.f.	*P*-value
Vegetative traits					
Biomass	High stress	−0.31	−2.07	40	0.045*
	Low stress	0.18	1.17	40	0.250
	Between sites	0.10	0.65	40	0.521
H	High stress	−0.29	−1.90	40	0.065†
	Low stress	0.27	1.76	40	0.086†
	Between sites	−0.05	−0.35	40	0.730
Reproductive traits					
CRI	High stress	−0.35	−2.38	40	0.022*
	Low stress	−0.52	−3.85	40	0.000***
	Between sites	−0.25	−1.65	40	0.108
TSFR	High stress	0.27	1.80	40	0.080†
	Low stress	0.49	3.42	37	0.002**
	Between sites	0.45	3.07	37	0.004**

r, Pearson's correlation, *t*, *t* statistic, d.f., degrees of freedom; CRI, cumulative reproductive investment; TSFR, threshold size for first reproduction.
Significant values ***< 0.001, **0.01, *0.05, †0.1.

attaining a larger size implies an increased lifetime reproductive output (Metcalf *et al.*, 2003). Although this relationship is clear in semelparous species, as they only have one reproductive event in their life (bang-bang strategy), in iteroparous species like trees the relationship is not straightforward because individuals allocate significant amounts of resources to maintenance each season throughout their lives (De Jong & Klinkhamer, 2005). For example, two trees with a similar adult size might differ in reproductive output due to differential investment in maintenance along their lives, whereas in annual plants, no differences in reproductive output are expected for similar-sized individuals (Weiner *et al.*, 2009a).

In plants, favourable environments for growth generally favour lower reproductive investment relative to size (Matyas & Varga, 2000; Ortiz *et al.*, 2011; Haymes & Fox, 2012). As these conditions are typically associated with increased competition (Grime, 1977), delayed reproduction in these environments could be driven both by a positive relationship between size at reproduction and lifetime reproductive investment, but also by a persistent allocation to growth and maintenance in crowded stands (Zhang, 2006).

At the population level, a low threshold size for reproduction was correlated with steeper slopes for the R–V relationship at both trial sites. Thus, genetic and environmental factors promoting allocation to reproduction acted consistently along the development of the trees in our experiment. Trade-offs between reproduction and growth are predicted to be more relevant in limiting conditions (Karlsson & Mendez, 2005). Accordingly, in the more stressful site, we found a negative correlation between vegetative traits and reproductive precocity (hence positive with TSFR), and correlation coefficients between reproductive allocation and size among populations were higher than in the less stressful site. However, contrary to our expectations, we found no differences for RE between sites; that is, delayed onset of reproduction in the low-stress site was not rewarded with a proportionally higher reproductive output. We did not extensively test for adaptive plasticity by inducing plants to express an inappropriate phenotype in a given environment (see Sultan, 2000). Nonetheless, our findings regarding enhanced reproduction at the more stressful site highlight the adaptive value of plasticity for reproduction (Anderson *et al.*, 2012) as they support theoretical expectations (Pigliucci, 2001). Indeed, if reproduction at the high-stress site had followed the same allometric trend as in the low-stress site, the risk of becoming locally extinct after a severe disturbance would be dramatically higher. However, at the same time, our results raise uncertainty about what the benefits of delayed reproduction are in environments favourable to vegetative growth. Also, as we did not consider male reproduction in our analysis as the trees were still young, we cannot rule out a possible trade-off between female and male reproduction, so that female reproduction is reduced in favourable environments, but male reproduction could be enhanced. To gain better insight into these uncertainties, data covering a longer time period for both sexual functions would be needed.

In our experiment, tree size was a weak predictor of reproductive output in both trial sites. Although our trees were young and the relationship may strengthen with time (Weiner *et al.*, 2009a), a loose, although significant, relationship between size and reproduction is common in perennial species, notably in trees (Climent *et al.*, 2008; Haymes & Fox, 2012). Population × site interaction was important for vegetative traits, but we found no evidence for larger plants corresponding to shorter environmental distances (Vergeer & Kunin, 2013). Actually, for the low-stress site, the trend was opposite, being larger plants from ecologically distant populations. Instead, reproductive output did show a negative relationship with the environmental distance of the original populations to each trial site. As reproductive output is closely linked to fitness, this suggests that populations have adapted to local climate conditions, and climate is important in controlling the expression of reproduction (Leimu & Fischer, 2008). Thus, we advise against using tree size as a proxy for fitness and encourage the use of reproductive output in tree evolutionary ecology studies.

Several additional factors might interact with raw reproductive output to define individual fitness (Braendle *et al.*, 2011). Within populations, some individuals remained nonreproductive well above their population TSFR, a phenomenon also described in biennials (Wesselingh & Klinkhamer, 1996), and the highest reproductive output was typically achieved by medium-sized individuals in consistency with other experiments in this species (Climent *et al.*, 2008). This pattern was more evident in the low-stress site, where a higher number of trees remained vegetative. A likely explanation for this observation is a diversifying bet-hedging strategy (Simons, 2007), with individuals reproducing according to a genetically determined allocation curve and others situated below that curve (Weiner, 2009a). If a disturbance occurred at either trial site, population and individual would be the most important factors determining the number of available seeds for the next generation. This would imply that if the primary reason for delaying reproduction was a larger future reward through increased size and greater potential future reproduction, many individuals would be making a nonprofitable investment. However, an enhanced allocation to growth would also increase fitness through an increased likelihood of survival (Zhang, 2006). The relative importance of these nonexclusive explanations deserves more attention to better understand adaptive responses in trees.

Contrary to expectations, reproductive output for the whole set of populations was very similar between the two contrasting environments. Plasticity for cumulative cone production was much lower (up to twofold) than that for biomass (up to 10-fold) (Fig. 2). Reproductive output emerged from a combination of plastic responses in growth (larger in the less stressful site) and allometry (higher reproduction for a given size in the more stressful site). An ecotypic trend of enhanced reproduction towards higher altitudes and more extreme temperatures, already described in Climent *et al.* (2008), was not related to population differentiation in plasticity. Interestingly, we found plasticity for both reproductive allometry and vegetative traits, but only genotype-by-environment interaction for vegetative traits.

Phenotypic plasticity is expected to arise in environments that change in a predictable fashion (Van Kleunen & Fischer, 2005). Within species, higher plasticity is generally expected in populations subject to greater interannual variance in precipitation and extreme temperatures and also those living in more patchy environments (Sultan & Spencer, 2002; Baythavong, 2011). In addition, traits might differ in their sensitivity to the environment, or may be constrained resulting in some being more plastic than others (Matesanz *et al.*, 2010), as is the case in our experiment, where not only phenotypic plasticity but also its variation among populations was higher for growth than for reproduction, consistent with findings in different plant genera (reviewed in Weiner *et al.*, 2009a). In our experiment, the lack of population differentiation for plasticity for reproductive allometry could be due to nonexclusive causes such as (i) a strong stabilizing selection for plasticity of reproductive allometry among populations, (ii) a canalization or total dependence of reproductive traits on vegetative traits, like internal cues, or (iii) the perception of environmental heterogeneity, selecting for plasticity, differing between reproductive and vegetative traits. For example, vegetative traits may be more dependent on fine-grain variability of soil depth or nutrient availability, but reproductive traits depend more on factors acting at a larger scale like climate and severe perturbations. In the high-stress site, variation in responses for vegetative traits was constrained, whereas in the low-stress site, among-population differences were neatly expressed, revealing cryptic genetic variation (Schlichting, 2008). Reproductive traits, however, displayed similar levels of variation at both trial sites, so in the environment with most limiting conditions, variation for reproductive traits was more relevant than that for vegetative traits.

Considering expectations of increased drought in the Mediterranean due to climate change (Lindner *et al.*, 2010) and assuming a high heritability of reproductive allometry (Santos-del-Blanco *et al.*, 2010 and in prep; Wesselingh & De Jong, 1995), we hypothesize that phenotypic plasticity coupled with subsequent natural selection on this trait (Anderson *et al.*, 2012; Chevin *et al.*, 2012) will play a relevant role in future adaptation of forest species.

Acknowledgments

The authors declare no conflict of interest. The data used in this research are part of the Spanish Network of Genetic Trials (GENFORED). Data are publicly available upon request through www.genfored.es. We thank E. Ballesteros, F. del Caño, D. Barba and E. Alamillo for fieldwork support. Luis Samperdo (CSIC), Jill T. Anderson (U. South Carolina) and two anonymous reviewers provided insightful comments that improved the manuscript. This study was funded by the Spanish Ministry of Economy and Competitiveness through the project Mitigenfor (RTA 2011-00016-00-00). Regina Chambel was granted by the Spanish Ministry of Economy and Competitiveness for technical support to GENFORED network.

References

Alexander, J.M., Edwards, P.J., Poll, M., Parks, C.G. & Dietz, H. 2009. Establishment of parallel altitudinal clines in traits of native and introduced forbs. *Ecology* **90**: 612–622.

Anderson, J.T., Lee, C.R. & Mitchell-Olds, T. 2011. Life-history QTLS and natural selection on flowering time in *Boechera stricta*, a perennial relative of *Arabidopsis*. *Evolution* **65**: 771–787.

Anderson, J.T., Inouye, D.W., McKinney, A.M., Colautti, R.I. & Mitchell-Olds, T. 2012. Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. *Proc. Biol. Sci.* **279**: 3843–3852.

Bates, D.M., Maechler, M. & Bolker, B. 2011. Package lme4: linear mixed-effects models using S4 classes. R package version 0.999375-42. http://CRAN.R-project.org/package=lme4.

Baythavong, B.S. 2011. Linking the spatial scale of environmental variation and the evolution of phenotypic plasticity: selection favors adaptive plasticity in fine-grained environments. *Am. Nat.* **178**: 75–87.

Bonser, S.P. & Aarssen, L.W. 2009. Interpreting reproductive allometry: individual strategies of allocation explain size-dependent reproduction in plant populations. *Perspect. Plant. Ecol.* **11**: 31–40.

S.P., Ladd, B., Monro, K., Hall, M.D. & Forster, M.A. 2010. The adaptive value of functional and life-history traits across fertility treatments in an annual plant. *Ann. Bot.* **106**: 979–988.

Braendle, C., Heyland, A. & Flatt, T. 2011. Integrating mechanistic and evolutionary analysis of life history variation. In: *Mechanisms of Life History Evolution: The Genetics and Physiology of Life History Traits and Trade-Offs* (T. Flatt & A. Heyland, eds), pp. 3–10. Oxford University Press, Oxford, UK.

Brophy, C., Gibson, D.J., Wayne, P.M. & Connolly, J. 2007. A modelling framework for analysing the reproductive output of individual plants grown in monoculture. *Ecol. Model.* **207**: 99–108.

Burd, M., Read, J., Sanson, G.D. & Jaffré, T. 2006. Age-size plasticity for reproduction in monocarpic plants. *Ecology* **87**: 2755–2764.

Chambel, M.R., Climent, J., Alía, R & Valladares, F. 2005. Phenotypic plasticity: a useful framework for understanding adaptation in forest species. *Inv. Agrar.: Sist. Rec. Forest.* **14**: 334–344.

Chevin, L.-M., Collins, S. & Lefèvre, F. 2012. Phenotypic plasticity and evolutionary demographic responses to climate change: taking theory out to the field. *Funct. Ecol.* doi: 10.1111/j.1365-2435.2012.02043.x.

Childs, D.Z., Metcalf, C.J.E. & Rees, M. 2010. Evolutionary bet-hedging in the real world: empirical evidence and challenges revealed by plants. *Proc. Biol. Sci.* **277**: 3055–3064.

Climent, J., Prada, M.A., Calama, R., Chambel, M.R., De Ron, D.S. & Alía, R. 2008. To grow or to seed: ecotypic variation in reproductive allocation and cone production by young female Aleppo pine (*Pinus halepensis*, *Pinaceae*). *Am. J. Bot.* **95**: 833–842.

De Jong, T.J. & Klinkhamer, P.G.L. 2005. *Evolutionary Ecology of Plant Reproductive Strategies*. Cambridge University Press, Cambridge.

Elston, D., Moss, R., Boulinier, T., Arrowsmith, C. & Lambin, X. 2001. Analysis of aggregation, a worked example: numbers of ticks on red grouse chicks. *Parasitology* **122**: 563–569.

Fang, W., Taub, D.R., Fox, G.A., Landis, R.M., Natali, S. & Gurevitch, J. 2006. Sources of variation in growth, form, and survival in dwarf and normal-stature pitch pines (*Pinus rigida*, *Pinaceae*) in long-term transplant experiments. *Am. J. Bot.* **93**: 1125–1133.

Franks, S.J. & Weis, A.E. 2008. A change in climate causes rapid evolution of multiple life-history traits and their interactions in an annual plant. *J. Evolution. Biol.* **21**: 1321–1334.

Girard, F., Vennetier, M., Guibal, F., Corona, C., Ouarmim, S. & Herrero, A. 2011. *Pinus halepensis* Mill. crown development and fruiting declined with repeated drought in Mediterranean France. *Eur. J. Forest Res.* **131**: 919–931.

Gonzalo-Jiménez, J. 2010. *Diagnosis fitoclimática de la España Peninsular: hacia un modelo de clasificación funcional de la vegetación y de los ecosistemas peninsulares españoles*. Organismo Autónomo de Parques Nacionales, Madrid, Spain.

Grime, J.P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and Evolutionary Theory. *Am. Nat.* **111**: 1169–1194.

Guo, H., Weiner, J., Mazer, S.J., Zhao, Z., Du, G. & Li, B. 2012. Reproductive allometry in *Pedicularis* species changes with elevation. *J. Ecol.* **100**: 452–458.

Hadfield, J.D. 2010. MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *J. Stat. Soft.* **33**: 1–22.

Haymes, K.L. & Fox, G.A. 2012. Variation among individuals in cone production in *Pinus palustris* (*Pinaceae*). *Am. J. Bot.* **99**: 1–6.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. 2005. Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.* **25**: 1965–1978.

Johnsen, Ø., Fossdal, C., Nagy, N., Mølmann, J., Gram Daehlen, O. & Skrøppa, T. 2005. Climatic adaptation in *Picea abies* progenies is affected by the temperature during zygotic embryogenesis and seed maturation. *Plant, Cell Environ.* **28**: 1090–1102.

Kagaya, M., Tani, T. & Kachi, N. 2009. Variation in flowering size and age of a facultative biennial, *Aster kantoensis* (*Compositae*), in response to nutrient availability. *Am. J. Bot.* **96**: 1808–1813.

Karlsson, P.S. & Mendez, M. 2005. The resource economy of plant reproduction. In: *Reproductive Allocation in Plants* (E.G. Reekie & F.A. Bazzaz, eds), pp. 1–40. Elsevier, Amsterdam, The Netherlands.

King, D. & Roughgarden, J. 1982. Graded allocation between vegetative and reproductive growth for annual plants in growing seasons of random length. *Theor. Popul. Biol.* **22**: 1–16.

Kozłowski, J. 1992. Optimal allocation of resources to growth and reproduction: implications for age and size at maturity. *Trends Ecol. Evol.* **7**: 15–19.

Lacey, E. 1988. Latitudinal variation in reproductive timing of a short-lived monocarp, *Daucus carota* (*Apiaceae*). *Ecology* **69**: 220–232.

Leimu, R. & Fischer, M. 2008. A meta-analysis of local adaptation in plants. *PLoS ONE* **3**: e4010.

Lindner, M., Maroschek, M., Netherer, S., Kremer, A., Barbati, A., Garcia-Gonzalo, J. *et al.* 2010. Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. *Forest Ecol. Manag.* **259**: 698–709.

Matesanz, S., Gianoli, E. & Valladares, F. 2010. Global change and the evolution of phenotypic plasticity in plants. *Ann. N.Y. Acad. Sci.* **1206**: 35–55.

Matyas, C. & Varga, G. 2000. Effect of intra-specific competition on tree architecture and aboveground dry matter allocation in Scots pine. *Forest Syst.* **9**: 111–119.

Matziris, D. 1997. Variation in growth, flowering and cone production in a clonal seed orchard of Aleppo pine grown in Greece. *Silvae Genet.* **46**: 224–228.

Mendez, M. & Karlsson, P.S. 2004. Between-population variation in size-dependent reproduction and reproductive allocation in *Pinguicula vulgaris* (*Lentibulariaceae*) and its environmental correlates. *Oikos* **104**: 59–70.

Metcalf, C.J.E., Rose, K. & Rees, M. 2003. Evolutionary demography of monocarpic perennials. *Trends Ecol. Evol.* **18**: 471–480.

Montero, G., Ruiz-Peinado, R. & Muñoz, M. 2005. *Producción de biomasa y fijación de CO2 por los bosques españoles*. Instituto Nacional de Investigacion y Tecnología Agraria y Alimentaria, Madrid, Spain.

Nagy, L. & Proctor, J. 1997. Plant growth and reproduction on a toxic alpine ultramafic soil: adaptation to nutrient limitation. *New Phytol.* **137**: 267–274.

Ne'eman, G., Gidi Goubitz, S. & Shirrinka Nathan, R. 2004. Reproductive traits of *Pinus halepensis* in the light of fire–a critical review. *Plant Ecol.* **171**: 69–79.

Ne'eman, G., Goubitz, S., Werger, M.J.A. & Shmida, A. 2011. Relationships between tree size, crown shape, gender segregation and sex allocation in *Pinus halepensis*, a Mediterranean pine tree. *Ann. Bot.* **108**: 197–206.

Nicholls, A.M. 2011. Size-dependent analysis of allocation to sexual and clonal reproduction in *Penthorum sedoides* under contrasting nutrient levels. *Int. J. Plant Sci.* **172**: 1077–1086.

Niklas, K.J. & Enquist, B.J. 2003. An allometric model for seed plant reproduction. *Evol. Ecol. Res.* **5**: 79–88.

Ortiz, O., Ojeda, G., Espelta, J.M. & Alcaniz, J.M. 2011. Improving substrate fertility to enhance growth and reproductive ability of a *Pinus halepensis* Mill. afforestation in a restored limestone quarry. *New Forest.* **43**: 365–381.

Pigliucci, M. 2001. *Phenotypic Plasticity: Beyond Nature and Nurture*. The Johns Hopkins University Press, Baltimore, USA.

Pinheiro, J.C. & Bates, D.M. 2000. *Mixed Effects Models in S and S-Plus*. Springer, New York.

R Development Core Team 2012. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.

Roff, D.A. 1992. Age and size at maturity. In: *The Evolution of Life Histories: Theory and Analysis* (D.A. Roff, ed.), pp. 179–241. Chapman and Hall, New York.

Roff, D.A. 2000. Trade-offs between growth and reproduction: an analysis of the quantitative genetic evidence. *J. Evol. Biol.* **13**: 434–445.

Rutter, M.T. & Fenster, C.B. 2007. Testing for adaptation to climate in *Arabidopsis thaliana*: a calibrated common garden approach. *Ann. Bot.* **99**: 529–536.

Santos-del-Blanco, L., Notivol, E., Zas, R., Chambel, M.R., Majada, J. & Climent, J. 2010. Variation of early reproductive allocation in multi-site genetic trials of Maritime pine and Aleppo pine. *Forest Syst.* **19**: 381–392.

Santos-del-Blanco, L., Climent, J., González-Martínez, S.C. & Pannell, J.R. 2012. Genetic differentiation for size at first reproduction through male versus female functions in the widespread Mediterranean tree *Pinus pinaster*. *Ann. Bot.* **110**: 1449–1460.

Schlichting, C.D. 1986. The evolution of phenotypic plasticity in plants. *Annu. Rev. Ecol. Syst.* **17**: 667–693.

Schlichting, C.D. 2008. Hidden reaction norms, cryptic genetic variation, and evolvability. *Ann. N.Y. Acad. Sci.* **1133**: 187–203.

Shmida, A., Lev-Yadun, S., Goubitz, S. & Ne'eman, G. 2000. Sexual allocation and gender segregation in *Pinus halepensis*, *P. brutia* and *P. pinea*. In: *Ecology, Biogeography and Management of Pinus halepensis* and *P. brutia* Forest Ecosystems in the Mediterranean Basin (G. Ne'Eman & L. Trabaud, eds), pp. 91–104. Backhuys Publisher, Leiden, The Netherlands.

Simons, A.M. 2007. Selection for increased allocation to offspring number under environmental unpredictability. *J. Evol. Biol.* **20**: 813–817.

Soto, A., Robledo-Arnuncio, J.J., González-Martínez, S.C., Smouse, P.E. & Alía, R. 2010. Climatic niche and neutral genetic diversity of the six Iberian pine species: a retrospective and prospective view. *Mol. Ecol.* **19**: 1396–1409.

Stearns, S.C. 1992. *The Evolution of Life Histories*. Oxford University Press, New York.

Sugiyama, S. & Bazzaz, F.A. 1998. Size dependence of reproductive allocation: the influence of resource availability, competition and genetic identity. *Funct. Ecol.* **12**: 280–288.

Sultan, S.E. 2000. Phenotypic plasticity for plant development, function and life history. *Trends Plant Sci.* **5**: 537–542.

Sultan, S.E. & Spencer, H. 2002. Metapopulation structure favors plasticity over local adaptation. *Am. Nat.* **160**: 271–283.

Thomas, S.C. 1996. Relative size at onset of maturity in rain forest trees: a comparative analysis of 37 Malaysian species. *Oikos* **76**: 145.

Van Kleunen, M. & Fischer, M. 2005. Constraints on the evolution of adaptive phenotypic plasticity in plants. *New Phytol.* **166**: 49–60.

Vergeer, P. & Kunin, W.E. 2013. Adaptation at range margins: common garden trials and the performance of *Arabidopsis lyrata* across its North-western European range. *New Phytol.* **197**: 989–1001.

Weiner, J. 2004. Allocation, plasticity and allometry in plants. *Perspect. Plant. Ecol.* **6**: 207–215.

Weiner, J., Campbell, L.G., Pino, J. & Echarte, L. 2009a. The allometry of reproduction within plant populations. *J. Ecol.* **97**: 1220–1233.

Weiner, J., Rosenmeier, L., Massoni, E.S., Vera, J.N., Plaza, E.H. & Sebastià, M.T. 2009b. Is reproductive allocation in *Senecio vulgaris* plastic? *Botany* **87**: 475–481.

Wesselingh, R.A. & De Jong, T.J. 1995. Bidirectional selection on threshold size for flowering in *Cynoglossum officinale* (hound's-tongue). *Heredity* **74**: 415–424.

Wesselingh, R.A. & Klinkhamer, P. 1996. Threshold size for vernalization in *Senecio jacobaea*: genetic variation and response to artificial selection. *Funct. Ecol.* **10**: 281–288.

Wesselingh, R.A., Klinkhamer, P.G.L., De Jong, T.J. & Boorman, L.A. 1997. Threshold size for flowering in different habitats: effects of size-dependent growth and survival. *Ecology* **78**: 2118–2132.

Willson, M.F. 1983. *Plant Reproductive Ecology*. John Wiley & Sons, New York, USA.

Zhang, D.Y. 2006. Evolutionary stable reproductive investment and sex allocation in plants. In: *The Ecology and Evolution of Flowers* (L.D. Harder & S.C.H. Barrett, eds), pp. 41–59. Oxford University Press, Oxford, UK.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 List and location of the *Pinus halepensis* populations comprised in the present study and planted at two trial sites with contrasting environmental conditions (high and low environmental stress).

Table S2 Vegetative and reproductive traits in 52 natural *Pinus halepensis* populations grown in a common garden placed at Valdeolmos (Madrid, Spain), referred as low stress site.

Table S3 Vegetative and reproductive traits in 52 natural *Pinus halepensis* populations grown in a common garden placed at Rincón de Ademuz (Valencia, Spain), referred as high stress site.

Table S4 Pearson correlation coefficients for the correlation at the population level between climatic variables and *Pinus halepensis* traits in each site, the low stress site above diagonal and the high stress site below the diagonal.

Received 13 December 2012; revised 13 April 2013; accepted 15 April 2013

Supplementary material

Plasticity in reproduction and growth among 52 range-wide populations of a Mediterranean conifer: adaptive responses to environmental stress

Authors: Santos-del-Blanco, Luis[1,2]*; Bonser, Stephen P.[3]; Valladares, Fernando[4]; Chambel, Maria Regina[1], Climent, José[1,2].

[1] INIA-CIFOR, Dpt. Forest Ecology and Genetics. Ctra A Coruña km 7.5, E-28040, Madrid, Spain

[2] Sustainable Forest Management Research Institute, INIA-University of Valladolid, http://sostenible.palencia.uva.es

[3] Evolution & Ecology Research Centre, School of Biological, Earth and Environmental Sciences, The University of New South Wales, NSW 2052, Sydney, Australia

[4] Laboratorio Internacional de Cambio Global LINC-Global, Museo Nacional de Ciencias Naturales, MNCN-CSIC, E-28006, Madrid, Spain

Running title:

Plasticity in plant reproductive traits

***corresponding author:**

e-mail address: santos.luis@inia.es

Tel +34 91 3476862,

Fax +34 91 3476767.

INIA-CIFOR, Dpt. Forest Ecology and Genetics.

Ctra A Coruña km 7.5,

E-28040, Madrid, Spain

TABLE S1. List and location of the *Pinus halepensis* populations comprised in the present study and planted at two trial sites with contrasting environmental conditions (high and low environmental stress).

Code	Population	Country	Altitude	Latitude	Longitude	AP	PDQ	AMT	MTWM	MTCM	CI
11	Cabanellas	Spain	258	977212	4693568	858	208	14.2	28.3	1.3	27.0
21	Tivissa	Spain	336	815989	4552144	566	75	15.3	30.6	3.2	27.4
31	Sant Salvador de Guardiola	Spain	318	896567	4624516	569	123	13.9	30.0	0.9	29.1
61	Zuera	Spain	576	672350	4642850	488	95	12.9	31.6	-0.1	31.7
82	Valdeconcha	Spain	837	510850	4477650	579	80	12.7	32.2	-1.2	33.4
83	Alcantud	Spain	1057	558120	4490600	819	100	10.5	30.9	-2.2	33.1
84	Colmenar de Oreja	Spain	692	471558	4437895	450	55	13.7	33.0	0.0	33.0
91	Cirat	Spain	445	715916	4436492	472	109	15.4	31.1	2.6	28.5
92	Tuéjar	Spain	729	657550	4409300	385	91	15.0	33.2	1.0	32.2
93	Enguidanos	Spain	990	615980	4388800	572	85	12.5	32.2	-0.7	32.9
101	Tibi	Spain	976	704994	4266071	503	58	13.0	28.7	1.5	27.2
102	Altura	Spain	662	704300	4407300	472	71	14.5	29.2	2.9	26.3
103	Villa de Ves	Spain	864	651250	4338000	489	82	13.5	30.6	2.0	28.6
104	Jarafuel	Spain	563	671850	4336700	518	71	14.7	30.5	3.5	27.0
105	Bicorp	Spain	587	685200	4330500	545	76	15.1	30.5	3.2	27.3
111	Benicasim	Spain	468	758019	4440766	699	90	14.2	26.3	4.4	21.9
112	Gilet	Spain	152	727450	4394300	562	69	16.3	28.9	5.3	23.6
131	Villajoyosa	Spain	126	735148	4264302	431	51	16.8	29.4	5.6	23.8
141	Ricote	Spain	688	637500	4222800	321	44	15.8	32.6	3.5	29.1
142	Monovar	Spain	820	678402	4250548	387	51	14.7	30.7	2.7	28.0
143	Monovar	Spain	601	681100	4251500	335	47	15.8	31.2	3.7	27.5
144	Paterna	Spain	1028	562950	4275800	552	67	13.2	32.2	-0.3	32.5
145	Abaran	Spain	657	651900	4237900	343	45	15.6	32.5	2.4	30.1
151	Quentar	Spain	1226	463550	4120300	657	29	13.2	31.6	-0.2	31.8
152	Benamaurel	Spain	908	523020	4172800	394	44	14.3	32.9	1.1	31.8
153	Velez Blanco	Spain	785	586700	4183100	357	37	14.2	32.6	1.2	31.4
154	Santiago de la Espada	Spain	842	546600	4231150	608	66	13.6	32.9	0.3	32.6
156	Lorca	Spain	831	592647	4178631	329	42	15.7	32.6	2.7	29.9
157	Alhama de Murcia	Spain	765	628924	4191849	433	47	14.9	29.8	3.9	25.9
158	Quesada	Spain	757	498321	4178582	510	33	15.3	34.1	2.1	32.0
171	Lentegi	Spain	363	438677	4075439	593	16	16.0	30.7	4.3	26.4
172	Carratraca	Spain	635	336500	4079000	774	35	14.3	30.4	2.9	27.5
173	Frigiliana	Spain	595	417900	4075100	696	26	15.2	28.9	4.3	24.6
182	Palma de Mallorca	Spain	32	1013545	4350235	554	38	16.8	28.7	6.9	21.8
183	Santanyí	Spain	19	1021743	4365904	568	40	16.8	28.7	6.9	21.8
184	Alcúdia	Spain	185	1027856	4431910	697	59	16.0	28.0	6.2	21.8
185	Calviá	Spain	243	971329	4396425	485	50	16.3	28.6	6.2	22.4
186	Alcotx	Spain	85	1112427	4449307	616	47	16.5	28.1	7.1	21.0
187	Atalix	Spain	67	1103104	4442252	606	45	17.0	28.5	7.5	21.0
191	Cala d'hort	Spain	329	868343	4312901	503	46	16.6	28.4	6.0	22.4
192	Ses Salines	Spain	10	881834	4308362	445	37	17.4	29.0	6.9	22.1
193	Ses Salandres	Spain	65	874946	4331016	507	50	16.3	28.2	5.8	22.4
211	Istaia-eyboia	Greece	53	2818134	4637783	506	35	17.4	32.5	5.3	27.2
212	Amfilohia	Greece	429	2618397	4595421	966	66	14.8	31.1	2.1	29
213	Tatoi-attica	Greece	253	2824738	4603335	619	49	14.1	29.7	2.1	27.6
214	Kassandra	Greece	402	2803882	4800501	518	63	14.1	29.2	1.5	27.7
221	Gemenos	France	391	1201790	4843798	712	102	12.4	25.9	1.0	24.9
231	Litorale Tarantino	Italy	204	2206311	4696011	550	76	15.2	29.5	3.9	25.6
232	Gargano Monte Pucci	Italy	382	2073826	4816115	553	97	13.4	26.0	3.4	22.6
233	Gargano Marzini	Italy		2075777	4774891	472	76	16.1	30.7	4.5	26.2
241	Thala	Tunisia	948	1558095	3999027	463	63	14.9	34.6	1.0	33.6
242	Tabarka	Tunisia	287	1583691	4108559	553	36	17.8	36.1	4.5	31.6

AP = annual mean precipitation (mm); PDQ = precipitation during the driest quarter (mm); AMT= annual mean temperature (ºC); MTWM = mean temperature of the warmest month (ºC); MTCM = mean temperature of the coldest month (ºC); CI = continentality index (ºC).

TABLE S2. Vegetative and reproductive traits in 52 natural *Pinus halepensis* populations grown in a common garden placed at Valdeolmos (Madrid, Spain), referred as low stress site.

Code	H (cm)	Biomass (kg)	TSFR (cm)	log(ARI) (n cones)	log(RA) (cones/kg biomass)	Intercept* (cones)	Slope* log(cones/kg biomass)
11	371±19	16.1±3.8		2.9±0.2	-0.36±0.56	6.19 (3.05-10.74)	0.043 (0.013-0.076)
21	323±21	9.4±4.4	221 (182-288)	2.1±0.2	-0.04±0.64	3.74 (2.08-8.25)	0.033 (-0.012-0.086)
31	342±20	11.5±4.1	202 (165-269)	2.0±0.2	-0.34±0.6	3.43 (1.68-6.36)	0.017 (-0.01-0.066)
61	325±20	8.6±4.1	149 (118-168)	3.2±0.2	1.09±0.6	10.61 (6.98-23.45)	0.058 (0.001-0.112)
82	357±19	10.6±3.8	220 (174-257)	2.3±0.2	-0.11±0.56	5.42 (2.75-9.08)	0.036 (-0.004-0.08)
83	348±20	10.8±4.1	174 (138-200)	2.4±0.2	-0.02±0.6	5.46 (2.93-10.37)	0.053 (-0.002-0.097)
84	304±20	6.4±4.1	116 (92-139)	3.5±0.2	2.47±0.6	17.39 (9.47-31.05)	0.078 (0.009-0.147)
91	338±22	9.2±4.5	172 (147-206)	3.6±0.2	1.03±0.66	13.89 (6.3-25.96)	0.068 (0.026-0.144)
92	294±20	5.4±4.2	202 (144-240)	1.5±0.3	0.02±0.62	2.54 (1.18-4.73)	0.015 (-0.035-0.102)
93	366±20	14.0±4.2		3.0±0.2	0.19±0.62	7.31 (3.24-11.83)	0.049 (0.009-0.07)
101	318±19	9.3±4.0	211 (193-248)	2.5±0.2	0.65±0.58	6.13 (3.64-11.47)	0.048 (0.000-0.087)
102	337±20	13.4±4.1	234 (182-276)	2.2±0.2	-0.17±0.6	4.28 (2.48-8.58)	0.025 (-0.007-0.062)
103	315±19	8.7±3.8	190 (131-263)	2.7±0.2	0.49±0.56	4.99 (2.99-9.75)	0.055 (0.011-0.096)
104	333±19	9.1±3.8	172 (169-176)	2.6±0.2	0.65±0.56	6.07 (3.1-10.48)	0.038 (-0.003-0.079)
105	336±20	11.5±4.2	205 (163-246)	2.8±0.2	0.24±0.62	6.55 (3.17-12.08)	0.037 (0.005-0.081)
111	325±20	7.3±4.2	212 (169-268)	1.7±0.2	-0.45±0.62	3.23 (1.44-5.77)	0.029 (-0.031-0.082)
112	365±20	32.9±4.2	312 (238-502)	2.0±0.2	-1.34±0.62	0.63 (0.28-1.71)	0.002 (-0.017-0.011)
131	331±20	8.1±4.2	236 (184-286)	2.1±0.2	-0.34±0.62	3.13 (1.47-6.94)	0.04 (-0.026-0.089)
141	308±20	6.6±4.2	215 (165-271)	2.3±0.2	-0.12±0.62	4.95 (2.23-9.16)	0.035 (-0.019-0.11)
142	345±19	11.6±3.8	209 (84-334)	2.1±0.2	-0.43±0.56	3.27 (1.68-5.77)	0.02 (-0.029-0.049)
143	311±22	8.1±4.5	236 (184-272)	1.9±0.2	-0.02±0.66	3.05 (1.49-7.41)	0.023 (-0.045-0.07)
144	379±21	23.4±4.4	180 (178-199)	3.2±0.2	0.05±0.64	7.26 (2.84-12.57)	0.028 (0.004-0.052)
145	383±19	15.2±4.0	150 (105-192)	3.0±0.2	0.03±0.58	6.67 (3.76-11.99)	0.046 (0.013-0.077)
151	301±24	7.8±5.0		3.5±0.2	1.86±0.73	13.15 (7.45-38.19)	0.06 (-0.004-0.135)
152	318±19	8.6±4.0	137 (94-160)	3.1±0.2	1.35±0.58	9.08 (5.92-19.59)	0.059 (0.011-0.095)
153	319±20	7.7±4.1	219 (177-259)	2.2±0.2	0.23±0.6	4.84 (2.39-8.67)	0.026 (-0.02-0.099)
154	343±19	14.3±4.0	173 (150-198)	2.9±0.2	0.42±0.58	6.33 (3.12-10.81)	0.038 (0.015-0.076)
156	328±19	7.6±4.0	177 (159-202)	2.4±0.2	0.58±0.58	5.63 (2.84-10.11)	0.051 (-0.022-0.09)
157	349±20	16.2±4.1	152 (129-181)	3.0±0.2	0.34±0.6	6.65 (3.04-10.91)	0.032 (0.011-0.063)
158	353±19	13.2±4.0	131 (123-142)	3.5±0.2	0.83±0.58	13.14 (7.04-22.96)	0.075 (0.038-0.117)
171	364±20	14.4±4.1	217 (184-263)	2.5±0.2	-0.22±0.6	3.96 (2.11-8.83)	0.022 (-0.004-0.066)
172	326±20	8.7±4.1	132 (106-159)	3.4±0.2	1.17±0.6	13.83 (6.78-21.65)	0.074 (0.023-0.129)
173	327±20	8.0±4.1	209 (169-241)	2.4±0.2	0.08±0.6	5.07 (3.1-10.42)	0.047 (-0.001-0.105)
182	339±20	13.3±4.1	214 (145-275)	1.8±0.2	-0.85±0.6	2.72 (1.3-5.02)	0.004 (-0.026-0.047)
183	346±25	10.9±5.2	272 (217-331)	2.0±0.3	-0.58±0.77	3.23 (1.09-8.58)	0.021 (-0.035-0.082)
184	350±20	9.3±4.2	251 (168-333)	1.8±0.2	-0.47±0.62	2.8 (1.59-6.2)	-0.014 (-0.053-0.062)
185	341±20	16.9±4.2	209 (206-218)	2.1±0.2	-0.51±0.62	2.22 (1.17-5.22)	0.023 (-0.006-0.045)
186	315±22	8.6±4.5	210 (145-296)	1.6±0.3	-0.14±0.66	2.68 (1.52-6.78)	0.033 (-0.046-0.082)
187	361±20	15±4.2	165 (82-217)	2.8±0.2	0.14±0.62	5.54 (2.58-10.55)	0.034 (0.000-0.066)
191	346±21	10.5±4.4	264 (207-318)	1.7±0.3	-0.75±0.64	2.74 (1.33-5.82)	0.011 (-0.045-0.061)
192	329±22	10.8±4.5	248 (194-307)	1.6±0.3	-0.82±0.66	2.18 (0.99-5.01)	0.018 (-0.020-0.060)
193	354±26	12.8±5.6	243 (201-321)	1.9±0.3	-0.5±0.83	3.01 (0.93-9)	0.023 (-0.035-0.083)
211	359±23	15.2±4.7	290 (242-362)	2.5±0.2	-0.76±0.69	2.77 (1.44-8.06)	0.038 (-0.010-0.070)
212	394±21	31.1±4.4	334 (248-496)	1.3±0.3	-1.88±0.64	1.03 (0.4-2.1)	-0.014 (-0.034-0.01)
213	378±19	26.0±3.8	169 (95-238)	2.5±0.2	-0.53±0.56	3.62 (1.67-6.03)	0.015 (-0.006-0.03)
214	328±20	10.8±4.2	254 (218-310)	1.7±0.2	-0.6±0.62	2.22 (1.09-4.76)	0.013 (-0.048-0.051)
221	379±20	14.5±4.1	269 (161-397)	1.8±0.2	-0.91±0.6	2.38 (1.11-5.23)	0.008 (-0.028-0.042)
231	332±22	13.1±4.5	166 (119-207)	4.0±0.2	1.31±0.66	16.76 (8.62-36.74)	0.074 (0.034-0.108)
232	366±21	15.0±4.4	235 (182-308)	3.1±0.2	0.21±0.64	5.88 (3.17-12.77)	0.047 (0.013-0.079)
233	325±21	10.7±4.4	189 (136-224)	3.5±0.2	0.36±0.64	6.51 (2.89-12.98)	0.076 (0.024-0.118)
241	323±21	7.0±4.4	134 (73-176)	4.5±0.1	2.62±0.64	42.63 (17.92-79.1)	0.078 (0.026-0.186)
242	314±24	7.0±5.0	140 (118-156)	4.6±0.2	2.5±0.73	44.23 (15.91-91.17)	0.074 (0.017-0.173)

* Intercept and slope were derived from independent models

TABLE S3. Vegetative and reproductive traits in 52 natural *Pinus halepensis* populations grown in a common garden placed at Rincón de Ademuz (Valencia, Spain), referred as high stress site.

Code	H (cm)	Biomass (kg)	TSFR (cm)	log(ARI) (n cones)	log(RA) (cones/kg biomass)	Intercept* (cones)	Slope* log(cones/kg biomass)
11	281±18	4.2±1.4	165 (156-174)	2.4±0.2	1.57±0.38	5.73 (3.41-9.09)	0.056 (0.015-0.131)
21	319±18	6.5±1.4	176 (156-231)	2.4±0.2	1.24±0.38	6.74 (3.82-9.51)	0.047 (-0.006-0.092)
31	288±19	4±1.5	149 (110-184)	2.5±0.2	1.81±0.4	8.57 (4.68-12.4)	0.087 (0.01-0.159)
61	274±17	4.5±1.4	118 (105-152)	3.0±0.1	2.05±0.36	9.03 (5.9-14.33)	0.113 (0.045-0.16)
82	260±18	2.8±1.5	122 (93-157)	2.2±0.2	2.12±0.39	5.67 (3.14-8.57)	0.08 (-0.003-0.156)
83	257±19	2.3±1.5	110 (92-165)	2.6±0.2	2.15±0.4	8.8 (5.49-14.2)	0.088 (0.005-0.179)
84	250±18	4.1±1.5	89 (69-118)	3.1±0.1	2.99±0.39	9.03 (6.11-15.62)	0.049 (0.018-0.108)
91	260±21	2.7±1.7	127 (92-136)	2.4±0.2	2.15±0.45	6.38 (3.72-11.46)	0.085 (-0.013-0.168)
92	272±18	3.2±1.4	170 (146-172)	2.5±0.2	1.72±0.38	6.67 (4.48-10.74)	0.087 (0.022-0.176)
93	284±19	4.6±1.5	225 (197-251)	2.3±0.2	1.47±0.4	5.69 (3.25-9.04)	0.031 (-0.043-0.085)
101	296±18	5±1.5	161 (155-167)	2.7±0.2	1.66±0.39	8.71 (4.8-11.91)	0.071 (0.021-0.133)
102	280±18	4±1.5	139 (117-170)	2.6±0.2	2.07±0.39	7.02 (4.95-12.3)	0.068 (0.014-0.146)
103	269±20	3.8±1.6	121 (80-167)	2.7±0.2	2.13±0.43	7.86 (4.88-14.07)	0.107 (0.029-0.165)
104	288±22	3.7±1.8	121 (76-155)	2.9±0.2	1.9±0.48	10.15 (5.46-17.53)	0.084 (0.017-0.188)
105	286±17	4.9±1.3	135 (102-168)	3.1±0.1	1.93±0.35	11.06 (7.3-15.66)	0.076 (0.026-0.124)
111	293±20	4.5±1.6	134 (122-155)	2.3±0.2	1.29±0.43	6.34 (3.38-9.57)	0.065 (0.001-0.136)
112	243±19	2.6±1.5	162 (122-164)	2.1±0.2	2.42±0.4	6.29 (3.58-9.45)	0.083 (-0.017-0.142)
131	248±22	2.4±1.8	147 (143-190)	2.3±0.2	2.15±0.48	7.14 (3.14-11.15)	0.072 (-0.011-0.168)
141	307±20	6.8±1.6	120 (83-166)	3.1±0.1	1.65±0.42	10.88 (5.46-15.11)	0.099 (0.046-0.149)
142	284±17	3.7±1.3	169 (127-205)	2.6±0.1	1.79±0.35	6.78 (4.77-10.35)	0.069 (0.015-0.147)
143	259±18	3.4±1.4	132 (108-162)	2.9±0.1	2.14±0.38	8.96 (6.04-14.55)	0.062 (-0.017-0.118)
144	270±17	3.4±1.3	128 (111-166)	2.2±0.2	1.5±0.35	5.24 (3.3-7.88)	0.089 (0.012-0.137)
145	268±16	3.7±1.3	131 (115-157)	2.7±0.1	2.04±0.35	8.75 (5.41-12.72)	0.081 (0.026-0.145)
151	299±22	5.4±1.8	119 (68-176)	3.9±0.1	2.09±0.48	15.32 (8.3-26.98)	0.1 (0.057-0.193)
152	258±20	2.9±1.6	114 (89-148)	2.8±0.2	2.3±0.43	8.97 (5.66-17.11)	0.091 (0.015-0.175)
153	261±18	4.7±1.4	160 (113-203)	1.9±0.2	1.31±0.38	3.23 (1.89-5.54)	0.037 (-0.008-0.08)
154	258±18	2.8±1.5	106 (70-135)	2.9±0.1	2.5±0.39	11.67 (6.89-17.87)	0.108 (0.033-0.194)
156	254±17	3.5±1.4	100 (85-124)	2.6±0.2	2.37±0.37	7.25 (5.02-12.25)	0.077 (0.012-0.145)
157	264±20	3.7±1.6	120 (100-143)	2.7±0.2	2.26±0.42	8.12 (4.44-13.14)	0.089 (0.02-0.156)
158	255±19	2.7±1.5	96 (83-121)	2.8±0.2	2.17±0.4	7.34 (4.89-12.63)	0.094 (0.015-0.181)
171	273±19	3.3±1.5	145 (120-176)	2.4±0.2	1.91±0.4	5.97 (3.8-10.78)	0.071 (0.017-0.157)
172	256±20	3.7±1.6	113 (96-143)	3.5±0.1	2.72±0.42	13.19 (9.83-26.95)	0.098 (0.045-0.186)
173	278±17	4.1±1.4	147 (128-174)	2.7±0.2	1.81±0.37	7.94 (4.73-11.6)	0.087 (0.022-0.151)
182	247±18	2.4±1.5	166 (146-174)	2.2±0.2	2.18±0.39	5.55 (4.1-10.11)	0.064 (-0.004-0.157)
183	259±22	2.9±1.8	176 (143-224)	2..0±0.2	1.57±0.48	4.04 (2.56-9.3)	0.067 (-0.01-0.16)
184	267±25	3.6±1.9	187 (93-216)	2.2±0.3	1.83±0.54	5 (2.55-12.37)	0.076 (-0.008-0.164)
185	291±20	4.7±1.6	148 (129-249)	2.7±0.2	1.47±0.43	6.46 (4.06-11.78)	0.094 (0.006-0.144)
186	244±18	2.2±1.5	118 (79-154)	2.4±0.2	2.74±0.39	8.65 (5.05-12.74)	0.093 (0.005-0.179)
187	237±22	2.3±1.8	105 (98-116)	3.0±0.2	2.91±0.48	9.9 (5.24-17.1)	0.089 (0.008-0.191)
191	314±22	6.2±1.8	226 (176-286)	2.1±0.2	1.15±0.48	4.57 (2.44-8.6)	0.024 (-0.036-0.09)
192	288±23	4.3±1.8	256 (195-330)	1.9±0.3	1.26±0.51	4.32 (2.15-8.57)	0.059 (-0.028-0.138)
193	292±18	3.7±1.5	218 (178-225)	1.9±0.2	1.26±0.39	4.51 (2.73-7.24)	0.058 (-0.039-0.115)
211	323±22	9.8±1.8	209 (203-224)	3.5±0.2	1.7±0.48	7.5 (5.24-16.55)	0.059 (0.023-0.099)
212	336±22	11.1±1.8	210 (177-222)	2.8±0.2	1.04±0.48	4.75 (2.28-7.93)	0.042 (-0.009-0.067)
213	300±23	6±1.8	139 (104-193)	3.0±0.2	1.84±0.51	7.84 (4.81-16.24)	0.074 (0.009-0.129)
214	292±25	3.9±1.9	283 (170-293)	2.1±0.3	1.57±0.54	6.27 (2.84-11.72)	0.068 (-0.015-0.149)
221	283±17	4.3±1.4	167 (142-202)	2.1±0.2	1.42±0.36	4.82 (3.10-7.57)	0.056 (-0.014-0.116)
231	278±17	4.2±1.3	120 (88-147)	3.4±0.1	2.59±0.35	15.87 (11.03-24.33)	0.118 (0.061-0.191)
232	256±18	2.6±1.4	116(86-143)	3.0±0.1	2.94±0.38	10.68(6.93-16.9)	0.100(0.038-0.200
233	304±19	5.8±1.5	135(111-190)	3.5±0.1	1.90±0.4	12.93(8.22-21.21)	0.113(0.066-0.174)
241	249±21	2.6±1.7	63(33-87)	4.2±0.1	3.69±0.45	27.69(16.75-51.32)	0.134(0.032-0.228)
242	243±26	2.8±2.1		3.7±0.2	3.68±0.59	14.11(5.61-28.13)	0.084(0.002-0.188)

* Intercept and slope were derived from independent models

TABLE S4 Pearson correlation coefficients for the correlation at the population level between climatic variables and *Pinus halepensis* traits in each site, the low stress site above diagonal and the high stress site below the diagonal. N = 52 populations. Values for climatic variables are the same for both trial sites as they refer to the environmental conditions found in the area where populations where sampled. Pale blue site-to-site correlations lie on the diagonal. Pale red, ecotypic trends with growth traits. Mid – red, ecotypic trends with reproductive traits. Red, trade-offs between reproduction and growth. Green, correlations with reproductive efficiency. Significant (p-value <0.05) correlations are indicated in boldface.

	CI	Alt	Long	Lat	AP	PDQ	ATM	TMWM	TMCM	Biom	H	TSFR	ARI_b	logRA	ARI	slope	RE
CI										-0.15	-0.22	**-0.48**	**0.38**	**0.50**	**0.36**	**0.34**	0.01
Alt										**0.40**	0.25	**0.32**	-0.17	-0.25	-0.02	-0.12	0.28
Long										0.23	0.25	0.26	-0.11	-0.20	-0.03	-0.07	0.24
Lat										0.15	0.27	0.09	0.01	-0.15	0.03	-0.04	-0.10
AP										0.04	0.17	-0.01	0.04	-0.09	0.09	0.08	0.14
PDQ										0.10	0.04	**0.30**	**-0.30**	**-0.31**	-0.27	**-0.29**	-0.04
ATM										0.03	-0.13	**-0.52**	**0.36**	**0.39**	**0.40**	**0.32**	0.18
TMWM										0.06	0.05	**0.41**	**-0.41**	**-0.40**	**-0.38**	**-0.36**	-0.12
TMCM										-0.02	-0.10	**-0.53**	**0.45**	**0.46**	**0.45**	**0.40**	0.17
Biom	0.08	0.15	0.13	-0.04	0.02	-0.08	-0.03	-0.08	0.03	0.28	**0.81**	-0.03	-0.12	**-0.40**	0.17	-0.21	-0.01
H	0.03	0.20	0.23	0.06	0.14	-0.12	-0.21	-0.07	-0.07	**0.87**	0.14	0.12	-0.18	**-0.55**	0.07	-0.25	-0.12
TSFR	**-0.38**	0.28	0.19	-0.01	0.02	0.23	**-0.31**	0.23	**-0.30**	0.24	**0.43**	**0.73**	**-0.74**	**-0.69**	**-0.72**	**-0.67**	-0.33
ARI_b	0.19	0.18	-0.05	0.08	-0.10	-0.07	**0.29**	-0.13	0.24	0.08	-0.04	**-0.55**	**0.75**	**0.84**	**0.88**	**0.82**	**0.76**
logRA	0.02	0.09	-0.11	0.01	-0.17	0.12	**0.32**	0.04	0.15	**-0.54**	**-0.71**	**-0.68**	**0.63**	**0.71**	**0.67**	**0.71**	**0.60**
ARI	0.27	0.13	-0.11	0.02	-0.14	-0.06	**0.37**	-0.15	**0.30**	0.20	0.02	**-0.60**	**0.94**	**0.59**	**0.71**	**0.83**	**0.82**
slope	0.12	0.11	0.03	0.03	-0.01	-0.07	0.17	-0.08	0.14	-0.22	-0.21	**-0.59**	**0.66**	**0.49**	**0.60**	**0.50**	**0.74**
RE	-0.04	**0.52**	0.27	-0.02	-0.09	-0.03	-0.02	-0.07	0.03	0.08	0.17	**0.47**	0.12	-0.03	0.06	0.02	0.20

CI, Continentality Index; Alt, Altitude; Long, Longitude; AP, Annual mean Precipitation; PDQ, Precipitation during the driest quarter; ATM, Annual mean Temperature; TMWM, Mean Temperature of the Warmest Month; MTCM, Mean Temperature of the Coldest Month. Biom, biomass; H height; TSFR, Threshold Size for First Reproduction (female); ARI_b Accumulated Reproductive Investment after accounting for plant size (biomass); logRA logReproductive Allocation; ARI, Accumulated Reproductive Investment; slope, slope associatied with biomass in the Reproductive-Vegetative size relationship (Poisson model); RE, Reproductive Efficiency.

APPENDIX IV

Correlated effects on reproductive traits derived from domestication of a forest tree are relevant for future forest resilience. **Santos-del-Blanco L**, Alía R, González-Martínez S, Sampedro L, Lario F, Climent, J. 2013.

Correlated effects on reproductive traits derived from domestication of a forest tree are relevant for future forest resilience

Santos-del-Blanco, L.[1,2,3], Alía, R. [1,2], González-Martínez, S.C.[1,2], Sampedro[4], L., Lario, F. [5], Climent, J. [1,2]

[1] Department of Forest Ecology and Genetics. INIA-CIFOR, Ctra A Coruña km 7.5, E-28040, Madrid, Spain.

[2] Sustainable Forest Management Research Institute, www.sostenible.palencia.uva.es

[3]Department of Ecology and Evolution. University of Lausanne. Biophore Building. CH-1015, Lausanne, Switzerland.

[4] Misión Biológica de Galicia - CSIC. Carballeira, 8, E-36143, Salcedo, Pontevedra, Galicia, Spain.

[5] Vivero de Maceda. Dirección Técnica. TRAGSA. Ctra.cesa Maceda-Valdrey km 2, E-32700, Maceda, Ourense, Spain.

Corresponding autor: Santos-del-Blanco, L., Department of Ecology and Evolution. Biophore Building. University of Lausanne. CH-1015. Lausanne, Switzerland Phone. +41(0)216924247, e-mail address Luis.SantosdelBlanco@unil.ch

Running title: Correlated effects of domestication in trees

BIOLOGICAL SCIENCES: Agricultural sciences

Abstract

Trade-offs between growth and reproduction are well established across the tree of life. According to allocation theory, artificial selection aimed at increasing timber yield in forest trees is expected to indirectly alter life-history reproductive traits, diverting resources from reproduction to growth. This hypothesis was tested in a long term artificial selection experiment with *Pinus pinaster* (Maritime pine), a widespread forest tree in its early stages of domestication. Growth and reproductive traits of progenies from phenotypically selected individuals were compared with those of progenies from control trees, not subjected to selection within the same population. Female and male threshold sizes for reproduction were much higher -up to above one meter- and reproductive investment relative to size for female and male functions were lower in the selected group compared with the control group. A single event of artificial selection proved sufficient to produce a significant change in traits fundamental for individual fitness and for population persistence. Importantly, the direction of this change towards delayed and reduced reproduction, opposes natural selective pressures derived from climate change. Widespread plantation of genetically growth-improved forest trees should therefore be done with caution, as they may constraint adaptive responses to climate change in natural or naturalised future forests.

Key Words: fitness traits, genetic change, adaptation, artificial selection, domestication syndrome.

\body

Introduction

Domestication of forest trees is still in its infancy compared to cultivated crops (1). The most advanced breeding programs have been running for only a few generations, and genetic breeding tools are still to be implemented (2). However, domestication of many widespread tree species is growing, as it is the area of planted forests in response to a rising demand of timber and cellulose (3). Managed forests occupy large extensions providing goods and services with an ecological relevance transcending the landscape scale. They constitute natural or naturalized ecosystems where improved tree genetic materials often interact with natural un-improved populations. As key pieces determining structure and function or extensive terrestrial ecosystems (4, 5), even slight changes in the adaptive ability of individual trees can have broad consequences. The incipient domestication of forest trees is probably interfering with their ability to adapt to the environment, but how and how much is currently unknown.

Common target traits in forest tree breeding are growth, timber yield, stem form and physical and chemical wood properties (6, 7). Unintended correlated responses to selection, already predicted by Darwin (8), are rarely reported in the forestry literature making it difficult to define a “domestication syndrome” for forest trees (9). Correlated responses to selection or unintended effects of management are well defined for other organisms. Fisheries and fish-farming have provided several paradigmatic examples(10). For instance, over-exploitation of fisheries has driven the evolution of slow growing and precocious individuals (11), revealing fundamental genetic correlations between growth and reproductive life-history traits. Life-history traits lay at the base of adaptive processes and are closely linked to individual fitness, effective population size and thus to population persistence. For example, micro-evolutionary changes of plant size at reproduction or reproductive phenology in response to climate change are well documented (12, 13), and a global trend towards earlier reproduction has been described (14).

Interestingly, monoecious forest trees allow the separate study of female and male sexual functions in the same individuals. Female reproduction is typically more costly in plants (15) and a different effect of domestication on male and female reproduction could reflect differential costs of reproduction (16). Therefore, separating investment in female and male reproductive functions, particularly at the onset of reproduction, is key to understand constrains to adaptive evolution for tree life-history traits. Size at maturity and reproductive allocation are key fitness traits defining contrasting life histories in all kind of organisms (17). As those traits imply shifting resource allocation between vegetative growth and reproduction, genetic breeding for allocation to growth is predicted to impact them.

Specifically, based on allocation theory, a decreased investment in reproduction would be expected if selection is focused solely on growth. Here we provide evidence for this hypothesis, for the first time in a forest tree, with a long term artificial selection experiment of *Pinus pinaster* (Maritime pine), a monoecious conifer widespread in southwestern Europe in its early stages of domestication. Growth and reproductive traits of the progenies from phenotypically selected individuals were compared with those from control trees, not subjected to selection within the same population, growing together in a common garden in North Spain. Indirect effects of selection for high allocation to stem growth delayed the ontogenetic program for reproduction in maritime pine. More specifically, we tested the hypothesis that progenies from selected trees would reproduce at larger sizes and/or produce fewer cones at a given size. Besides, considering the expected greater cost in terms of growth for female reproduction, we hypothesised that female function of progenies selected for high stem growth should show a greater indirect change, as compared to male function.

Results

Direct and indirect effects of domestication Progenies of trees selected for timber yield (hereafter ‘selected group’) were on average 11.7 cm higher at age 10 than those sampled at random from the wild base population (hereafter ‘control group’) (Table 1). As expected, phenotypic selection on mother trees lead also to better stem form scores in the selected than in the control group ($P < 0.001$; Table 1). Trees from the selected group were consistently taller than those in the control group for juvenile, vegetative, female and cosexual groups (all $P < 0.05$; Fig. 1). However, height of male trees did not significantly differ between groups and showed large variability within groups (Fig. 1, Table S1).

Selection for timber yield established large differences in reproductive traits between the selected and control group, leading to delayed reproduction both in age and size terms in the former (Table 1). The proportion of reproductive trees was higher in the control group (66%) than in the selected one (51%) (X^2_1 14.3 $P < 0.001$). In both groups, trees started their reproductive phase most commonly as females, followed by cosexuals and then males (Table S1). Among reproductive individuals, the selected group had a lower proportion of cosexuals (36%) (X^2_1 13.2 $P < 0.001$) and higher proportion of males (24%) (X^2_1 14.3 $P < 0.007$). compared to the control group (54% and 13% respectively) (Table S1).Differences in reproductive traits between both selected and control groups were highly significant ($p<0.001$, Table 1). The selected group had significantly greater female and male threshold size for first reproduction

(TSFR), and showed a consistently lower allocation to reproduction at a given size (reproductive – vegetative allocation, female and male R-V; Table 1). The maximum change in TSFR was recorded for the female function, which increased in 106.9 cm in height in the selected population (Fig. 2). In the control group, female TSFR was significantly lower than male TSFR (Fig. 2, Table S1), but both parameters did not differ in the selected group as selection had a greater effect on female TSFR (Fig. 2, Table S1). The control group showed the smallest reproductive individual and significantly lower male TSFR than any of the 31 selected progenies. Regarding the female TSFR, all but two selected families showed point estimates above the control, but wider credible intervals made those differences non-significant for about half of the selected families (Fig. S1).

Quantitative genetic parameters of growth and reproductive traits

Models were improved by including genetic relatedness for height (Δ Deviance Information Criterion, DIC 7.3). Additive genetic variance for height was very low compared to total variance. Thus its heritability was centred at zero, although with wide credible intervals (Table 2). Heritability for stem form was 0.13 and significantly different from zero. Male and female reproductive functions expressed sharp differences in genetic control between selected and control groups. While narrow sense heritabilities for female TSFR and relative reproductive investment (R-V) were very high (> 0.53), those for male function were indistinguishable from zero (Table 3).

In the selected group we found significant negative genetic correlations between female TSFR and female relative reproductive investment (r = - 0.82; Table S2a) indicating that precocious trees were also more prolific at this stage. Male and female thresholds for first reproduction were positively correlated (r = 0.39) but not the relative investment in male and female functions (Table S2a). Within the 31 selected families, we only found evidence of genetic trade-offs between height and reproduction for male function, but not for female function (Table S2a). This showed that taller families tended to have a delayed male size for first reproduction (r = 0.38; Table S2).

Discussion

In this study we show how a single event of artificial selection aiming at domestication of a forest tree interfered with adaptive potential of a widespread conifer, *Pinus pinaster*. Our study is, to our knowledge, the first to report on the correlated effects in reproductive life-history traits caused by selection for increased timber yield in a forest tree.

In our experiment, phenotypic selection in natural stands pursued an ideotype of tall, thick straight trees with short horizontal branches and high apical dominance. There was a positive phenotypic correlation between growth and absolute female and male reproduction (Table S2). However, due to “unconscious selection” (8) of traits genetically correlated with high stem allocation, female and male threshold sizes for reproduction were both increased and male and female reproductive allocation decreased in the selected group, revealing an underlying negative genetic correlation between allocation to growth and reproduction in the base population (18). Differences in ontogenetic development were also reflected in a higher proportion of reproductive individuals in the control group compared to the selected group. Considering actual growth rates, two to three years would be needed for the selected group to achieve a similar reproductive status as the control group. Thus indirect selection effects on male and female reproductive traits caused delayed reproduction both in age and size terms.

Altogether, direct and indirect effects of selection contributed to define a “domestication syndrome” for timber selection in trees (19).

A few artificial selection experiments in plants have focused on reproductive life-history traits, such as the threshold size for reproduction (20, 21) and flowering phenology (22, 23), revealing abundant additive genetic variation for these traits. Narrow-sense heritabilities reported in those studies are moderate, and close to the lower bound of our estimates for female reproduction in *Pinus pinaster*. Numerous studies in forest trees, in particular in conifers, also report high broad-sense heritabilities for cone and pollen production in clonal seed orchards, usually in the range of 0.25-0.80 (24).

Female reproduction has been usually found to be more costly than male reproduction (15). In agreement with this idea, we found smaller average size for protogynous than protrandrious trees in maritime pine, indicative of higher female reproductive costs (Fig 1). Despite we were not able to detect negative genetic correlations between reproduction and growth in the selected group, our results evidence that strong negative genetic association between growth and early reproductive effort existed in the native original population subjected to selection. At the genetic level, greater costs of female reproduction could be thought of as stronger negative correlations for growth (25), thus, a more intense indirect change of the threshold size for female reproduction, compared to the male threshold, fitted our expectations. The change due to a single selection event implied an increase close to half of the species range of the threshold size for female reproduction and similar to the species male range of the threshold size for male reproduction (1.0 m female, 0.8 m male). Our results partially support greater genetic costs of female reproduction, as the change in female function was more important in absolute terms (greater change in female function), but not in relative terms (greater change in male function).

Implications for adaptation of forest trees and forest management in a changing environment

The observed correlated genetic effects of breeding for timber production on reproductive life-history traits are closely linked to individual fitness, but may also impact effective population size at a given age, and thus on population "*in situ*" persistence and resilience against disturbance. Climate warming has led to dramatic increases of severe droughts and more frequent forest fires in many areas such as the Mediterranean basin (26). Under forecasts of increased disturbance frequency, and thus reduced life expectancy, precocious trees could be naturally selected for (27), as mounting evidence indicates for non-woody species (12–14, 27). Indeed, under climate change scenarios, both survival *in situ* and potential for migration and extended gene flow rely on enhanced early allocation to reproductive function(28).

Present-day population differentiation along climate clines can also help us to infer responses to changing climate. In that sense, genetically-based phenotypic clines are common in forest trees (4). Particularly, poorer growth conditions of origin have been related to earlier female reproduction in several pine species, including *P. pinaster* (27, 28, 29), suggesting that populations facing increased environmental stress would benefit from enhanced early reproduction. However, correlated genetic effects observed after a single artificial selection event for increased timber yield lie in the opposite direction of the putatively adaptive trend observed in natural populations evolving in the wild. In a context of changing environment, and taking into account the long life cycles and extensive pollen dispersal, intensive breeding of native forest species could be compromising the adaptation of managed forest and also of natural surrounding forests interacting genetically with them.

The impact of forest management on genetic resources of forest trees is a declared matter of concern for conservation practices (29). Silvicultural activities such as selective logging (30), clear cuttings (31) and seed tree regeneration (32) have not been not found to strongly impact, within limits, forest stands in terms of neutral genetic diversity. Also, assuming a positive correlation between neutral and adaptive diversity, production of genetically improved material in seed orchards ideally seeks a maximization of genetic diversity as assessed by neutral markers (33). However, breeding, by definition implies genetic changes that are reflected in phenotypes. The fitness consequences of deployment of material improved for increased allocation to growth (this study) or selective harvesting in natural tree stands (30, 34) can be of great importance, as has also been demonstrated in fisheries and fish-farming (10). Indeed, growth traits should not be considered alone as adaptive traits of forest trees (35, 36). Due to long life cycles, extensive distribution areas and key environmental relevance, reproductive traits and tolerance to biotic and abiotic stressors must be also taken into account to assess adaptation of forest trees in the long term. An excessive focus on growth traits might imply a correlated negative response in reproductive investment, with detrimental consequences on forest regeneration and long-term survival, in particular under environmental stressful conditions.

In spite of impressive recent development, with first full-genome draft sequences underway, molecular genetic tools are still not accurate enough to describe adaptive variation in forest trees, particularly in species with huge genomes like pines. Screening for differences between domesticated and wild populations of forest trees might be a powerful tool to unveil the genetic basis of adaptation to both natural and human environments (19) and further improve domestication (2). Meanwhile, quantitative genetic tools cannot be dismissed for policy guidance in the sustainable management of forest genetic resources aimed at ensuring their evolutionary potential.

Acknowledgements

This research has been supported by projects PSS-310000-2008-4, RTA 2011-00016-00-00 and AGL2012-40151-C03-02. The *Pinus pinaster* Montaña de Soria-Burgos genetic breeding program is part of a collaborative work between former DGCONA (currently in Spanish Ministry of Agriculture, Food and Environment), ETSIM-Universidad Politécnica de Madrid, INIA and Junta de Castilla y León. L. Gil, S. Martín, E. Notivol and O. Cisneros are thanked for their collaboration in the project. B. Sada, E. Ballesteros, A. Calle and F. del Caño assisted in field work. The authors declare no conflict of interest.

LITERATURE CITED

1. Neale DB, Kremer A (2011) Forest tree genomics: growing resources and applications. *Nat Rev Genet* 12:111–122.

2. Harfouche A et al. (2012) Accelerating the domestication of forest trees in a changing world. *Trends Plant Sci* 17:64–72.

3. Food and Agriculture Organization of the United Nations (2010) *Global Forest Resources Assessment 2010* (FAO Forestry Paper 163).

4. Petit RJ, Hampe A (2006) Some evolutionary consequences of being a tree. *Annu Rev Ecol Syst* 37:187–214.

5. Whitham TG et al. (2006) A framework for community and ecosystem genetics: from genes to ecosystems. *Nat Rev Genet* 7:510–523.

6. Gapare WJ et al. (2011) Genetic parameters and provenance variation of Pinus radiata D. Don. “Eldridge collection” in Australia 1: growth and form traits. *Tree Genet Genomes* 8:391–407.

7. Lepoittevin C et al. (2011) Genetic parameters of growth, straightness and wood chemistry traits in *Pinus pinaster*. *Ann For Sci* 68:873–884.

8. Darwin C (1859) *On the origin of species, or the preservation of favoured races in the struggle for life* (Murray, J., London, UK).

9. Cornelius J (1994) The effectiveness of plus-tree selection for yield. *Forest Ecol Manag* 1127:23–34.

10. Hutchings JA, Fraser DJ (2008) The nature of fisheries- and farming-induced evolution. *Mol Ecol* 17:294–313.

11. Olsen EM et al. (2004) Maturation trends indicative of rapid evolution preceded the collapse of northern cod. *Nature* 428:932–935.

12. Anderson JT, Inouye DW, McKinney AM, Colautti RI, Mitchell-Olds T (2012) Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. *Proc R Soc London Ser B* 279:3843–3852.

13. Franks SJ, Sim S, Weis AE (2007) Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proc Natl Acad Sci U S A* 104:1278–1282.

14. Munguía-Rosas MA, Ollerton J, Parra-Tabla V, De-Nova JA (2011) Meta-analysis of phenotypic selection on flowering phenology suggests that early flowering plants are favoured. *Ecol Lett* 14:511–521.

15. Obeso JR (2002) The costs of reproduction in plants. *New Phytol* 155:321–348.

16. McDowell SCL, McDowell NG, Marshall JD, Hultine K (2000) Carbon and nitrogen allocation to male and female reproduction in Rocky Mountain Douglas-fir (*Pseudotsuga menziesii* var. *glauca*, *Pinaceae*). *Am J Bot* 87:539–546.

17. Roff DA (1992) *The evolution of life histories: theory and analysis* (Chapman and Hall, New York, USA).

18. Schluter D (1996) Adaptive radiation along genetic lines of least resistance. *Evolution* 50:1766–1774.

19. Ross-Ibarra J, Morrell PL, Gaut BS (2007) Plant domestication, a unique opportunity to identify the genetic basis of adaptation. *Proc Natl Acad Sci U S A* 104:8641–8648.

20. Wesselingh RA, de Jong TJ (1995) Bidirectional selection on threshold size for flowering in *Cynoglossum officinale* (hound’s-tongue). *Heredity* 74:415–424.

21. Wesselingh RA, Klinkhamer PGL (1996) Threshold size for vernalization in *Senecio jacobaea*: genetic variation and response to artificial selection. *Funct Ecol* 10:281–288.

22. Burgess KS, Etterson JR, Galloway LF (2007) Artificial selection shifts flowering phenology and other correlated traits in an autotetraploid herb. *Heredity* 99:641–648.

23. Van Dijk H (2009) Evolutionary change in flowering phenology in the iteroparous herb *Beta vulgaris* ssp. *maritima*: a search for the underlying mechanisms. *J Exp Bot* 60:3143–3155.

24. Prescher F et al. (2007) Female fertility variation in mature *Pinus sylvestris* clonal seed orchards. *Scand J For Res* 22:280–289.

25. Reznick D (1985) Costs of reproduction: an evaluation of the empirical evidence. *Oikos* 44:257–267.

26. Lindner M et al. (2010) Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. *Forest Ecol Manag* 259:698–709.

27. Shaw RG, Etterson JR (2012) Rapid climate change and the rate of adaptation: insight from experimental quantitative genetics. *New Phytol* 195:752–765.

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

29. Lefèvre F (2004) Human impacts on forest genetic resources in the temperate zone: an updated review. *Forest Ecol Manag* 197:257–271.

30. Borges Silva M, Kanashiro M, Yamaguishi Ciampi A, Thompson I, Magno Sebbenn A (2008) Genetic effects of selective logging and pollen gene flow in a low-density population of the dioecious tropical tree *Bagassa guianensis* in the Brazilian Amazon. *Forest Ecol Manag* 255:1548–1558.

31. Valbuena-Carabaña M, Gonzaléz-Martínez SC, Gil L (2008) Coppice forests and genetic diversity: A case study in *Quercus pyrenaica* Willd. from Central Spain. *Forest Ecol Manag* 254:225–232.

32. Savolainen O, Kärkkäinen K (1992) Effect of forest management on gene pools. *New Forests* 6:329–345.

33. Lindgren D, Cui J, Son SGU, Sonesson J (2004) Balancing seed yield and breeding value in clonal seed orchards. *New Forests* 28:11–22.

34. Sokol K (2004) Impacts of long-term diameter-limit harvesting on residual stands of red spruce in Maine. *North J Appl For* 21:69–73.

35. Koskela J et al. (2013) Translating conservation genetics into management: Pan-European minimum requirements for dynamic conservation units of forest tree genetic diversity. *Biol Conserv* 157:39–49.

36. Lefèvre F et al. (2013) Considering evolutionary processes in adaptive forestry. *Ann For Sci*:d.o.i. 10.1007/s13595–013–0272–1.

37. Bucci G et al. (2007) Range-wide phylogeography and gene zones in *Pinus pinaster* Ait. revealed by chloroplast microsatellite markers. *Mol Ecol* 16:2137–2153.

38. Zas R, Sampedro L, Prada E, Fernández-López J (2005) Genetic variation of *Pinus pinaster* Ait. seedlings in susceptibility to the pine weevil *Hylobius abietis* L. *Ann For Sci* 62:681–688.

39. Santos-del-Blanco L et al. (2010) Variation of early reproductive allocation in multi-site genetic trials of Maritime pine and Aleppo pine. *Forest Syst* 19:381–392.

40. Raymond CA, Cotterill PP (1990) Methods of assessing crown form of *Pinus radiata*. *Silvae Genet* 39:67–71.

41. Galera R et al. (1997) *Manual de selección de masas productoras de semillas. Evaluación de caracteres*. (Instituto Nacional de Investigacion y Tecnología Agraria y Alimentaria, Madrid, Spain).

42. Montero G, Ruiz-Peinado R, Muñoz M (2005) *Producción de biomasa y fijación de CO2 por los bosques españoles* (Instituto Nacional de Investigacion y Tecnología Agraria y Alimentaria, Madrid, Spain).

43. Nakagawa S, Schielzeth H (2010) Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biol Rev Camb Philos Soc* 85:935–956.

44. Hadfield JD (2010) MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *J Stat Soft* 33:1–22.

45. Kotze DJJ, O'Hara RB (2010) Do not log-transform count data. *Meth Ecol Evol* 1:118–122.

46. Mendez M, Karlsson PS (2004) Between-population variation in size-dependent reproduction and reproductive allocation in *Pinguicula vulgaris* (*Lentibulariaceae*) and its environmental correlates. *Oikos* 104:59–70.

47. Santos-del-Blanco L, Climent J, González-Martínez SC, Pannell JR (2012) Genetic differentiation for size at first reproduction through male versus female functions in the widespread Mediterranean tree *Pinus pinaster*. *Ann Bot* 110:1449–1460.

48. Spiegelhalter DJ, Best NG, Carlin BP, van der Linde A (2002) Bayesian measures of model complexity and fit. *J Roy Statist Soc Ser B* 64:583–639.

49. Reid JM, Arcese P, Sardell RJ, Keller LF (2011) Additive genetic variance, heritability, and inbreeding depression in male extra-pair reproductive success. *Am Nat* 177:177–187.

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

Methods

Studied species and selection experiment

Pinus pinaster is a Western Mediterranean monoecious conifer, with a disjoint distribution in South-Western France, Iberian Peninsula (Portugal and Spain), Italy and North Africa (Morocco, Algeria and Tunisia). A complex phylogeographic history has been postulated for this species based on different molecular markers (37), and significant within population variation has been reported for growth, wood properties and stem form (7), defence (38), and also for reproductive life-history traits (39).

A long term selection experiment aimed at increasing timber yield was carried out in the 'Montaña de Soria-Burgos' provenance area, Central-North Spain, during the 1990's. Thirty one plus trees were selected in natural stands according to their superior phenotypes for growth and stem form, i.e. timber production. Altogether, less than 1 out of 10,000 trees were selected as plus trees. Then, in 2001, their progeny was planted in a common garden together with the progeny from an unselected control lot randomly selected within the same population. The common garden was placed in the vicinity of the surveyed stands (Latitude 41º 55' 15" N; Longitude 3º 11' 35" W; 1,153 m a.s.l.; Fig. 3). A detailed description of the selection protocol and common garden design can be found in the Supplementary Material (ST1).

A total of 1,087 trees were included in this study, 833 belonging to progenies of selected trees (selected group) and 254 to progenies of unselected trees (control group). Mortality at ten years was very low (2.4%) and did not differ between selected and control groups ($X^2_1 = 0.266$, $P = 0.606$). Some trees showing damage caused by insects or pathogens were excluded from the study, and thus the final sample size was 868 trees.

Field measurements and variable description

Two measurements were carried out in late May in 2009 and 2011, when the trees were eight and ten years old, respectively. In both dates, we measured tree height below the elongating bud (this is, previous winter height) and diameter at breast height (for those trees $\geq$ 1.3 m) and female cones were counted for the following cohorts: female strobili (red-coloured and placed at the end of the current year flush), one-year old immature conelets and two-year old and above mature cones (SFig. 2). Male (pollen) cone abundance was categorically scored from 0 (absent) to 3 (very abundant). Stem form and branching habit was assessed through an ordinal scoring from 1 to 6 in 2009 (40). A higher score implied high apical dominance and straight stem with few branches, inserted at high angles (closer to horizontal position) (41). Total biomass was estimated from diameter at breast height using standard formulae in Montero *et al.* (2005).

Considering its reproductive status, each tree was classified as either reproductive or non-reproductive. According to their first reproductive event, trees were assigned to female (F, those that started their reproductive life as females, i.e. protogynous), male (M, those that started their reproductive life as males, i.e. protrandious) or cosexual (C, those that started their reproductive life with both female and male strobili) groups. Non-reproductive trees were further classified as juveniles (J, size below the family or group-specific smallest reproductive individual) or vegetative (V, size above the family or group-specific smallest reproductive individual).

Statistical analyses

We used Bayesian approaches for fitting quantitative genetic models as they provide more flexibility and accuracy in estimating errors of model parameters and their combinations, e.g. heritability, than Maximum Likelihood procedures (43). We computed posterior mode and 95% credible intervals (95% CIs) for fixed effects, variance components, threshold sizes for reproduction, heritabilities and phenotypic correlations across a variety of models. A detailed description of priors used can be accessed in the Supplementary Material (ST2). Analyses were run in R, version 2.15.2 (R Development Core Team, Vienna), using the package MCMCglmm, version 2.01 (44).

Tree height at ten years was modelled as normal distributed traits with Gaussian errors including block as a random factor. Tree form was modelled as an ordinal trait with a generalised model with probit link and residual variance (V_R) fixed to one by convention. Stem form model included block as a random factor. Fixing V_R to a particular value affects proportionally all variance components in a model, and thus heritability estimates are independent of the V_R value.

Threshold size for first reproduction (TSFR) was calculated as minus the result of dividing intercept by slope estimates from a generalised binomial model with logit link, where categorical reproduction (present or absent) was the response variable. Height was included as a covariate (20). V_R was fixed to one in binomial models by convention. Cumulative quantitative female reproduction, i.e. number of cones produced throughout a tree's life, was modelled as a Poisson generalised model with log link, using log biomass as a continuous predictor (Female R-V). This was equivalent to standard log-log allometric Reproductive vs. Vegetative size (R-V) regressions but benefited from the inclusion of zeroes in the response variable (non-reproductive individuals), otherwise commonly discarded or transformed (45). Only data from non-juvenile trees were used in R-V models in order to avoid zero inflation (46). Male reproduction at ten years, an ordinal trait, was modelled analogously to stem form, but including tree log biomass as a covariate (Male R-V). Inclusion of size (height or biomass) as a covariate in TSFR and R-V models accounts for small scale environmental variation in common gardens (47), and so, block effects were not considered.

Direct and indirect effects of early domestication

We first tested for differences between progenies from the selected (hereafter “selected group”) and non-selected (hereafter “control group”) mother trees. We analysed height, stem form, threshold for first reproduction (TSFR), and reproductive-vegetative (R-V) size relationships for female and male reproduction by fitting independent univariate models and using fixed effects (selected – control) 95% credible intervals (95% CIs) to evaluate the significance of their difference. Average sizes per reproductive group (juvenile, vegetative, female, male or cosexual) were also calculated. For TSFR and R-V models we evaluated the significance of separate additive (intercept) and multiplicative (slope) selection effects.

Genetic control and correlation among traits

To evaluate the quantitative genetic basis of variation in traits affected by early domestication, we fitted ‘animal models’ to data from the selected group to estimate additive genetic variance. A detailed description of model specification and estimation of quantitative genetic parameters can be accessed in the Supplementary Material (ST3). As 95% CIs for variance components cannot overlap 0, it is not possible to test the null hypothesis of zero variance. Instead, significance of variance components was assessed by means of Deviance Information Criteria (DIC), comparing DIC values of nested models (48). However, this was done only for Linear Mixed Models (height) as interpretations of DIC in Generalised Linear Mixed Models with latent variables may not be clear. In these cases (binomial, Poisson and ordinal models), parameter posterior distribution was used to illustrate the magnitude of variance components (49).

Genetic correlations were estimated as the Pearson's correlation between traits for family means derived from mixed models where family was coded as random (50). Phenotypic correlations were estimated by fitting bivariate models to data from the selected and control groups.

TABLES

TABLE 1. Effects of a single artificial selection event aimed at improving growth and timber yield in a *Pinus pinaster* wild population on growth and reproductive function of the following generation. Selected and unselected groups were grown in a common garden close to the original population in Central-North Spain. Effect size and 95% credible interval, CI, are shown. For models where a covariate was used (height or biomass), we indicate both add., additive (intercept) and mult., multiplicative (slope) effects of selection. Results are reported on the corresponding latent linear scale.

Trait		**Effect size**	**95% CI**		**p-value**
Height		11.7	1.9	27.7	**0.024**
Stem form		0.375	0.218	0.618	**<0.001**
Female TSFR	add.	-1.242	-1.452	-0.893	< **0.001**
	mult.	-0.003	-0.006	0.001	0.198
Female R-V	add.	-1.627	-1.918	-1.197	**<0.001**
	mult.	0.123	-0.128	0.301	0.45
Male TSFR	add.	-1.616	-1.957	-1.272	< **0.001**
	mult.	-0.002	-0.007	0.003	0.422
Male R-V	add.	-0.886	-1.278	-0.605	**<0.001**
	mult.	-0.073	-0.274	0.176	0.764

TSFR, Threshold size for first reproduction; R-V, relative Reproductive – Vegetative effort obtained from a Poisson (female) or ordinal (male) model with number of cones (female) or qualitative pollen production (males) as the response variable and log(biomass) as a covariate

TABLE 2. Posterior modes (h^2) and credible intervals (95% CI) of narrow sense heritabilities for growth and reproductive traits recorded on *Pinus pinaster* trees. Values for stem form, TSFR and RV are reported on the corresponding latent linear scale.

trait	**h^2**	**95% CI**	
H	0.00	0.00	0.19
Stem form	0.13	0.03	0.36
Female TSFR	0.53	0.35	0.91
Female R-V	0.73	0.42	0.81
Male TSFR	0.00	0.00	0.24
Male R-V	0.00	0.00	0.43

TSFR, Threshold size for first reproduction; R-V, relative Reproductive – Vegetative effort obtained from a Poisson (female) or ordinal (male) model with number of cones (female) or qualitative pollen production (males) as the response variable and vegetative size as a covariate.

FIGURE 1. Effect of a single event of artificial selection for growth and timber yield on the height of the progeny from selected *Pinus pinaster* trees compared to an unselected control group. Bars represent average height at age 10 years for selected progenies (dark bars: N = 656) and the control, unselected group, representing the base population mean (grey bars; N = 195). Results are presented per each expressed sexual function. Error bars represent credible intervals. 'Vegetative' refers to those non-reproductive trees taller than the smallest reproductive tree for a given group.

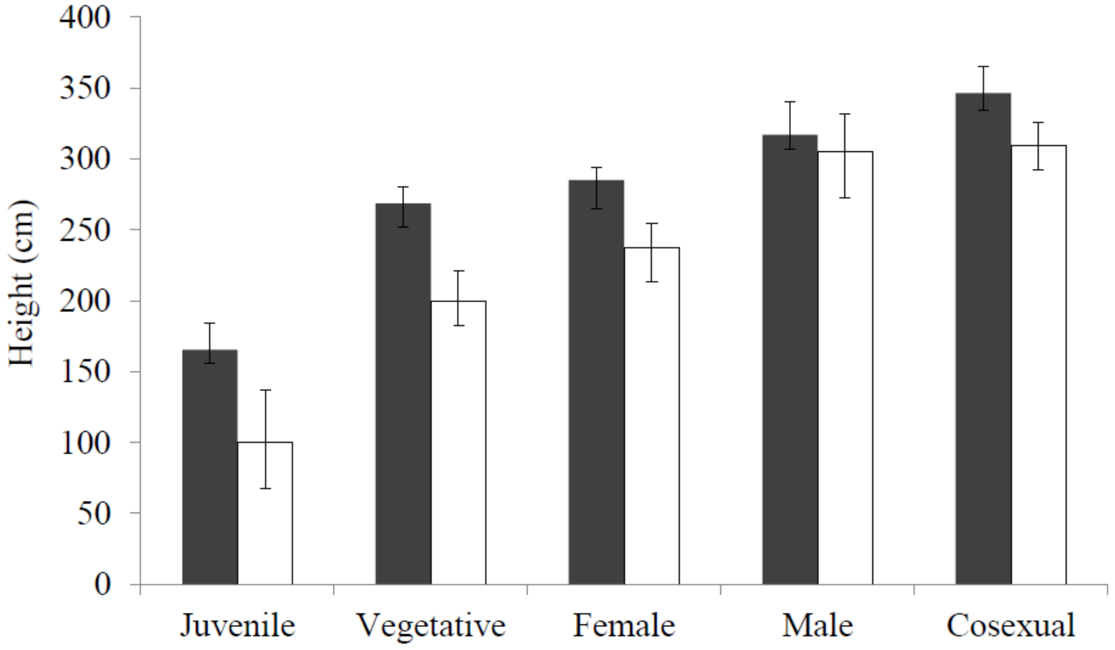


FIGURE 2. Comparison of height density distribution and threshold size (height) for first reproduction between *Pinus pinaster* progenies of trees either selected (S) or not selected (C, control) for timber production after one generation. Bell-shaped lines represent height probability distribution at age 10 yrs. Control group, orange lines; selected group, blue lines. Vertical lines represent threshold sizes for reproduction. Solid line, female function; broken line, male function. 95% Credible intervals for threshold sizes are represented by horizontal lines. Arrows show changes in threshold size for reproduction in male and female function due to selection. Control group, left; selected group, right.

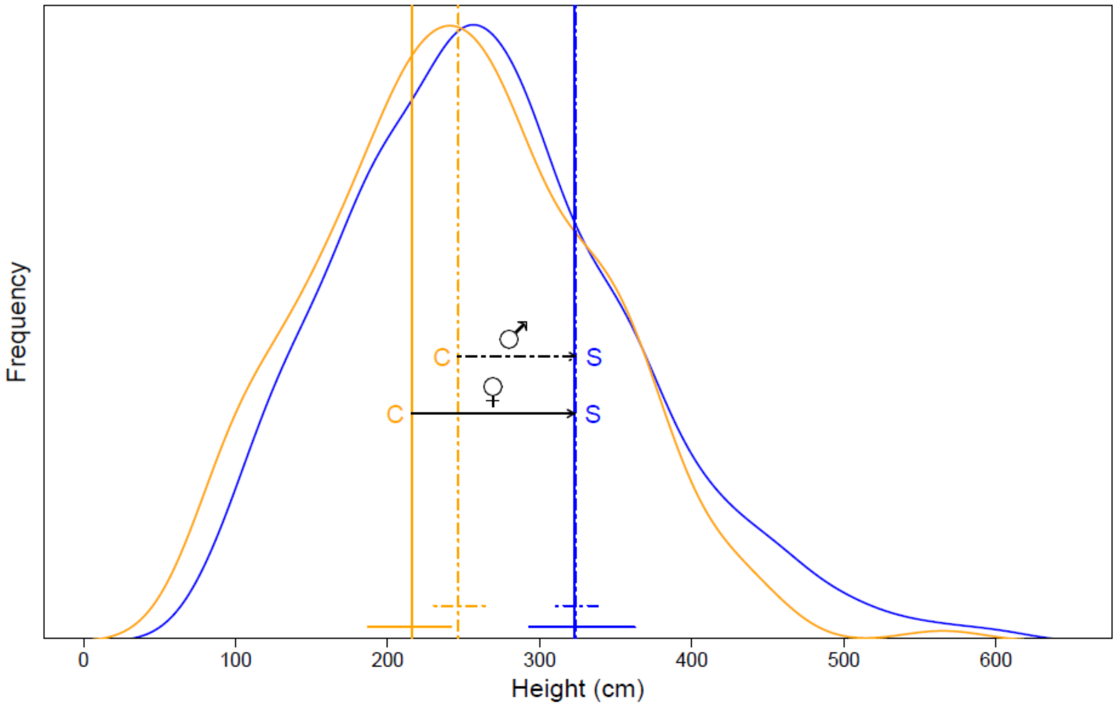
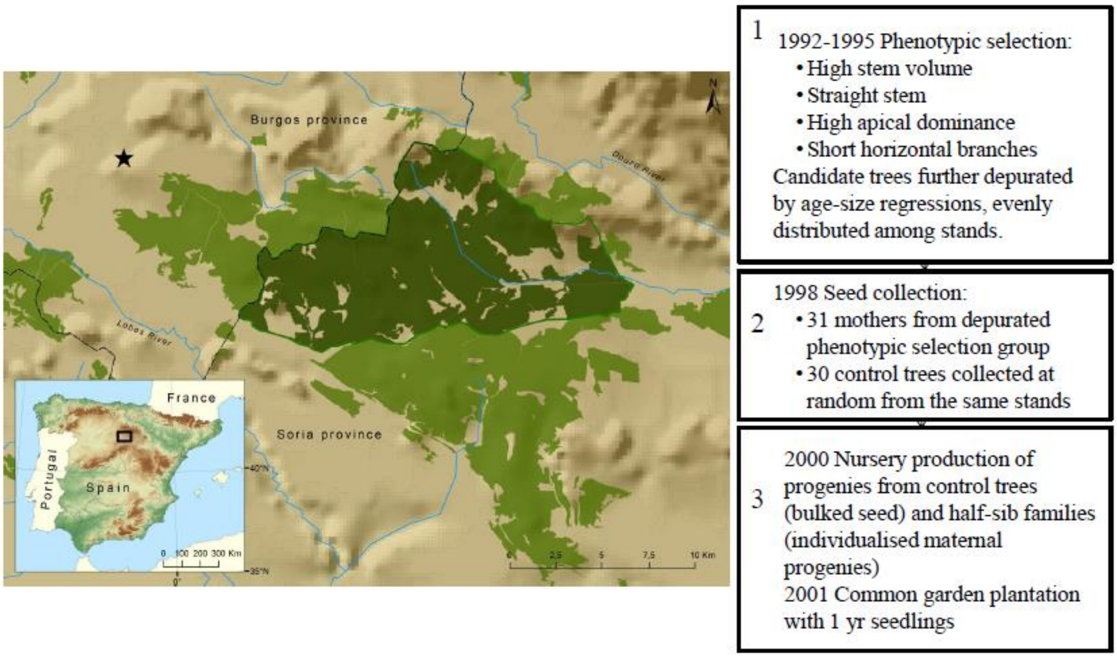


FIGURE 3. Location of the *Pinus pinaster* region of provenance Montaña de Soria-Burgos (light green), and the forest stand (dark green) where artificial selection for growth and timber yield was carried out. Boxes 1-3 describe the breeding program from phenotypic selection to common garden establishment. Star denotes the location of the common garden.



1 1992-1995 Phenotypic selection:
- High stem volume
- Straight stem
- High apical dominance
- Short horizontal branches

Candidate trees further depurated by age-size regressions, evenly distributed among stands.

2 1998 Seed collection:
- 31 mothers from depurated phenotypic selection group
- 30 control trees collected at random from the same stands

3 2000 Nursery production of progenies from control trees (bulked seed) and half-sib families (individualised maternal progenies)
2001 Common garden plantation with 1 yr seedlings

Correlated effects on reproductive life-history traits derived from domestication of a forest tree are relevant for future forest resilience

Santos-del-Blanco, L., Alía, R., González-Martínez, S.C., Sampedro, L., Lario, F., Climent, J.

SUPPLEMENTARY INFORMATION

SUPPLEMENTARY TEXT 1. Phenotypic selection in the wild and sampling

A breeding program for the *Pinus pinaster* region of provenance Montaña de Soria-Burgos in Northern Spain (1) was designed in the 1990's. The first stage involved phenotypic selection of plus trees growing in natural stands. Best valued traits were high stem volume growth, straight stem, high apical dominance and short branches inserted at high angles (closer to horizontal position) (2). Trees were selected between 1992 and 1995 by subjective comparison with neighbouring individuals. In order to avoid biasing the selection of best phenotypes towards favourable sites, a geographical subdivision of the stands was made, such that best scoring individuals of all subdivisions were chosen for progeny testing. All individuals were above 28 years old (at breast height), between 20 and 28 m tall and between 35 and 60 cm of diameter over bark at breast height. Some candidate plus trees had to be discarded as they did not produce enough seed for progeny testing. Considering the area of sampled stands and the average tree density, less than 1 out of 10,000 trees were selected as plus trees. The history of forest management in the area and the age of all mother trees allowed us to discard seed transfers from unknown origins.

Ripe cones of 31 selected plus trees were collected in autumn 1998. Also, a control seed lot was built the same year from cones collected randomly from 30 trees felled during forest management activities. This ensured that control seed lot was representative of the average phenotype of the study stand, avoiding a frequent bias of commercial seed lots collected from standing trees, therefore over-representing highly reproductive mothers (3). The control seed lot was built in the same study area where plus tress were selected. This ensured that comparisons were not biased by fine-scale local adaptation and that pooled data from these 31 plus tree progenies gathered a priori a similar genetic variability than the control seed lot.

All cones harvested from selected and control trees were processed together, including their opening in an oven at 60º C after being air-dried. Seeds were extracted manually, and kept dry at 4ºC until sowing in 2000. Seedlings were produced in 250 cc containers with a mixture of fertilized peat and vermiculite following best nursery practices for the species and planted in the common garden in 2001. Plants were placed at the intersections of a 3 x 3 m grid following a resoluble alpha design with 28 complete blocks and single-tree plots.

The common garden for progeny testing was set in a former marginal arable land, within the area where phenotypic selection was carried out (Figure 1). The plot is placed at 1.153 m above sea level, with approximately 10% of slope facing eastwards. The mother rock is siliceous sandstone and conglomerates, the predominant soil type in the ‘Montaña de Soria-Burgos’ area, but soil impoverishment and erosion from inadequate previous farming practices were evident.

SUPPLEMENTARY TEXT 2. Prior specification for statistical models

Default priors for fixed effects followed a normal distribution centred around cero and had a large (10^8) variance. Non-informative diffuse proper priors were used for variance components. By default, we used inverse-Wishart priors with parameter V = 1 and a degree of belief of 0.002. However, given the null or very low value of some variance components, parameter expanded priors were also used in order to improve chain mixing. For height mixed model, the working parameter prior was normally distributed, with a mean of 0 and a variance of 1000, and the location effect prior was inverse-Wishart distributed, forming a scaled non-central F distribution, with a degree of belief parameter and a limit variance of one. In the case of binomial (male and female threshold size for reproduction) and ordinal models (stem form and male reproduction) residual variance was fixed to 1, causing inflation in heritability estimates due to high additive genetic variances driven by long tails in prior distribution. In order to keep additive genetic variance within expected boundaries, we used a Chi-square distributed prior with one degree of freedom for variance components (4). All MCMC analyses were run yielding in every case a total of 1,000 data points for each analysis and ensuring autocorrelations below 0.1.

SUPPLEMENTARY TEXT 3. Model specification and estimation of quantitative genetic parameters

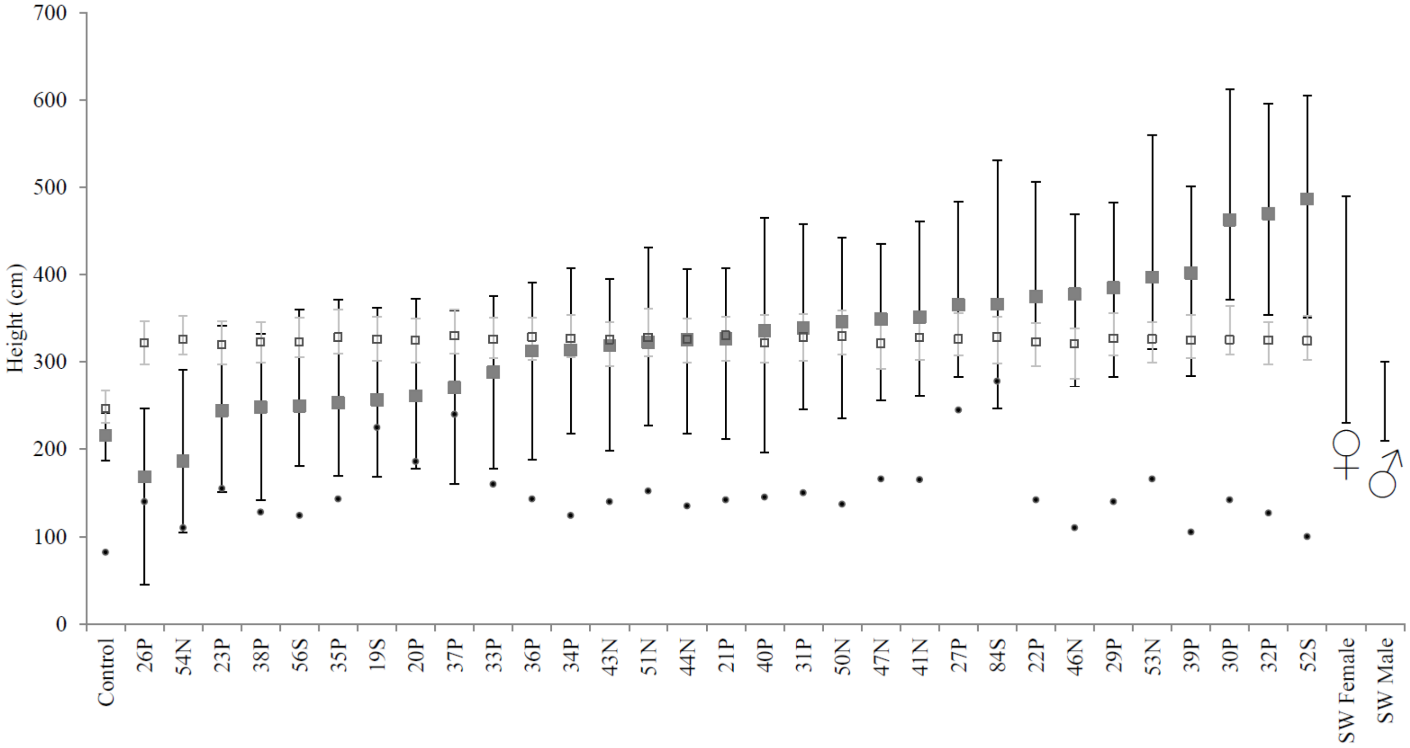
Animal models use pairwise coefficients of relatedness (0.25 between half-sibs) among individuals to define a matrix that is proportional to the variance-covariance structure of additive genetic effects. In these models, individual (animal) effects estimate the variance in phenotypes that can be related to additive genetic variance (V_A). For stem form and TSFR generalised models, an additional individual effect was added to account for overdisperssion, i.e. individual variance (V_I) larger than that specified by the model link due to causes other than additive genetic effects.

The heritability of normally distributed growth variables (height and biomass) was calculated by dividing V_A by total variance (V_A + residual variance, V_R). Latent scale heritabilities can be interpreted as the heritability of a continuous, directly immeasurable variable that describes a variation in a discontinuous trait. For example, increasing continuous values of liability for reproduction are translated into two phenotypes (reproduction absent or present) separated by a threshold. Heritability of tree form and male reproduction on the underlying (probit) latent scale, was calculated as: $h^2_{\text{form latent}}$: $V_A / (V_A+V_I+V_R+V_L)$, and $h^2_{\text{RVm latent}}$: $V_A / (V_A+V_R+V_L)$, respectively, with $V_R = 1$ by convention and latent scale variance $V_L = 1$ as the probit variance. Heritability of the threshold size for female and male reproduction was also calculated on the underlying (logit) latent scale as: $h^2_{\text{TSFR latent}}$: $V_A / (V_A+V_I+V_R+V_L)$, with $V_R = 1$ by convention and $V_L = \pi2/3$ as the logit variance. Heritability on the underlying (Poisson) latent scale for female reproductive investment after accounting for size was calculated as:

$h^2_{RVf\ latent}$: V_A / $\{V_A+V_R+\log[1/\exp(\beta_0)+1]\}$, where β_0 is the predicted trait value given certain fixed effects (5). We calculated $h^2_{RVf\ latent}$ taking β_0 as the average cone production per tree.

Genetic correlations were estimated as the Pearson's correlation between traits for family means derived from mixed models where family was coded as random (6, 7). Genetic correlations were only calculated for those variables with significant genetic variance or heritability. Phenotypic correlations were estimated by fitting bivariate models to data from the selected and control groups. Models were fitted with two variables at each time given that there were slight differences in number of observations per variable due to the existence of juveniles regarding male or female function. The variables included were height, biomass, stem form and male (qualitative scale) and female (cone counts) reproduction. Height and biomass were considered Gaussian variables while quantitative female reproduction was treated as Poisson. Originally, stem form and male reproduction were recorded according to an ordinal distribution, but here were considered also as count data (Poisson error) in order to enable the calculation of phenotypic correlations (8). Genetic and phenotypic correlations thus do not represent the same set of traits as phenotypic correlations for male and female reproductive data were not corrected for size effects.

SUPPLEMENTARY FIGURE 1. Effect of a single event of artificial selection for timber yield within a *Pinus pinaster* population on the threshold size for first reproduction of the progeny of selected plus trees and control, unselected, base population. Smallest reproductive individual (black dots), and threshold size for female (grey squares) and male first reproduction (empty squares plus) per family and control group are shown. Family or group mean ± 95% credible intervals are shown. Control, control group; 26P-52S codes, half-sib family codes; SW Female and Male: female and male Species-Wide ranges for the threshold size for first reproduction (reported by Santos-del-Blanco L, Climent J, González-Martínez SC, Pannell JR (2012) Genetic differentiation for size at first reproduction through male versus female functions in the widespread Mediterranean tree *Pinus pinaster*. Ann Bot 110:1449–1460)



SUPPLEMENTARY FIGURE 2. Description of *Pinus pinaster* seed cone development. Top left, current year female strobilus. Mid-left, first year female cone. Mid-right, second year mature female cone. Top right, whorls of serotinous female cones older than two years attached to the main stem.

SUPPLEMENTARY TABLE 1. Posterior modes (mode) and credible intervals (95% CI) of height (cm) per reproductive class (juvenile, vegetative, female, male or cosexual) and experimental group (selected, control) in *Pinus pinaster* trees. TSFR indicates the median threshold size for first reproduction per sex. Cells marked with an asterisk (*) indicate significant (P<0.05) differences between selected and control groups.

		selected					control					
	Reproductive class	n	%	mode	95% CI		n	%	mode	95% CI		sig.
Height	Juvenile	167		165.4	156.1	184.1	15		100.0	67.2	137.3	*
	Vegetative	155		268.6	251.7	280.2	51		199.7	182.5	221.2	*
	Female	134	40	285.1	264.7	294.1	42	33	237.6	213.4	254.5	*
	Male	81	24	316.8	306.6	340.2	17	13	305.3	272.5	331.9	
	Cosexual	119	36	346.3	334.3	365.2	70	54	308.9	292.0	325.5	*
	Total	656	51				195	66				
TSFR	Female			322.6	293.0	362.4			215.7	187.1	241.7	*
	Male			323.9	310.6	338.2			246.5	230.5	267.7	*

n, number of trees per class. Total, total number of trees per group and age. %, referred to F, M and C represents the percentage of those classes respect to reproductive trees. % referred to total represent the percentage of reproductive trees. Pines are monoecious species and individual trees usually produce both female and male cones, in our study, Female and Male trees represent protogynous and protrandrious individuals. Vegetative trees where those that could reproduce according to their size, but they did not.

SUPPLEMENTARY TABLE 2. Genetic and phenotypic correlations between vegetative and reproductive traits measured in *Pinus pinaster* trees grown in a common garden in North Spain. Significant correlations ($P < 0.05$) in bold face.

a) Half-sib family correlations (above diagonal) and corresponding P-values (below diagonal) among traits in the selected group for timber production.

		Female		Male		Vegetative	
		TSFR	R-V	TSFR	R-V	Height	Stem form
Female	TSFR		**-0.82**	**0.39**	-0.19	0.12	**-0.38**
	R-V	<0.001		**-0.52**	0.00	-0.14	0.25
Male	TSFR	0.03	0.00		0.02	**0.38**	-0.31
	R-V	0.31	0.99	0.90		0.06	0.02
Vegetative	Height	0.51	0.45	0.04	0.74		-0.36
	Stem form	0.04	0.17	0.09	0.92	0.05	

TSFR, Threshold size for first reproduction; R-V, reproductive investment relative to size.

b) Phenotypic correlations among raw traits for the selected (above diagonal) and control, unselected, (below diagonal) groups. 95% credible intervals in brackets.

	Female	Male	H	Stem Form
Female		0.62 (-0.06-0.85)	**0.35** (0.27-0.46)	0.15 (-0.13-0.46)
Male	**0.76** (0.53-0.92)		**0.52** (0.44-0.58)	0.12 (-0.24-0.33)
Height	**0.28** (0.10-0.36)	**0.45** (0.45-0.66)		**0.52** (0.31-0.54)
Stem Form	0.23 (-0.18-0.54)	0.12 (-0.26-0.49)	**0.52** (0.44-0.58)	

Female, absolute female reproduction; Male, qualitative male reproduction (scale 0-3).

REFERENCES CITED IN THE SUPPLEMENTARY INFORMATION

1. Alía R et al. (1996) *Las regiones de procedencia de Pinus pinaster Aiton.* (Ministerio de Medio Ambiente, Madrid, Spain).

2. White TL, Adams WT, Neale DB (2007) *Forest Genetics* (CABI Publishing).

3. Varghese M, Kamalakannan R, Harwood CE, Lindgren D, McDonald M (2009) Changes in growth performance and fecundity of Eucalyptus camaldulensis and E. tereticornis during domestication in southern India. *Tree Genet Genomes* 5:629–640.

4. De Villemereuil P, Gimenez O, Doligez B (2013) Comparing parent-offspring regression with frequentist and Bayesian animal models to estimate heritability in wild populations: a simulation study for Gaussian and binary traits. *Meth Ecol Evol* 4:260–275.

5. Nakagawa S, Schielzeth H (2010) Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biol Rev Camb Philos Soc* 85:935–956.

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

7. Sakai AK et al. (2008) Sexual dimorphism and the genetic potential for evolution of sex allocation in the gynodioecious plant, Schiedea salicaria. *J Evolution Biol* 21:18–29.

8. Hadfield JD (2010) MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. *J Stat Soft* 33:1–22.

9. Santos-del-Blanco L, Climent J, González-Martínez SC, Pannell JR (2012) Genetic differentiation for size at first reproduction through male versus female functions in the widespread Mediterranean tree *Pinus pinaster*. *Ann Bot* 110:1449–1460.

APPENDIX V

Somatic and genetic costs of reproduction in a conifer tree. **Santos-del-Blanco L**, Climent J.

Somatic and genetic costs of reproduction in a conifer tree.

Luis SANTOS-DEL-BLANCO[1,2,3*], José CLIMENT[2,3]

[1] Department of Ecology and Evolution, University of Lausanne, Biophore Building, CH-1015, Lausanne, Switzerland.

[2] Departamento de Ecología y Genética Forestal, CIFOR-INIA, Carretera de La Coruña km 7.5, 28040 Madrid, Spain

[3] Instituto Universitario de Gestión Forestal Sostenible www.sostenible.uva.es

*Corresponding author:
Luis Santos-del-Blanco
Departamento de Sistemas y Recursos Forestales, CIFOR-INIA, Carretera de La Coruña km 7.5, 28040 Madrid, Spain
Telephone: +34 91 3473904
Fax: +34 91 3476767

ABSTRACT

Costs of reproduction lie at the core of basic ecological and evolutionary theories, and their existence is commonly invoked to explain adaptive processes. Despite their sheer importance, empirical evidence for the existence and quantification of costs of reproduction in tree species, comes mostly from correlational studies and more comprehensive approaches remain missing. Manipulative experiments are a preferred approach to study cost of reproduction, as they allow controlling for otherwise inherent confounding factors like size or genetic background. Here, we conducted a manipulative experiment in a *Pinus halepensis* common garden comprising Spanish populations, removing developing cones from a group of trees and comparing growth and reproduction after treatment with a control group. Manipulated trees grew slightly more than control trees just after treatment, but not significantly. Larger differences were observed for the number of female cones initiated one year after treatment, with an increase of 70% more cones in the manipulated group. Phenotypic and genetic correlations between vegetative size and female reproduction were also calculated, providing no evidence for costs of reproduction.

INTRODUCTION

Life history theory assumes that costs, or trade-offs, exist between functions linked to fitness, namely survival, growth and reproduction (Roff, 1992). The study of the costs of reproduction in terms of growth, survival or subsequent reproduction has a long tradition in evolution and ecology, but the approaches used by both disciplines usually differ and focus on different conceptions of costs of reproduction (Reznick, 1992). When considering evolutionary change, it is only genetically-based costs that are relevant to future adaptation. In turn, when considering plastic, environmentally-driven responses within genotypes, physiological costs are those that matter.

Four approaches have been used to illustrate costs of reproduction (Reznick, 1985): phenotypic correlations, experimental manipulations of reproduction, genetic correlations and selection experiments. Among these approaches, only genetic correlations and selection experiments are valid to test the assumptions of life history theory (Reznick, 1985). On the other hand, phenotypic correlations and, preferably, experimental manipulations illustrate physiological costs of reproduction provided that confounding factors such as environmental and genetic variability are controlled (Obeso, 2002).

The study of costs of reproduction in trees is more complex and variable than in short lived taxa, as investment in growth, maintenance and reproduction might change along time (Thomas, 2011). Available evidence for tree species highlights a physiological trade-off between reproduction and growth, with several recent papers (Sánchez-Humanes *et al.*, 2011; Alla *et al.*, 2011; Sala *et al.*, 2012; Barringer *et al.*, 2013; Żywiec & Zielonka, 2013) and a review (Thomas, 2011) helping to define mechanistic links between both functions. Reproduction has been related to reduced growth in terms of stem diameter and shoot elongation, but other traits like leaf size and chemistry (Leal & Thomas, 2003), and photosynthesis (Obeso, 1998) have been also used to illustrate costs of reproduction in trees. Despite reflecting a more basic trade-off than that between reproduction and growth, negative correlations between current and future reproduction are not always explored, and evidence in plants remains only marginal (Obeso, 2002).

Studies reporting on physiological costs of reproduction in trees are mainly based on correlational studies (Thomas, 2011). The most basic approach consists on relating a variable of interest, usually growth, with naturally-occurring variable levels of reproduction (or vice versa) at different hierarchical levels, from shoots to whole the tree (Sánchez-Humanes *et al.*, 2011), and in one or several populations or species (Barringer *et al.*, 2013) along one or several growth seasons (Pulido *et al.*, 2013). Some studies have used manipulation experiments, helping to generate more variability than naturally available for water or nutrient resources (Sánchez-Humanes & Espelta, 2011; Montesinos *et al.*, 2012; Pulido *et al.*, 2013). Moreover, direct manipulation of reproduction is widely considered a more straightforward method for highlighting costs of reproduction (Reznick, 1992; Obeso, 2002), but it has so far been reported only once in a tree species, with non-conclusive results (Fox, 1995). Indeed, manipulation of reproduction in trees poses great practical challenges in such large organisms.

Genetically-based costs of reproduction in trees are also scarcely reported in the plant ecology literature given that genetic trials must be planted and cared after at least until trees reach reproductive maturity, and then there is the issue of defining the variables that best represent investment in growth and in reproduction. There are however some examples in the forestry literature reporting on negative genetic correlations between reproductive and vegetative traits (Schmidtling, 1981; El-Kassaby & Barclay, 1992; Santos-del-Blanco *et al.*, 2010). Non-significant or positive correlations between current reproduction and growth or future reproduction often come from studies where environmental confounding factors like tree size are not controlled (Almqvist *et al.*, 2001; Hannerz & Aitken, 2001; Bilir *et al.*, 2002). Moreover, the use of genotypes selected for enhanced growth in breeding programs can incorporate a severe bias that limits a broad interpretation of results (El-Kassaby & Barclay, 1992). Data from tree breeding programs could nonetheless be used to illustrate genetic costs of reproduction by comparing reproductive traits in progenies from trees submitted and not submitted to phenotypic selection. This, however, has not yet been done and there is only indirect evidence for genetic costs of reproduction derived from artificial selection (Varghese *et al.*, 2009).

Overall, it seems that a comprehensive study of costs of reproduction in trees is impaired mainly by logistic reasons. In this study we tried to increase our knowledge of costs of reproduction both at the phenotypic and genetic levels by focusing on a precocious Mediterranean pine whose reproductive ecology is becoming reasonably well understood, the Aleppo pine (*Pinus halepensis* Ait.) (Ne'eman *et al.*, 2004, 2011; Climent *et al.*, 2008; Santos-del-Blanco *et al.*, 2013). We specifically (1) test the existence of a physiological trade-off between current reproduction and subsequent growth and reproduction at the whole tree level by experimentally manipulating reproduction in young *Pinus halepensis* trees, and (2) provide estimates of phenotypic and genetic correlations between vegetative and reproductive traits from common garden experiments, indicative of costs of reproduction.

MATERIAL AND METHODS

Study site

Our experiment was carried out in a provenance-progeny common garden planted in 1995 with one year old seedlings. Size and female reproduction was periodically registered for all trees in the common garden since establishment. Details can be accessed in (Santos-del-Blanco *et al.*, 2010) and www.genfored.es. .

Study species

Pinus halepensis has several features that make it a suitable species for the study of costs of (female) reproduction. First, it is a precocious species, producing female cones as early as three years old. Second, it has a relatively low size at maturity, which at early ages enables manipulation without need of special equipment like scaffolds or elevator platforms. Third, it has a high reproductive allocation and produces cones regularly along its life, which facilitates the detection of costs of reproduction (Grivet *et al.*, 2013).. And fourth, it is a wide-spread species, with populations putatively adapted to varying ecological conditions that might reveal compromises between reproduction and other life history traits (Climent *et al.*, 2008).

Pinus halepensis has a two year female cone developmental cycle, thus at any time there are potentially two cohorts of developing female cones (Pardos *et al.*, 2003) (Figure 1). Female floral induction of vegetative buds takes place at the end of the summer and female cones emerge the following spring (Enescu, 1987), generally from vigorous branches of the upper half of the crown (Shmida *et al.*, 2000). Pollination then takes place in spring but ovule fecundation and seed and cone development are arrested until the following year. First year female cones then fully grow along the season completing their development two years after pollination. Developed cones are attached to their bearing branches through a very thick peduncle so they rarely drop after final development (Ne'eman *et al.*, 2004). Female cones in *Pinus halepensis* trees typically precede male cones in one or several years at the onset of reproductive life. Therefore female reproduction is clearly more relevant during early stages in this species (Ne'eman *et al.*, 2011), unlike other related pine species like *P. pinaster* (Santos del Blanco et al 2012) or *P. pinea* (Shmida *et al.*, 2000).

Manipulative experiment

In May 2010, when trees where 16 years old, a manipulative experiment was carried out. At that moment, still about half of the trees in the common garden remained vegetative or had produced a low number of cones (less than five). We imposed a minimum threshold of five cones in the previous two years for the trees to be eligible for the experiment. The aim was to increase power in detecting significant differences. Among those with at least five cones, we selected trees evenly in the range of 5-61 cones per tree by subdividing them into three categories, low, medium and high reproduction. In total, 110 trees were selected, randomly assigning half of them to the treatment group and leaving the other half as a control group (CTR group hereafter). We ensured that there were no statistical differences in size or female reproduction between groups previous to treatment.

All developing female cones, both of current year (strobili) and previous year (conelets) were counted and removed from trees in the treatment group (FCR group hereafter). A whole-tree assessment of the treatment effects was preferred since compensation between plant parts could mask processes at the branch level (Karlsson & Mendez, 2005), as suggested by a previous correlational study in *P. halepensis* (Ne'eman *et al.*, 2011). Special care was taken when removing the cones as not to damage the branches. Current and previous year female cones were also counted in trees from the control group.

We did not assess costs of male reproduction for two reasons. Firstly because of the above mentioned prevalence of female gender during early reproductive stages (Ne'eman *et al.*, 2011) and, second, because a similar removal of male cones in pines is not possible without severely damaging vegetative structures of the affected shoot

At the end of 2011autumn growth season, thus two years after treatment a radial wood microcore was extracted at breast height from each tree. Wood microcores were mounted on a frame, sanded and radial growth for years 2009, 2010 and 2011 measured with a tree-ring measurement station. Basal area for each tree and year was calculated from diameter at breast height in late 2011and yearly radial growth increments. Current year female cones were also counted in 2011. Basal area and female reproduction provided information to assess costs of reproduction with a holistic approach, focusing on their phenotypic outcome but not on their proximate causes.

Data analysis.

Differences in vegetative growth one and two years after treatment were evaluated with linear mixed models which included treatment as a fixed factor and basal area previous to treatment as a covariate. Model term significance was evaluated by means of Likelihood Ratio Tests. The interaction between treatment and covariate was non-significant and therefore it was removed from final models. The provenance (population of origin) of each individual was included as a random factor in the model in order to avoid pseudoreplication.

Count data for female cones was analysed with Poisson Generalised Linear Mixed Models. We first tested for differences between treatment and control groups in absolute number of cones produced after the treatment (counted in late 2011). As before, treatment was defined as fixed but we used no covariate. Population was included as a random factor as well as an observation-level random factor in order to account for overdisperssion in our data

(Elston *et al.*, 2001). Finally, in order to check for differences in reproductive allometry between treatments, we included the logarithm of basal area in 2011 as a covariate in the model.

Complementarily, correlations between reproduction and size, indicative of phenotypic and genetic costs of reproduction were calculated with measurements at 16 years from the entire trial (28 populations 144 families, 1305 trees). We calculated correlations between biomass in 2009 (as data were available for all individuals) and absolute (reproductive investment) or relative (reproductive allocation) reproduction until 2009. Reproductive allocation was calculated by directly dividing the number of cones into biomass, at the individual level (Karlsson & Mendez, 2005). Finally, we also calculated correlations between biomass growth between 2005 and 2009 and number of cones produced during that period, each variable reflecting investment in vegetative growth or reproduction, respectively. Phenotypic correlations were estimated as Pearson’s correlation coefficients between phenotypic values after correcting for population effects. As a proxy for genetic correlations we calculated Pearson’s correlation coefficients between family corrected means. All models and tests were performed with R packages lme4 v. 0.999999-0 and lmerTest v.1.2-0.

RESULTS

Manipulated trees, i.e. those with removed current and first year female cones, FCR group, grew slightly more than control trees after treatment, but differences were only marginally significant in 2010 (P X^2_1 3.33 = 0.07) and this small significance disappeared the following year (Table 1, Figure 2).

By contrast, the female cone removal treatment showed a greater effect in subsequent reproduction. FCR trees produced, on average about 70% more female cones than control trees (Table 1, Figure 3). We found only additive effects of treatment on reproductive allometry, indicating a similar effect of experimental treatment across tree sizes (P X^2_1 6.05 = 0.014).

The phenotypic correlation between size and reproduction, either absolute or relative, was positive and significant, i.e. bigger trees, or those that grew more, yielded more female cones (Table 2). The correlation between size and number of cones per size unit (RA) was however negative. Among-family genetic correlations between size and reproductive investment were positive and significant but lower than phenotypic correlations, while those with reproductive allocation were negative and significant (Table 2).

DISCUSSION

Experimental removal of developing female cones in *Pinus halepensis* resulted in enhanced production of female cones one year later, even when treatment had only a minor effect on vegetative growth. To our knowledge, this is the first account on somatic costs of reproduction in a forest tree as assessed by a manipulative experiment.

Induction of vegetative meristems into meristems giving rise to female cones takes place after summer (Enescu, 1987), and induction depends on nutrient content on branches, such that vigorous branches with higher nitrogen content are more likely to bear female cones (Ne'eman *et al.*, 2011). Then, fruit development takes place partially at the expenses of stored N and P, that in the case of the masting species *Pinus albicaulis* can be even depleted (Sala *et al.*, 2012) but with no apparent effect on vegetative growth. By removing developing female cones, trees in our experiment were probably left with a surplus of readily available resources that could be allocated to other functions or stored. Thus, cone induction in the following year could be influenced in two ways. First, indirectly by the investment of those resources into vegetative growth that could help to acquire and store more resources. And second, directly by readily stored-extra resources the previous year. Measurement of nutrient (Ne'eman *et al.*, 2011; Sala *et al.*, 2012) and carbohydrates (Ichie *et al.*, 2013) at the branch and tree level could help elucidate between those alternatives. Our results suggest that manipulation of reproduction can have greater carry-over effects on reproduction compared to immediate effects on vegetative growth.

Experimental manipulation of reproduction proved critical in the detection of costs of reproduction in our study. Indeed, manipulative experiments have been recommended for the study of costs of reproduction in plants, while controlling for confounding factors like size and genetic background (Obeso, 2002). For example, large trees invest more in both vegetative and reproductive growth, leading to positive correlations (de Jong & Klinkhamer, 2005) which indeed are the base of allometric relationships between size and reproduction (Niklas & Enquist, 2003). Such an example is also provided by our data at the phenotypic level. Therefore only rarely a negative correlation between absolute size and reproduction is described but see (Hansen *et al.*, 2013). Besides size effects, other environmental factors can blur the detection of costs of reproduction, but also produce false positives (Knops *et al.*, 2007). Experimental manipulations are therefore recommended whenever possible.

Despite the high number of trees and contrasted origin of the families included in our study, we only found mixed support for phenotypic and genetic costs of reproduction in this species, indicative of evolutionary constraint. A negative relationship of growth and reproduction was only evident at the individual and family level when comparing absolute size and reproductive allocation, also reported in (Santos-del-Blanco *et al.*, 2010). Such comparisons have raised concern about spurious correlations (Klinkhamer & de Jong, 1990), however they also describe a biological reality, and the problem might be not the use of such correlations but their straightforward interpretation as evidence for costs of reproduction.

In our common garden study, smaller trees invested a higher proportion of their resources in reproduction, compared to larger trees (Table 3), a pattern also described in (Climent *et al.*, 2008). This could be caused by costs of reproduction, dragging the growth of those trees that started to reproduce at smaller sizes (Climent *et al.*, 2008) or just by specific allometric patterns that might determine a more intense relative reproduction at young ages. To the extent that the allometry of reproduction is genetically determined for a species, one could interpret the difference between the theoretical reproductive allocation and the actual reproductive allocation as driven by costs of reproduction, i.e. the predicted reproductive allocation, or that of a control group, can provide a reference by which decide whether the relationship between size and the actual reproductive allocation can or cannot be influenced by costs of reproduction. Also here, manipulative experiments would be a preferred tool.

Even if present, several factors could have affected our ability to detect such costs. First, family estimates had high errors as the number of trees per family was relatively low. Second, neutral genetic variability in Spanish populations is low, following recent range expansion (Grivet *et al.*, 2011). Thus, although neutral and adaptive genetic variability need not be correlated, and despite our comprehensive sampling effort, Spanish populations might lack enough variation. And third, tree phenotypes are complex and lack of correlation is not enough proof against it, also because there might be third variables indirectly influencing the expression of trade-offs, like variability in resource acquisition (Friedman & Barrett, 2011).

Reproduction in trees has been an overlooked trait in studies addressing adaptation, while the relationship between fitness and reproduction is much more direct than that with vegetative traits. A deeper knowledge on tree reproductive ecology is thus still needed to understand adaptive processes in forest stands. Our experiment has shown that as long as reliable approaches and adequate model organisms are chosen, simple methods can be useful to illustrate costs of reproduction in trees.

REFERENCE LIST

Alla AQ, Camarero JJ, Maestro-Martínez M, Montserrat-Martí G. **2011**. Acorn production is linked to secondary growth but not to declining carbohydrate concentrations in current-year shoots of two oak species. *Trees* **26**: 841–850.

Almqvist C, Jansson G, Sonesson J. **2001**. Genotypic correlations between early cone-set and height growth in *Picea abies* clonal trial. *Forest Genetics* **8**: 197–204.

Barringer BC, Koenig WD, Knops JMH. **2013**. Interrelationships among life-history traits in three California oaks. *Oecologia* **171**: 129–139.

Bilir N, Kang KS, Zang D, Lindgren D. **2002**. Fertility variation and gene diversity in clonal seed orchards of *Pinus brutia*, *Pinus nigra* and *Pinus sylvestris* in Turkey. *Silvae Genetica* **51**: 112–115.

Climent J, Prada MA, Calama R, Chambel MR, de Ron DS, Alía R. **2008**. To grow or to seed: ecotypic variation in reproductive allocation and cone production by young female Aleppo pine (*Pinus halepensis, Pinaceae*). *American Journal of Botany* **95**: 833–842.

El-Kassaby YA, Barclay HJ. **1992**. Cost of reproduction in Douglas-fir. *Canadian Journal of Botany* **70**: 1429–1432.

Elston D, Moss R, Boulinier T, Arrowsmith C, Lambin X. **2001**. Analysis of aggregation, a worked example: numbers of ticks on red grouse chicks. *Parasitology* **122**: 563–569.

Enescu V. **1987**. Climate and the choice of seed orchard sites. *Forest Ecology and Management* **19**: 257–265.

Fox JJ. **1995**. Shoot demographic responses to manipulation of reproductive effort by bud removal in a willow. *Oikos* **72**: 283–287.

Friedman J, Barrett SCH. **2011**. Genetic and environmental control of temporal and size-dependent sex allocation in a wind-pollinated plant. *Evolution* **65**: 2061–2074.

Grivet D, Climent J, Zabal-Aguirre M, Neale DB, Vendramin GG, González-Martínez SC. **2013**. Adaptive evolution of Mediterranean pines. *Molecular Phylogenetics and Evolution* **68**: 555–566.

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

Hannerz M, Aitken SN. **2001**. Inheritance of strobili production and genetic correlation with growth in lodgepole pine. *Forest Genetics* **8**: 323–329.

Hansen CF, García MB, Ehlers BK. **2013**. Water availability and population origin affect the expression of the tradeoff between reproduction and growth in *Plantago coronopus*. *Journal of Evolutionary Biology* **26**: 993–1002.

Ichie T, Igarashi S, Yoshida S, Kenzo T, Masaki T, Tayasu I. **2013**. Are stored carbohydrates necessary for seed production in temperate deciduous trees? *Journal of Ecology* **101**: 525–531.

Iwasa Y, Satake A. **2004**. Mechanisms inducing spatially extended synchrony in mast seeding: The role of pollen coupling and environmental fluctuation. *Ecological Research* **19**: 13–20.

De Jong TJ, Klinkhamer PGL. **2005**. *Evolutionary ecology of plant reproductive strategies*. Cambridge: Cambridge University Press.

Karlsson PS, Mendez M. **2005**. The resource economy of plant reproduction. In: Reekie EG, Bazzaz FA, eds. Reproductive allocation in plants. Amsterdam, The Netherlands: Elsevier, 1–40.

Klinkhamer PGL, de Jong TJ. **1990**. Effects of plant size, plant density and sex differential nectar reward on pollinator visitation in the protandrous Echium vulgare (Boraginaceae). *Oikos* **57**: 399–405.

Knops JMH, Koenig WD, Carmen WJ. **2007**. Negative correlation does not imply a tradeoff between growth and reproduction in California oaks. *Proceedings of the National Academy of Sciences of the United States of America* **104**: 16982–16985.

Leal DB, Thomas SC. **2003**. Vertical gradients and tree-to-tree variation in shoot morphology and foliar nitrogen in an old-growth *Pinus strobus* stand. *Canadian Journal of Forest Research* **33**: 1304–1314.

Montesinos D, Villar-Salvador P, García-Fayos P, Verdú M. **2012**. Genders in J*uniperus thurifera* have different functional responses to variations in nutrient availability. *New Phytologist* **193**: 705–712.

Ne'eman G, Gidi Goubitz S, Shirrinka Nathan R. **2004**. Reproductive traits of *Pinus halepensis* in the light of fire–a critical review. *Plant Ecology* **171**: 69–79.

Ne'eman G, Goubitz S, Werger MJA, Shmida A. **2011**. Relationships between tree size, crown shape, gender segregation and sex allocation in *Pinus halepensis*, a Mediterranean pine tree. *Annals of Botany* **108**: 197–206.

Niklas KJ, Enquist BJ. **2003**. An allometric model for seed plant reproduction. *Evolutionary Ecology Research* **5**: 79–88.

Obeso JR. **1998**. Sex ratios, size distributions, and sexual dimorphism in the dioecious tree *Ilex aquifolium* (*Aquifoliaceae*). *American Journal of Botany* **85**: 1602–1608.

Obeso JR. **2002**. The costs of reproduction in plants. *New Phytologist* **155**: 321–348.

Pardos M, Climent J, Gil L, Pardos JA. **2003**. Shoot growth components and flowering phenology in grafted *Pinus halepensis* Mill. *Trees-Structure and Function* **17**: 442–450.

Pulido F, Moreno G, Garcia E, Obrador JJ, Bonal R, Diaz M. **2013**. Resource manipulation reveals flexible allocation rules to growth and reproduction in a Mediterranean evergreen oak. *Journal of Plant Ecology*: 1–9.

Reznick D. **1985**. Costs of reproduction: an evaluation of the empirical evidence. *Oikos* **44**: 257–267.

Reznick D. **1992**. Measuring the costs of reproduction. *Trends in Ecology & Evolution* **7**: 1990–1993.

Roff DA. **1992**. *The evolution of life histories: theory and analysis*. New York, USA: Chapman and Hall.

Sala A, Hopping K, McIntire EJB, Delzon S, Crone EE. **2012**. Masting in whitebark pine (*Pinus albicaulis*) depletes stored nutrients. *New Phytologist* **196**: 189–199.

Sánchez-Humanes B, Espelta JM. **2011**. Increased drought reduces acorn production in *Quercus ilex* coppices: thinning mitigates this effect but only in the short term. *Forestry* **84**: 73.

Sánchez-Humanes B, Sork VL, Espelta JM. **2011**. Trade-offs between vegetative growth and acorn production in *Quercus lobata* during a mast year: the relevance of crop size and hierarchical level within the canopy. *Oecologia* **166**: 101–110.

Santos-del-Blanco L, Bonser SP, Valladares F, Chambel MR, Climent J. **2013**. Plasticity in reproduction and growth among 52 range-wide populations of a Mediterranean conifer: adaptive responses to environmental stress. *Journal of Evolutionary Biology* **26**: 1912–1924.

Santos-del-Blanco L, Notivol E, Zas R, Chambel MR, Majada J, Climent J. **2010**. Variation of early reproductive allocation in multi-site genetic trials of Maritime pine and Aleppo pine. *Forest Systems* **19**: 381–392.

Schmidtling RC. **1981**. The inheritance of precocity and its relationship with growth in Loblolly pines. *Silvae Genetica* **30**: 188–192.

Shmida A, Lev-Yadun S, Goubitz S, Ne'eman G. **2000**. Sexual allocation and gender segregation in Pinus halepensis, P. brutia and P. pinea. In: Ne'Eman G, Trabaud L, eds. Ecology, biogeography and management of *Pinus halepensis* and *P. brutia* forest ecosystems in the Mediterranean basin. Leiden, The Netherlands: Backhuys Publisher, 91–104.

Thomas SC. **2011**. Age-related changes in tree growth and functional biology: the role of reproduction. In: Meinzer FC, Lachenbruch B, Dawson TE, eds. Size-and age-related changes in tree structure and function. Springer Netherlands, 33–64.

Varghese M, Kamalakannan R, Harwood CE, Lindgren D, McDonald MW. **2009**. Changes in growth performance and fecundity of *Eucalyptus camaldulensis* and *E. tereticornis* during domestication in southern India. *Tree Genetics & Genomes* **5**: 629–640.

Żywiec M, Zielonka T. **2013**. Does a heavy fruit crop reduce the tree ring increment? Results from a 12-year study in a subalpine zone. *Trees*.

TABLES

Table 1. Least square mean values per trait and p-value for the test of differences between *Pinus halepensis* trees subjected to removal of developing cones (FCR) and control (CTR) groups.

Trait	FCR	CTR	p-value
Basal area 2010 (mm^2)	6188±43	6088±43	0.07
Basal area 2011 (mm^2)	7638±112	7551±113	0.77
Female cones 2011	16.8±2.0	9.2±2.1	<0.01

Table 2. Phenotypic and genetic (family) correlations between vegetative and reproductive size in *Pinus halepensis*. RI correlation between absolute vegetative and reproductive sizes. RA correlation between absolute vegetative size and reproductive allocation. R-G correlation between growth in the period 2005-2009 and the number of female cones produced.

	Phenotypic n= 1305	p-value	Family n=144	p-value
RI	0.46 (0.42-0.50)	<0.001	0.38 (0.23-0.51)	<0.001
RA	-0.40 (-0.44- -0.35)	<0.001	-0.43 (-0.56- -0.29)	<0.001
R-G	0.31 (0.27 – 0.36)	<0.001	0.21 (0.05 – 0.36)	0.010

FIGURES

Figure 1. Developmental stages of female strobili in *Pinus halepensis*. a) Female strobili in spring, soon after budburst. b) Female conelets in spring, one year after budburst. c) Fully-developed and ripe two-year old female cones.

Figure 2. Scatterplots showing the relationship between basal area preivous to treatment (2009) and a) one or b) two years after treatment. Red dots, control group; Blue dots, female cone removal treatment.

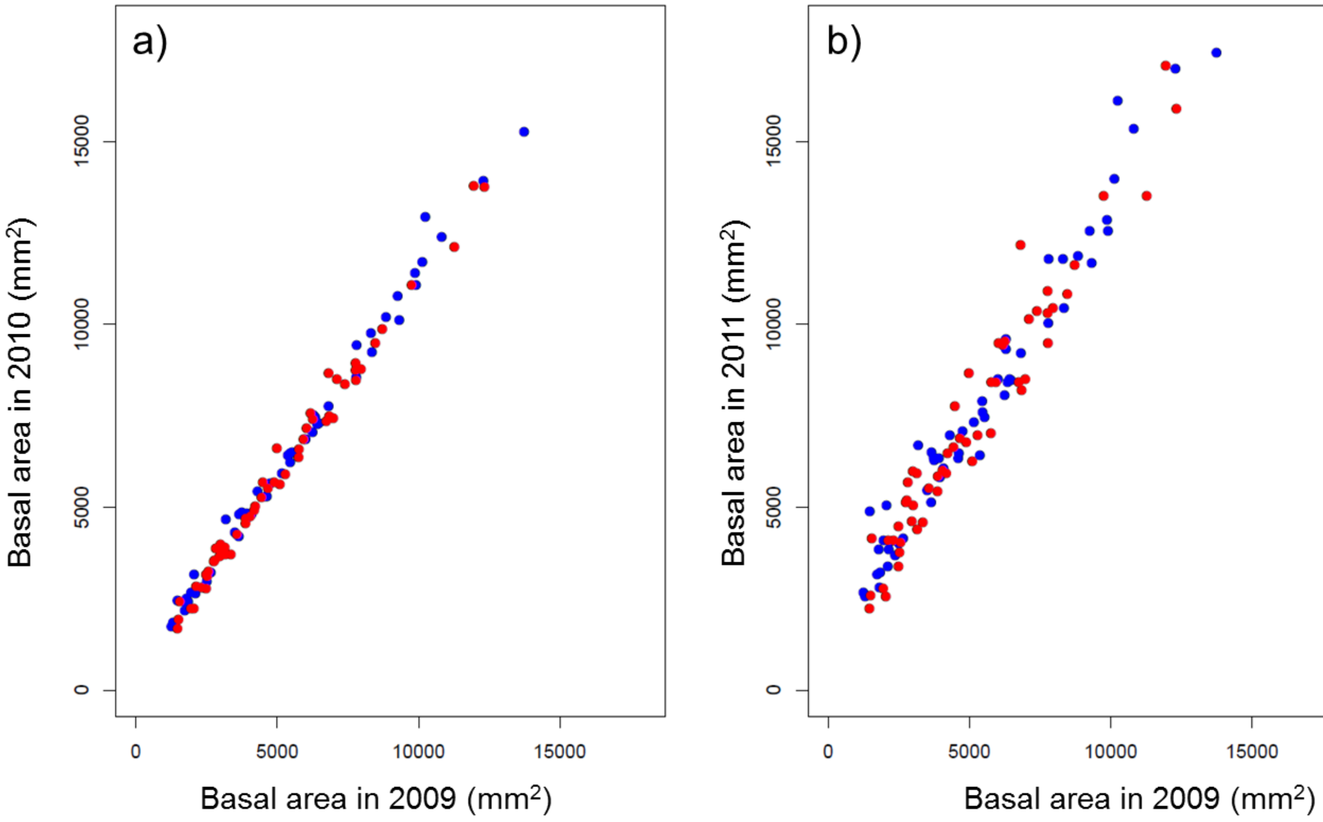
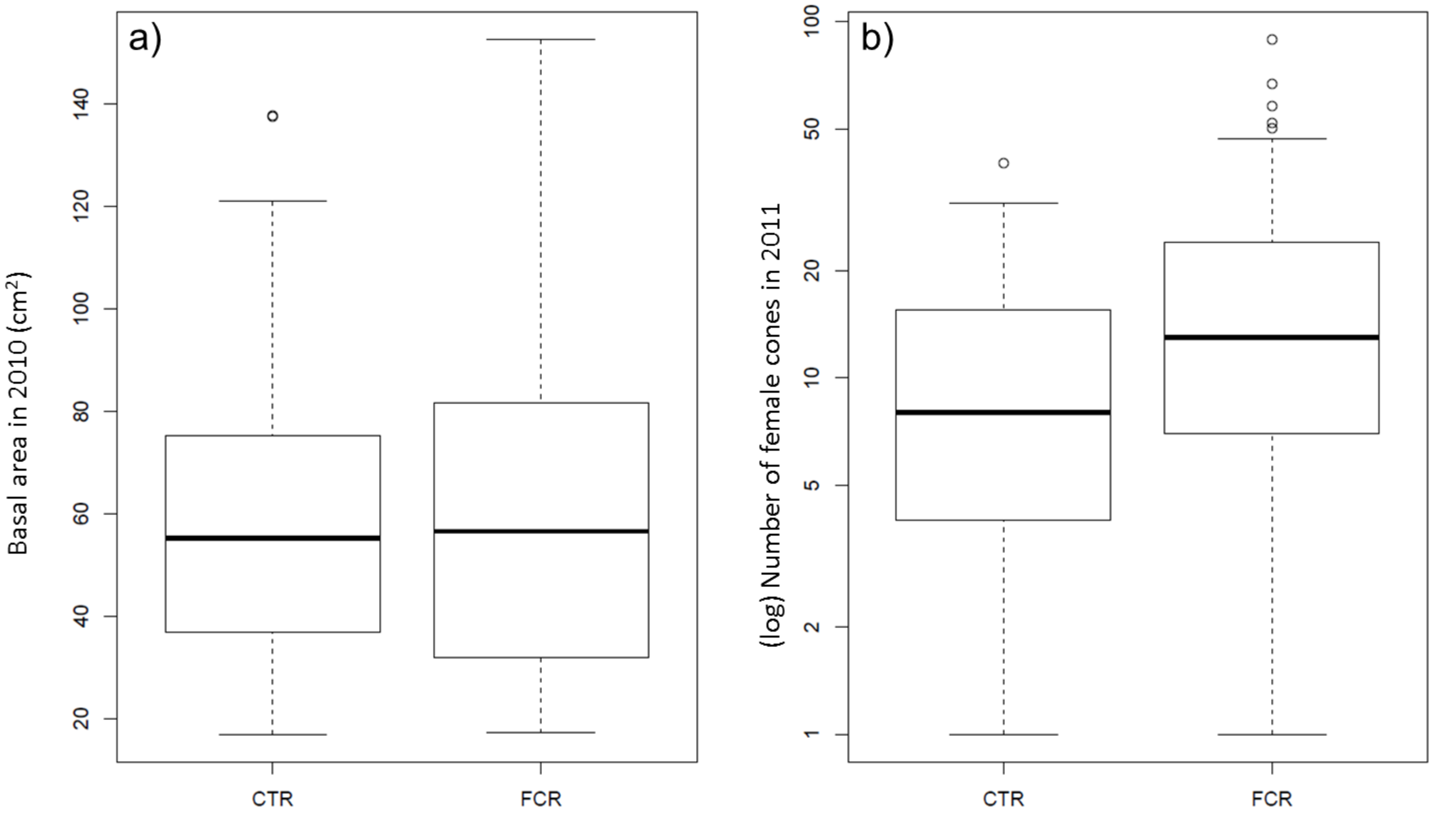


Figure 3. Boxplots of a) basal area in 2010, one growth season after experimental manipulation, and b) number of female cones in 2011, one reproductive season after experimental manipulation comparing control Pinus halepensis individuals (CTR) with others subjected to experimental removal of developing female cones (FCR).



APPENDIX VI

Guidelines for estimation of quantitative genetic parameters of reproductive traits. **Santos-del-Blanco L**, Climent J.

Guidelines for the estimation of quantitative genetic parameters for reproductive traits in trees

Luis SANTOS-DEL-BLANCO[1,2,3], José CLIMENT[12]

[1] Departamento de Ecología y Genética Forestal, CIFOR-INIA, Carretera de La Coruña km 7.5, 28040 Madrid, Spain

[2] Instituto Universitario de Gestión Forestal Sostenible http://sostenible.palencia.uva.es

3 Department of Ecology and Evolution. University of Lausanne. Biophore Building. CH-1015, Lausanne, Switzerland

INTRODUCTION

Reproductive strategies lie at the core of adaptive evolutionary processes and are studied in depth in evolutionary ecology (de Jong & Klinkhamer, 2005). Reproductive traits in forest trees are often neglected and the focus is instead placed on growth, physiological and defence traits and survival. However, phenotypic selection on putatively adaptive traits will be evolutionarily neutral unless traits covary genetically with fitness (Robertson, 1966; Price, 1970). Given a direct link between fitness and reproduction, reproductive strategies are key to understand adaptive processes.

Trees are long lived organisms and have a considerably long reproductive period from maturity to senescence (Petit & Hampe, 2006). This enables trees to face variable environmental conditions during many seasons, increasing the odds to find suitable conditions for seedling establishment. Therefore, at any given time, it seems more rewarding in terms of fitness to focus on traits related to survival rather than to reproduction. However, when thinking in adaptation to long-term changes like those predicted by climatic models, reproductive traits cannot be dismissed. In fact, enhancing and anticipating natural recruitment has been suggested as a tool for forest adaptation to climate change (Aitken *et al.*, 2008), but we need a much deeper knowledge of reproduction than we currently have in forest trees.

Reproductive traits have been extensively studied in seed orchards from breeding programs in a few commercially interesting forest trees (El-Kassaby & Cook, 1994; Burczyk & Chalupka, 1997; Nikkanen & Ruotsalainen, 2000; Bilir *et al.*, 2002; Lindgren *et al.*, 2004; Prescher *et al.*, 2007). Since panmixia (equilibrated inter-crossing among genotypes) is a basic assumption in seed orchard managent, deviations from panmixia either via male of female sexual functions are a matter of concern as they lower the genetic variability in the offspring (Kang *et al.*, 2003). As the final contribution to the next generation will be determined by the absolute number of seeds produced and sired by each tree, the effect of tree size in reproductive output is usually neglected in seed orchard studies, not least because trees in seed orchards are usually pruned, such that tree size can be artificially homogenised.

In natural stands, however, the relationship between size and reproduction, i.e. the allometry of reproduction, determines key life history traits (Thomas, 2011). First, the threshold size for first reproduction defines the onset of the reproductive stage for either male, female or both functions. Both under a migration scenario or when life expectancy lowers (for example due to disturbance), threshold size for reproduction is a key adaptive trait (Kozłowski, 1992). Second, during the adult stage, the relationship between Vegetative and Reproductive size defines the proportion of resources devoted to reproduction. According to allocation theory, growth, reproduction and defence trade-off against each other as a result of drawing resources from a common source (Reznick, 1992). Selection acting in a given environment will ultimately define which optimal share of resources, and at which moment, will be devoted to each function in order to maximize fitness (Roff, 1992). For example, a recent a review has described a global pattern towards earlier reproduction in plants as a result of climate change confirming that reproductive traits are central to adaptive micro-evolution (Munguía-Rosas *et al.*, 2011).

Thus considering or not the relationship between size and reproduction will ultimately depend on the questions to be answered. Here we define precocity either as the proportion of reproductive trees from a group (e.g. family or population) at a given time, irrespective of size. It is important to distinguish ontogenetic precocity along the developmental program from phenological precocity within years. Given that the onset of reproduction in plants is more strongly determined by size than by age (de Jong &

Klinkhamer, 2005; Weiner *et al.*, 2009), it seems adequate to try to separate to which extent onset of reproduction in a tree is determined by changes in size or in allometry. This can be achieved simply by including size as a covariate in statistical models, and is useful to standardise estimations of precocity between studies for which growth rates might differ.

Similarly, we define fruitfulness as the total reproductive output of an individual at a given time, irrespective of size. With cumulative reproductive investment we refer to the accumulated reproductive output throughout a certain period of the life of an individual. Total reproductive output will be largely determined by size, but it is possible for two equally sized trees to have a different investment in reproduction (Santos-del-Blanco *et al.*, 2013). Reproductive allocation is defined as the amount of resources devoted to reproduction relative to total resources (Karlsson & Mendez, 2005). For a given size, a greater investment in reproduction implies greater reproductive allocation. However, unless the relationship between reproduction and size (as a proxy to available resources) has a slope of 1, reproductive allocation will not be constant but also dependent on size, and again, differences in reproductive allocation between two individuals can be driven by size alone (de Jong & Klinkhamer, 2005). Log (reproduction) – log (size) allometric models, commonly known as RV models, have been recommended to model the relationship between reproduction and size due to their simplicity and flexibility (Klinkhamer *et al.*, 1992).

Interestingly, many conifer species used in forest breeding are monoecious, bearing independent male and female reproductive organs on single trees. This enables the separate study of both sexual functions, as well as the ratio of male to total reproduction (sex allocation) within individual trees, and to test ecological theories predicting differences in timing or intensity between sexual functions (Fox, 1993). For example, it is commonly assumed that wind-pollinated plants will tend to have more male-biased reproduction at larger sizes as tall plants are able to disperse pollen more efficiently (Klinkhamer *et al.*, 1997).

However, an added difficulty in the analysis of reproductive traits is their discontinuous nature. In the case of precocity or threshold for reproduction, individual trees either reproduce or not, following a binary variable. In the case of fruitfulness or RV models, reproduction is sometimes coded as counts of fruits or seeds if not in weight, and is typically strongly asymmetrical. Also when analysing sex ratio data, problems also arise from non-normal distribution of proportions (Wilson & Hardy, 2002). The analysis of discontinuous variables is easily accommodated by Generalised Linear Models (GLM) using logit or probit link functions for binary or proportions data or logarithmic or inverse gamma link functions for count data. GLMs can also be readily extended into Generalised Linear Mixed Models (GLMM) that include random variables. GLMMs are suitable for the analysis of hierarchical designs while avoiding pseudo-replication and also for variance partition, for example in genetic trials (Zuur, 2009; Nakagawa & Schielzeth, 2010). A major constraint in the use of GLMMs in the estimation of quantitative genetic parameters has been that standard formulas need to be modified, and have been rarely used until quite recently (Nakagawa & Schielzeth, 2010). However, these drawbacks should be solved if we are to calculate quantitative genetic parameters of reproductive traits, as cornerstones of evolutionary ecology providing relevant information about the genetic basis of phenotypic variation, the possibility for traits to be modified by natural or artificial selection, and the degree of current genetic differentiation among groups.

Along these guidelines we review the literature on quantitative genetics of reproduction in forest trees and propose a methodology for its standardised study, providing a case study with data from a *Pinus pinaster* provenance-progeny trial.

MATERIAL AND METHODS

Analysis of binary, proportion and count data

Binary data are frequent in field genetic trials and convey information about the presence or absence of relevant features like survival (Aparicio *et al.*, 2012), disease (Vivas *et al.*, 2011) or reproduction (Santos-del-Blanco *et al.*, 2013). Binary data are considered to have a low information content, as under the assumption of a polygenic basis of inheritance of such traits, we can only record two alternative phenotypes. The underlying continuous trait controlling the binary variable is known as liability, and cannot be observed directly. Instead, values of the liability above or below a certain threshold produce contrasting phenotypes, and thus they are also known as threshold traits (Falconer, 1989; Lynch & Walsh, 1998).

Due to their relevance, various methods for the analysis of binary data from genetic trials have been available since early times. Here, for the analysis of reproductive data we advocate the use of Generalised Linear Mixed Models as they can readily accommodate several explanatory covariates, like size, as well as confounding variables. In such models reproduction, coded as binary, is the response variable. In a typical half-sib design, family needs to be defined as a random variable for the estimation of additive genetic variance. Additive genetic variance in half-sib designs is estimated as four times the family variance, just as in models with Gaussian data. It is also possible to fit an individual-level variable instead and define a pedigree, as to obtain a matrix that is proportional to the variance-covariance structure of additive genetic effects. These models are known as animal models, as they have been mainly developed by animal breeders, and allow a direct estimation of additive genetic effects from the model. Other variables can also be included in the model in order to account for pseudoreplication e.g. block effects, to obtain variance estimates used in quantitative genetic formulae e.g. population effects for Qst or to model overdisperssion.

An important difference of binary models with respect to Gaussian models is that the error term is fixed to an arbitrary value depending on the statistical software used. Although this avoids a direct comparison of the values of variance estimates across studies, has little effect on variables that are derived from ratios of variance components like repeatability, heritability or Qst. Total variance, will thus be determined by the sum of variance components of the model (population, family, block, overdisperssion, error…), together with the fixed variance associated with the link function. For the analysis of binary data it is sensible to choose between logit or probit link functions. The variance of a logit distribution is π2/3, while that of a probit distribution is one. Heritability in the transformed (latent) scale in GLMMs is therefore calculated analogously to Gaussian models dividing additive genetic variance into total variance. Qst is calculated dividing population variance into the sum of population variance plus two times the additive genetic variance.

Proportion data are a special case of binary data. Proportions are commonly used to report on, for example, germination rates or herbivore or pathogen damage. When applied to the proportion of male relative to total reproduction (sex allocation or sex ratios), they are a powerful tool to test ecological theories. Proportion data can be arcsin or square root transformed to achieve normality, but using a single estimate might be statistically inefficient as important information regarding sample size is lost. For example, a proportion of 0.25 can be achieved with the germination of one out of four seeds or of a hundred out of four hundred, being in this last case the estimation more precise. GLMMs make use of all information by considering all untransformed data and thus are less prone to bias. As argued before, within-individual errors can be estimated from proportion data, and thus are not fixed in the model.

After the onset of reproduction, trees start producing a low number of reproductive structures and there is typically strong asymmetry among individuals. Poisson or inverse gamma models with logarithmic error distribution are commonly recommended to deal with such data. Quantitative genetic parameters derived from Poisson models are however more difficult to obtain as they depend on the average value of the dependent variable, e.g. average number of cones per individual in the data. Heritability in the latent scale with additive overdisperssion is calculated according to the formula:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2 + \ln(1/(\exp(\beta_0) + 1)}$$

Where σ_a^2 is the additive genetic variance, σ_e^2 corresponds to additive overdisperssion variance and β_0 is the model intercept on the link scale i.e. untransformed. β_0 informs about the average value of the variable, and thus heritability estimates depend on average trait values. In models with covariates, e.g. reproductive allometry models, β_0 informs about the expected reproductive value for an individual of size equal to 0. In this cases, it is possible to substitute β_0 by the actual average reproductive value in the population (Santos del Blanco et al in prep).

A worked example with *Pinus pinaster*

Data for the present study were obtained from the *Pinus pinaster* provenance-progeny common garden described in (Santos del Blanco et al 2012). The common garden comprises 2,767 trees from 194 open-pollinated families and 23 natural populations. Of the 2,767 trees initially planted, 2,240 trees were still alive at last measurement in 2010. Reproductive and growth-related traits (Table 1) were recorded in 2007, 2009 and 2010, covering the transitional period towards reproductive maturity in most of the trees.

General and Generalised Linear Mixed Models were fitted for size and reproductive data, respectively (Table 2). Bayesian inference as implemented in MCMCglmm R package, was preferred over restricted maximum likelihood methods as it allows a more accurate and intuitive estimation of errors of parameters that are derived from ratios of other parameters like heritability, Qst or the median threshold size for first reproduction. Animal models were used to estimate additive genetic variance directly from the model. Pedigree was constructed assuming that mothers (dams) within population were unrelated and that progenies were true half sibs. For vegetative size data (height, diameter at breast height and biomass) the inclusion of block by family interaction resulted in a high correlation between animal (individual tree) and error terms and model instability. Vegetative size data was instead modelled by substituting animal by family terms. Additive genetic variance was estimated as four times the family variance.

Different models were run with varying chain lengths, as necessary to achieve a population of 1000 independent (autocorrelation < 0.1) estimates per parameter. Lack of trends and convergence of the model was checked graphically and by using the Gelman-Rubin statistic comparing the results of two models that had the same specification. Here we report modes and 95% credible intervals for model parameters (Table 3).

Literature review

As a previous step to a meta-analysis on quantitative genetic parameters of reproductive traits in trees, we surveyed the scientific literature in search of articles reporting broad-sense or narrow-sense heritability, genetic correlations, additive coefficients of variation or Qst estimates. The search was aimed to be as through as possible and also included cited references in relevant articles, mostly in the forestry literature. A total of 29 articles were retrieved, most of them reporting on broad-sense heritabilities of conifer species, and particularly *Pinus spp*. obtained in seed orchards (Table 4). Studied traits were very diverse and included diverse estimates of male and female reproductive investment (fruitfulness), reproductive allocation, reproductive phenology and precocity.

TABLES

Table 1. Variables and measurement dates

Variable		Description
H	Height (cm)	Total height of each tree in 2007, 2009 and 2010
DBH	Diameter at breast Height	Diameter at breast height (1.30 m) measured in 2007, 2009 and 2010
Biomass	Biomass	Inferred from DBH (REF)
F	Female reproduction	Presence or absence of female cones in each tree, yearly from 2007
Fq	Cuantitative female reproduction	Number of seed cones in each tree, yearly from 2007
M	Male reproduction	Presence or absence of pollen cones in each tree in 2009 and 2010
Mq	Cuantitative male reproduction	Number of male cone clusters per tree counted in 15 seconds and modified by a factor proportional to cluster size. 2010

Table 2. Model specification

Response variable	Type	Concept modelled	Fixed terms	Random terms
H, DBH, Biomass	Gaussian	Growth	-	Population, Family, Block, Family by Block
F, M	Binary	Precociousness relative to size	Size (H, DBH or Biomass)	Population, animal, individual tree
$F_{(2008\text{-}2010)}$,$M_{(2009,2010)}$	Binary	Precociousness at a given age	-	Population, animal
Fq,Mq	Poisson	Fruitfulness relative to size (R-V)	Size (Biomass)	Population, additive overdisperssion, animal
Fq,Mq	Poisson	Fruitfulness (accumulated until 2010)	-	Population, additive overdisperssion, animal
Fq,Mq	Proportion	Sex allocation relative to size	Size (H,DBH, Biomass)	Population, additive overdisperssion, animal

Table 3. Narrow-sense heritability (h^2) and Q_{st} estimates for several vegetative and reproductive traits in *Pinus pinaster*. 95% CI, 95% credible interval. CE, covariate effect. ns, not significant.

	h^2		Q_{st}		CE
	mode	95% CI	mode	95% CI	
Growth					
H	0.13	(0.01-0.30)	0.21	(0.08-0.64)	
DBH	0.13	(0.03-0.34)	0.14	(0.04-0.48)	
Biomass	0.12	(0.02-0.28)	0.12	(0.03-0.42)	
Precociousness relative to size					
Female	0.43	(0.27-0.67)	0.13	(0.06-0.23)	+
Male	0.42	(0.28-0.53)	0.07	(0.02-0.14)	+
Precociousness at a given age					
F_{2008}	0.38	(0.07-0.59)	0.25	(0.07-0.61)	
F_{2009}	0.34	(0.16-0.54)	0.14	(0.05-0.35)	
F_{2010}	0.32	(0.15-0.50)	0.10	(0.03-0.26)	
M_{2009}	0.59	(0.41-0.74)	0.08	(0.04-0.20)	
M_{2010}	0.51	(0.3-0.70)	0.08	(0.03-0.19)	
Fruitfulness relative to size					
F RV	0.34	(0.19-0.62)	0.15	(0.06-0.34)	+
M RV	0.38	(0.23-0.63)	0.09	(0.04-0.23)	+
Fruitfulness 2010					
F Fr	0.36	(0.17-0.56)	0.11	(0.04-0.29)	
M Fr	0.48	(0.30-0.70)	0.09	(0.05-0.22)	
Sex allocation					
Sex allocation H	0.29	(0.12-0.43)	0.17	(0.08-0.38)	+
Sex allocation DBH	0.30	(0.13-0.45)	0.20	(0.09-0.38)	ns
Sex allocation Biomass	0.28	(0.15-0.47)	0.16	(0.08-0.36)	+

Table 4. Summary of heritability (narrow-sense for family, F, trials; broad-sense for clonal, C, trials) estimates reported in the literature for several tree species.

Species	Trait and (age)	Trial	Heritability (range)	Reference
Picea abies	Cone production (6 / 14)	C	0.14-0.24 / 0.37-0.55	(Almqvist *et al.*, 2001)
Picea abies	Male / female flowering abundance (not reported)	C	0.37 / 0.38	(Nikkanen & Ruotsalainen, 2000)
Pinus elliottii	Cone yield (14-17) / Flower production (4-6)	C	0.50 / 0.49	(Varnell *et al.*, 1967)
Pinus halepensis	Female flowering (3-4) / cone production (8-9)	C	0.86-0.83 / 0.43-0.41	(Matziris, 1997)
Pinus halepensis	Cone production (9-10)	F	0.68-0.57	(Matziris, 2000)
Pinus halepensis	Female reproductive allocation (11-15)	F	0.29-0.63	(Santos-del-Blanco *et al.*, 2010)
Pinus koraiensis	Male / female strobili (15-18)	C	0.34-0.56 / 0.20-0.46	(Choi *et al.*, 2004)
Pinus nigra	Cone production (11-13)	C	0.82-0.88	(Matziris, 1993)
Pinus pinaster	Male / female cone abundance (8-11)	C	0.39-0.59 / 0.76-0.82	(Merlo & Fernández López, 2004)
Pinus pinaster	Female reproductive allocation (5)	F	0.32-0.47	(Santos-del-Blanco *et al.*, 2010)
Pinus pinea	Logcone yield (1-10)	C	0.15	(Mutke *et al.*, 2005)
Pinus pinea	Logcone yield (9)	C	0.19	(Mutke *et al.*, 2003)
Pinus sylvestris	Cone production (several seed orchards, review)	C	0.24-0.78	(Prescher *et al.*, 2007)
Pinus sylvestris	Log male flowers, log female flowers, log females cones (13-15)	C	0.70 / 0.52-0.54 / 0.37-	(Sivacioglu *et al.*, 2009)
Pinus sylvestris	Male and female pollen and cone production (17-31)	C	0.36-0.54	(Savolainen *et al.*, 1993)
Pinus sylvestris	Male and female strobili number (10-19)	C	(0.00-0.15)- (0-0.16)	(Bilir *et al.*, 2006)

Pinus sylvestris	Sex ratio (male/female)	C	0.56	(Burczyk & Chalupka, 1997)
Pinus radiata	Male and female onset, ending and duration of flowering phenology (3-5)	C	Male 0.92 / 0.82 / 0.32 Female 0.93 / 0.84 / 0.83	(Codesido *et al.*, 2005)
Pinus taeda	Precocity (3-4) / Square root female cone production (2-8)	F	0.47-0.65 / 0.61	(Schmidtling, 1981)
Pinus taeda	Square root +0.5 Male and female cone production (6-18)	C	0.5-0.4	(Schmidtling, 1983)
Pinus taeda	Cone production (4-10), (8-20)	C	0.35-0.40	(Byram *et al.*, 1986)
Pinus contorta	Male and female flowering (11-29 / 10-11-29)	F	0.40-0.64 / 0.13-0.42	(Hannerz & Aitken, 2001)
Pinus virginiana	Fruitfulness	F	0.59-0.65	Bramlett 1971 cited in (Schmidtling, 1981)
Pseudotsuga menziesii	Cone / seed production (19)	C	0.38 / 0.40	(El-Kassaby & Cook, 1994)
Cryptomeria japonica	Male cones (7)	F	0.78-1.05	(Tsubomura *et al.*, 2012)
Eucalyptus cladocalyx	Precocity (3)	F	0.51	(Mora *et al.*, 2009)
Eucalyptus cladocalyx	Flowering intensity (8)	F	0.48	(Cané-Retamales *et al.*, 2010)
Eucalyptus globulus	Precocity (2-4)	F	0.21-0.93	(Jordan *et al.*, 1999)
Eucalyptus globulus	Presence or absence of reproduction (4)	F	0.47	(Chambers *et al.*, 1997)
Prunus avium	Advanced flowering / flower number / cherry number (6-7)	C	0.82 / 0.90 / 0.64	(Díaz & Merlo, 2008)

REFERENCE LIST

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

Almqvist C, Jansson G, Sonesson J. **2001**. Genotypic correlations between early cone-set and height growth in *Picea abies* clonal trial. *Forest Genetics* **8**: 197–204.

Aparicio A, Zuki S, Pastorino M, Martinez-Meier A, Gallo L. **2012**. Heritable variation in the survival of seedlings from Patagonian cypress marginal xeric populations coping with drought and extreme cold. *Tree Genetics & Genomes* **8**: 801–810.

Bilir N, Kang KS, Zang D, Lindgren D. **2002**. Fertility variation and gene diversity in clonal seed orchards of *Pinus brutia, Pinus nigra* and *Pinus sylvestris* in Turkey. *Silvae Genetica* **51**: 112–115.

Bilir N, Prescher F, Ayan S, Lindgren D. **2006**. Growth characters and number of strobili in clonal seed orchards of *Pinus sylvestris*. *Euphytica* **152**: 293–301.

Burczyk J, Chalupka W. **1997**. Flowering and cone production variability and its effect on parental balance in a Scots pine clonal seed orchard. *Annales des Sciences Forestières* **54**: 129–144.

Byram TD, Lowe WJ, Mcgriff JA. **1986**. Clonal and annual variation in cone production in loblolly pine seed. *Forest Science* **32**: 1067–1073.

Cané-Retamales C, Mora F, Vargas-Reeve F, Perret S, Contreras-Soto R. **2010**. Bayesian threshold analysis of breeding values, genetic correlation and heritability of flowering intensity in *Eucalyptus cladocalyx* under arid conditions. *Euphytica* **178**: 177–183.

Chambers P, Potts BM, Tilyard P. **1997**. The genetic control of flowering precocity in Eucalyptus globulus ssp . globulus. *Silvae Genetica* **46**: 207–214.

Choi W, Kang KS, Han S, Hur S. **2004**. Estimation of heritabilities and clonal contribution based on the flowering assessment in two clone banks of *Pinus koraiensis* Sieb. et Zucc. Breeding and genetic resources of five-needle pines:growth, adaptability and pest resistance.172–180.

Codesido V, Merlo E, Fernandez-Lopez J. **2005**. Variation in reproductive phenology in a *Pinus radiata* D. Don seed orchard in Northern Spain. *Silvae Genetica* **54**: 4–5.

Díaz R, Merlo E. **2008**. Genetic variation in reproductive traits in a clonal seed orchard of *Prunus avium* in northern Spain. *Silvae Genetica* **57**: 110–118.

El-Kassaby YA, Cook C. **1994**. Female reproductive energy and reproductive success in a Douglass-fir seed orchard and its impact on genetic diversity. *Silvae Genetica* **43**: 243–246.

Falconer D. **1989**. *Introduction to quantitative genetics.* New York. Longman.

Fox JJ. **1993**. Size and sex allocation in monoecious woody plants. *Oecologia* **94**: 110–113.

Hannerz M, Aitken SN. **2001**. Inheritance of strobili production and genetic correlation with growth in lodgepole pine. *Forest Genetics* **8**: 323–329.

De Jong TJ, Klinkhamer PGL. **2005**. *Evolutionary ecology of plant reproductive strategies*. Cambridge: Cambridge University Press.

Jordan GJ, Potts BM, Wiltshire RJER. **1999**. Strong, independent, quantitative genetic control of the timing of vegetative phase change and first flowering in *Eucalyptus globulus* ssp. *globulus* (Tasmanian blue gum). *Heredity* **83**: 179–187.

Kang KS, Bila AD, Harju AM, Lindgren D. **2003**. Estimation of fertility variation in forest tree populations. *Forestry* **76**: 329–344.

Karlsson PS, Mendez M. **2005**. The resource economy of plant reproduction. In: Reekie EG, Bazzaz FA, eds. Reproductive allocation in plants. Amsterdam, The Netherlands: Elsevier, 1–40.

Klinkhamer PGL, G L De Jong TJ, J Metz H. **1997**. Sex and size in cosexual plants. *Trends in Ecology and Evolution* **12**: 260–265.

Klinkhamer PGL, Meelis E, de Jong TJ, Weiner J. **1992**. On the analysis of size-dependent reproductive output in plants. *Functional Ecology* **6**: 308–316.

Kozłowski J. **1992**. Optimal allocation of resources to growth and reproduction: Implications for age and size at maturity. *Trends in Ecology and Evolution* **7**: 15–9.

Lindgren D, Cui J, Son SGU, Sonesson J. **2004**. Balancing seed yield and breeding value in clonal seed orchards. *New Forests* **28**: 11–22.

Lynch M, Walsh B. **1998**. *Genetics and Analysis of Quantitative Traits*. Sinauer Associates, Incorporated.

Matziris D. **1993**. Variation in cone production in a clonal seed orchard of black pine. *Silvae Genetica* **42**: 136–141.

Matziris D. **1997**. Variation in growth, flowering and cone production in a clonal seed orchard of Aleppo pine grown in Greece. *Silvae Genetica* **46**: 224–228.

Matziris D. **2000**. Genetic variation and realized genetic gain from Aleppo pine tree improvement. *Silvae Genetica* **49**: 2 – 3.

Merlo E, Fernández López J. **2004**. Análisis del balance parental reproductivo en un huerto semillero de *Pinus pinaster* Ait. *Investigación Agraria, Sistemas y Recursos Forestales* **13**: 387–398.

Mora F, Gleadow R, Perret S, Scapim CA. **2009**. Genetic variation for early flowering, survival and growth in sugar gum (*Eucalyptus cladocalyx* F. Muell) in southern Atacama Desert. *Euphytica* **169**: 335–344.

Munguía-Rosas MA, Ollerton J, Parra-Tabla V, De-Nova JA. **2011**. Meta-analysis of phenotypic selection on flowering phenology suggests that early flowering plants are favoured. *Ecology letters* **14**: 511–521.

Mutke S, Gordo F, Gil L. **2005**. Cone yield characterization of a stone pine (*Pinus pinea* L .) clone bank. *Silvae Genetica* **5**: 189–197.

Mutke S, Sada B, Iglesias S, Gil L. **2003**. Evaluación de la producción individual de piña en un banco clonal de pino piñonero (*Pinus pinea* L .) en Madrid. *Investigación Agraria, Sistemas y Recursos Forestales* **12**: 149–157.

Nakagawa S, Schielzeth H. **2010**. Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. *Biological Reviews of the Cambridge Philosophical Society* **85**: 935–956.

Nikkanen T, Ruotsalainen S. **2000**. Variation in flowering abundance and its impact on the genetic diversity of the seed crop in a Norway spruce seed orchard. *Silva Fennica* **34**: 205–222.

Petit RJ, Hampe A. **2006**. Some evolutionary consequences of being a tree. *Annual Review of Ecology, Evolution, and Systematics* **37**: 187–214.

Prescher F, Lindgren D, Almqvist C, Kroon J, Lestander TA, Mullin TJ. **2007**. Female fertility variation in mature *Pinus sylvestris* clonal seed orchards. *Scandinavian Journal of Forest Research* **22**: 280–289.

Price G. **1970**. Selection and covariance. *Nature* **227**: 520–521.

Reznick D. **1992**. Measuring the costs of reproduction. *Trends in Ecology & Evolution* **7**: 1990–1993.

Robertson A. **1966**. A mathematical model of the culling process in dairy cattle. *Animal Production* **8**: 95–108.

Roff DA. **1992**. *The evolution of life histories: theory and analysis*. New York, USA: Chapman and Hall.

Santos-del-Blanco L, Bonser SP, Valladares F, Chambel MR, Climent J. **2013**. Plasticity in reproduction and growth among 52 range-wide populations of a Mediterranean conifer: adaptive responses to environmental stress. *Journal of Evolutionary Biology* **26**: 1912–1924.

Santos-del-Blanco L, Notivol E, Zas R, Chambel MR, Majada J, Climent J. **2010**. Variation of early reproductive allocation in multi-site genetic trials of Maritime pine and Aleppo pine. *Forest Systems* **19**: 381–392.

Savolainen O, Karkkainen K, Katri Harju A, Anni Nikkanen T, Rusanen M. **1993**. Fertility variation in Pinus sylvestris: a test of sexual allocation theory. *American Journal of Botany* **80**: 1016–1020.

Schmidtling RC. **1981**. The inheritance of precocity and its relationship with growth in Loblolly pines. *Silvae Genetica* **30**: 188–192.

Schmidtling RC. **1983**. Genetic variation for fruitfulness in a Loblolly Pine (*Pinus taeda* L.) seed orchard. *Silvae Genetica* **32**: 76–80.

Sivacioglu A, Ayan S, Sezgin Çelik D. **2009**. Clonal variation in growth, flowering and cone production in a seed orchard of Scots pine (*Pinus sylvestris* L.) in Turkey. *African Journal of Biotechnology* **8**: 4084–4093.

Thomas SC. **2011**. Age-related changes in tree growth and functional biology: the role of reproduction. In: Meinzer FC, Lachenbruch B, Dawson TE, eds. Size-and age-related changes in tree structure and function. Springer Netherlands, 33–64.

Tsubomura M, Fukatsu E, Nakada R, Fukuda Y. **2012**. Inheritance of male flower production in *Cryptomeria japonica* (sugi) estimated from analysis of a diallel mating test. *Annals of Forest Science* **69**: 867–875.

Varnell RJ, Squillace E, Bengtson GW. **1967**. Variation and heritability of fruitfulness in slash pine. *Silvae Genetica* **16**: 125–128.

Vivas M, Zas R, Solla A. **2011**. Screening of Maritime pine (Pinus pinaster) for resistance to *Fusarium circinatum*, the causal agent of Pitch Canker disease. *Forestry* **85**: 185–192.

Weiner J, Campbell LG, Pino J, Echarte L. **2009**. The allometry of reproduction within plant populations. *Journal of Ecology* **97**: 1220–1233.

Wilson K, Hardy ICW. **2002**. Statistical analysis of sex ratios: an introduction. In: Wardy ICW, ed. Sex ratios: concepts and research methods. Crambridge, U.K.: Cambridge University Press, 48–93.

Zuur AF. **2009**. *Mixed effects models and extensions in ecology with R.*

APPENDIX VII

Ecología evolutiva de la reproducción en dos pinos mediterráneos: *Pinus pinaster* y *Pinus halepensis*. **Santos-del-Blanco L**, Chambel R, Notivol E. Alía R, Climent J. Proceedings 6º Congreso Forestal Español. Sociedad Española de Ciencias Forestales, Vitoria, 2013.

6º CONGRESO FORESTAL ESPAÑOL

6CFE01-192

Montes: Servicios y desarrollo rural
10-14 junio 2013
Vitoria-Gasteiz

Edita: Sociedad Española de Ciencias Forestales
Vitoria-Gasteiz, 10-14 junio de 2013
ISBN: 978-84-937964-9-5

Ecología evolutiva de la reproducción en dos pinos mediterráneos: *Pinus pinaster* y *Pinus halepensis*

SANTOS DEL BLANCO, L.[1,2], CHAMBEL, R.[1], NOTIVOL, E.[3], ALÍA, R.[1,2] y CLIMENT, J.[1,2]

[1] Departamento de Ecología y Genética Forestal, INIA-CIFOR,
[2] Instituto Universitario de Gestión Forestal Sostenible Uva-INIA
[3] Unidad de Recursos Forestales, Centro de Investigación y Tecnología Alimentaria de Aragón

Resumen

La adaptación de los árboles a su entorno está ligada a una adecuada programación de la reproducción, que a su vez está interrelacionada con el crecimiento y mantenimiento. Los ensayos genéticos forestales permiten testar las hipótesis de diferenciación y plasticidad para caracteres adaptativos tan relevantes como el tamaño umbral de reproducción y la alometría de la reproducción. Los resultados de varios ensayos de *Pinus pinaster* y *P. halepensis* en condiciones ambientales contrastadas corroboran una diferenciación entre procedencias y la existencia de variabilidad genética para caracteres reproductivos en ambas especies. En general, los ambientes de origen con un alto grado de continentalidad se relacionaron con una reproducción femenina precoz y más intensa en relación al tamaño. A su vez, unas condiciones más limitantes del ambiente de ensayo también tendieron a acelerar la reproducción. En *P. pinaster*, además, encontramos marcadas diferencias en la asignación sexual entre procedencias. Estos resultados demuestran compromisos entre crecimiento y reproducción, constatados a nivel genético mediante correlaciones genéticas negativas y a nivel fisiológico mediante experimentos de retirada de conos. Esta información es clave para entender la adaptación local y orientar el uso y conservación de los recursos genéticos de ambas especies.

Palabras clave

Genética forestal, diversidad genética, asignación reproductiva, adaptación local, caracteres de historia vital.

1. Introducción

Los caracteres de historia vital son aquellos que definen los patrones de desarrollo en cuanto a crecimiento, reproducción y supervivencia de un organismo (ROFF 1992). Estos caracteres están estrechamente ligados a la adaptación de los organismos al medio, principal materia de estudio de la ecología evolutiva y de gran relevancia en la gestión forestal sostenible. En el caso de especies de árboles forestales, existe un patrón general de ciclo vital definido por altas tasas de mortalidad juvenil, edad de reproducción tardía y alta longevidad, pero dentro de este patrón, existe un amplio abanico de variaciones íntimamente ligado con el ambiente (THOMAS 2011).

Los pinos mediterráneos, y especialmente *Pinus halepensis* Mill. y *P. pinaster* Ait., constituyen un modelo de estudio muy adecuado tanto desde puntos de vista teóricos como aplicados, de cara a conocer los procesos de adaptación al medio, pasados y futuros, de las especies forestales en España (LEV-YADUN & SEDEROFF 2000). Ello se debe a una serie de características de la biología de estas especies (NE'EMAN et al. 2004, 2011): edad temprana de reproducción, que permite evaluar caracteres reproductivos de forma relativamente precoz; asignación reproductiva elevada, que facilita la detección de costes de reproducción; escasa variabilidad interanual de la reproducción en comparación con otras especies más veceras, lo que posibilita hacer seguimientos con series de datos más continuas y con mejores propiedades estadísticas; mantenimiento variable de los conos femeninos cerrados (serotinia), lo que posibilita la reconstrucción de la reproducción en temporadas pasadas; separación entre reproducción masculina (estróbilos productores de polen) y femenina (conos o estróbilos), lo que hace posible testar hipótesis específicas respecto al papel de la reproducción masculina o femenina en estas especies; reproducción obligatoria por semillas, y no vegetativa, lo que facilita la asociación entre partes del árbol y funciones, e incrementando el valor adaptativo de la reproducción sexual; arquitectura de copa simple y por último, baja longevidad en comparación con otras especies forestales, mejorando las correlaciones entre la inversión reproductiva real a lo largo de toda la vida de un individuo y la estimada en una serie menor de años.

También son destacables las características ecológicas de estas especies, como su amplio nicho ecológico, sobre todo en *P. pinaster* (FADY 2012), que se traduce en un amplio rango de distribución y la posibilidad de testar la relación entre caracteres fenotípicos y ambientales. Otro factor importante de la ecología de estas especies es su relación con regímenes de perturbaciones, específicamente incendios forestales, que han dejado su impronta en adaptaciones como el espesor de corteza o la serotinia (KEELEY 2012).

Por último, la gran superficie ocupada por estas especies en España tanto de forma natural como por reforestación, pone de manifiesto su importancia ecológica. Estas masas, sin embargo, se ven afectadas por incendios forestales cada vez más virulentos y frecuentes lo que precisamente ha motivado varios estudios científicos sobre la ecología reproductiva de *P. halepensis* (DE LAS HERAS et al. 2007, ESPELTA et al. 2008, GONZALEZ-OCHOA et al. 2004, IRAIMA y ESPELTA 2004, MOYA et al. 2007, ORTIZ et al. 2011, VERKAIK y ESPELTA 2006) . Desde el punto de vista de la gestión forestal, se pretenden conocer los tratamientos selvícolas y condiciones en las que se produce una mayor cantidad de piñas de forma más precoz. De esta forma, asumiendo que una gran proporción de piñas en edades jóvenes son serótinas, se construye un banco de semillas aéreo suficiente para garantizar la regeneración post-incendio en caso de corto periodo de recurrencia del fuego. Las principales conclusiones de estos estudios son que las condiciones que aceleran el crecimiento de los árboles como clareos o podas (DE LAS HERAS et al. 2007, MOYA et al. 2007), fertilización artificial o alta calidad de estación (MOYA et al. 2007, ORTIZ et al. 2011), aceleran también la producción de piñas. Sin embargo, una producción de piñas mayor por árbol en masas poco densas, puede verse compensada por un mayor número de pies en masas más densas (MATYAS & VARGA 2000).

A pesar del indudable valor para la gestión forestal proporcionado por los citados estudios, su aportación al campo de la ecología se ve limitado por varios factores. En primer lugar, se considera la producción de piñas en valor absoluto por árbol o por superficie independientemente del tamaño del árbol. Así los árboles de mayor tamaño son los que normalmente producen más piñas, sin embargo la producción de piñas por unidad de biomasa es mayor en árboles pequeños (ORTIZ et al. 2011). El segundo problema se da en estudios que comparan diferentes procedencias de regeneración natural. En este caso, no es posible discriminar entre causas genéticas y/o ambientales que expliquen las correlaciones de la reproducción con variables climáticas. Para conocer de forma sistemática si existe variación genética entre procedencias en caracteres reproductivos, una muestra representativa de árboles de las procedencias a comparar deben crecer en condiciones ambientales lo más homogéneas posible.

Los ensayos genéticos forestales de ambiente común (*common gardens*) cumplen precisamente la función de proporcionar condiciones ambientales homogéneas, bajo las que se comparan entradas genéticas que pueden ser de rango poblacional (ensayo de procedencias), familiar (ensayo de progenies) o individual (ensayo clonal). Estos ensayos pueden llevarse a cabo en condiciones de campo o de invernadero. Los ensayos de progenies, además, permiten el cálculo de parámetros de genética cuantitativa que describan la arquitectura y grado de determinación genéticos de los caracteres estudiados.

Los caracteres más comúnmente estudiados en pinos mediterráneos en ensayos de ambiente común han sido por un lado el crecimiento (ALÍA et al. 1995, CHAMBEL et al. 2007) y por otro, caracteres fisiológicos de valor adaptativo, como la resistencia al frío (CLIMENT et al. 2009), a la sequía (ARANDA et al. 2010, VOLTAS et al. 2008) o fenología (CODESIDO y FERNANDEZ-LOPEZ 2009). Desde un punto de vista evolutivo, un carácter se considera adaptativo si tiene una influencia positiva en el número de descendientes producidos por un organismo. De ello se deriva la gran importancia de cuantificar también los caracteres reproductivos, situados de esta forma en un nivel jerárquico superior de relevancia.

La evidencia empírica sobre la estrecha relación entre condiciones ambientales naturales o influidas por el hombre y caracteres reproductivos es abundante en numerosos tipos de organismos (ROFF 1992, STEARNS 1992). Los pinos mediterráneos *P. pinaster* y *P. halepensis*, no parecen ser una excepción, y varios trabajos describen caracteres reproductivos de historia vital en ambas especies (CLIMENT et al. 2008, SANTOS-DEL-BLANCO et al. 2010, 2012, TAPIAS et al. 2004).

2. Objetivos

Los objetivos del presente trabajo son testar las hipótesis básicas de la teoría de historia vital aplicadas al caso de especies mediterráneas forestales como son *P. pinaster* y *P. halepensis* y hacer una lectura práctica de los resultados de cara a la gestión forestal. En concreto, pretendemos (1) cuantificar la diferenciación entre procedencias para caracteres de historia vital relacionados con la reproducción (tamaño umbral de reproducción, alometría reproductiva, esfuerzo reproductor), (2) comprobar la relación entre los citados caracteres y variables ambientales en términos de variación, (3) cuantificar la existencia de variación genética aditiva dentro de procedencias para caracteres de historia vital mediante herramientas de genética cuantitativa y (4) estudiar patrones de compensación (*tradeoffs*) entre caracteres de historia vital reproductivos y caracteres de crecimiento vegetativo

3. Metodología

3.1 Ensayos de campo

Para *Pinus halepensis* utilizamos un ensayo de procedencias y otro de procedencias-progenies. El ensayo de procedencias fue instalado en 1997, estando replicado en seis localidades, dos de las cuales se incluyen en este trabajo (Valdeolmos, Madrid –P24OLM- y Rincón de Ademuz, Valencia-P24ADE-). En ellos están representadas 52 procedencias nativas de la especie a lo largo de todo su rango de distribución en España continental, Islas Baleares, Italia, Francia, Túnez y Grecia. (ver CLIMENT et al. 2008 para más detalles). Los sitios elegidos presentan condiciones ambientales contrastadas. El sitio de ensayo OLM tiene veranos cálidos e inviernos suaves, siendo el suelo arenoso y profundo. Por el contrario, en ADE los inviernos son fríos y el suelo es rocoso y poco profundo. Debido a la combinación de factores climáticos y edáficos, el sitio de ensayo de ADE es mucho más limitante para el crecimiento vegetativo que OLM.

El ensayo de procedencias-progenies fue instalado en 1995, estando replicado en dos localidades, ambas incluidas en este trabajo (Megeces, Valladolid —F24MEG—, y Montañana, Zaragoza —F24MON—). En ellos están representadas 148 familias de polinización abierta de 32 procedencias repartidas por el rango de distribución de la especie en la Península Ibérica e Islas Baleares. Aquí también existen diferencias entre ambos sitios: F24MEG está situado en un suelo calizo poco profundo y en ligera pendiente, mientras que F24MON está situado en un valle aluvial fértil y con disponibilidad hídrica durante el verano (ver SANTOS-DEL-BLANCO et al. 2010 para más detalles). Adicionalmente tanto en el ensayo de procedencias como en el de procedencias-progenies, se incluyeron progenies de tres rodales de origen desconocido plantados en la provincia de Valladolid que no han sido considerados en el presente trabajo.

Para *Pinus pinaster* hicimos uso de un ensayo de procedencias-progenies instalado en 2005 y replicado en cinco sitios de ensayo, utilizando uno de ellos en este trabajo (A Merca, Orense –F26MER). El ensayo contiene 194 familias de polinización abierta de 23 procedencias naturales distribuidas sobre gran parte del rango de la especie, incluyendo Península Ibérica Atlántica, Francia Atlántica, Córcega, España Mediterránea y Marruecos. Las condiciones ambientales del ensayo se consideran intermedias respecto al nicho ecológico de la especie, pues se sitúa en una zona de transición entre clima Mediterráneo y Atlántico (ver SANTOS-DEL-BLANCO et al. 2012 para más detalles). Todos los ensayos utilizados en este trabajo pertenecen a la red nacional de ensayos genéticos forestales GENFORED.

3.2 Toma de datos

Se tomaron datos de variables representativas del crecimiento vegetativo (altura total, diámetro normal) y reproductivas en los diferentes ensayos de campo a edades que variaron entre los 5 y los 15 años (Tabla 1). La altura total se midió con pértiga telescópica y el diámetro normal con forcípula. Dependiendo del ensayo, se discriminaron las piñas por cohortes en función de su tamaño y color: los estróbilos femeninos formados en la primavera del año en curso son de color rojo y de pequeño tamaño (aprox. 1 cm), al comienzo de la primavera siguiente aumentan su tamaño y toman un color verde (aprox 2-3 cm) y al final de la segunda primavera han completado su desarrollo final pero conservan el color verde, que perderán poco a poco para ser color marrón brillante al comienzo de la tercera primavera. A partir de entonces, las zonas de las piñas más expuestas al sol irán degradando su color tomando colores grisáceos cada vez más claros (GIL et al. 2009). Dado el aún limitado desarrollo de los árboles en la mayoría de los ensayos (F26MER, F24MEG, P24OLM, P24ADE), fue posible distinguir las piñas por cohortes. En MON, sin embargo, el elevado porte y las copas adyacentes de los árboles impidieron distinguir entre cohortes y se estimó el número total de piñas por árbol contadas en 15 segundos (KNOPS & KOENIG 2012). La reproducción masculina sucede a la femenina en el desarrollo de *Pinus halepensis* (NE’EMAN et al. 2004) pero no así en *P. pinaster* (SANTOS-DEL-BLANCO et al. 2012), indicando un mayor interés de la reproducción masculina precoz en esta última especie. Se recogieron datos semi-cuantitativos de reproducción masculina en los ensayos F26MER y F24MEG. El método aplicado fue el conteo de agrupaciones de conos masculinos en 15 segundos y paralelamente aplicar una escala cuantitativa con valores del 1 al 5 dependiendo del tamaño y densidad de dichas agrupaciones. Esta valoración cualitativa se utilizó para ponderar los datos cuantitativos.

A partir de las coordenadas geográficas de las procedencias incluidas en los ensayos, se obtuvieron variables climáticas de temperatura mediante los modelos de clima de GONZALO-JIMÉNEZ (2010) para procedencias de España Peninsular y de HIJMANS et al. (2005) para el resto de procedencias.

Tabla 1. Ensayos de campo de Pinus pinaster y P. halepensis utilizados en este trabajo, y caracteres medidos con edad de medición entre paréntesis

Especie	Ensayo	Variables medidas (edad)
Pinus pinaster	F26MER	Altura total (6), diámetro normal (6), reproducción masculina (5) y femenina anuales cuantitativas (5)
Pinus halepensis	F24MEG	Altura total (15), diámetro normal (15), reproducción femenina anual cuantitativa (15)
Pinus halepensis	F24MON	Altura total (15), diámetro normal (15), reproducción femenina total cuantitativa (15)
Pinus halepensis	P24OLM	Altura total (13), diámetro normal (13), reproducción femenina anual cuantitativa (13)
Pinus halepensis	P24ADE	Altura total (12), diámetro normal (12), reproducción femenina anual cuantitativa (12)

La biomasa total de los árboles se calculó a partir de ecuaciones alométricas (MONTERO et al. 2005). El tamaño umbral de reproducción a nivel de procedencia se derivó combinando medidas de crecimiento vegetativo y presencia o ausencia de reproducción masculina o femenina en modelos binomiales (SANTOS-DEL-BLANCO et al. 2010, 2012).

3.3 Análisis de datos

Para testar la hipótesis de diferenciación entre procedencias para los caracteres de interés, ajustamos modelos mixtos generales para las variables continuas (altura total y diámetro normal) y generalizados para las discontinuas (binomial para reproducción en forma categórica; Poisson para reproducción en forma cuantitativa). De estos modelos se extrajeron las estimaciones medias por procedencia y/o familia para el cálculo de correlaciones ambientales y entre caracteres.

La relación entre valores fenotípicos medios por procedencia y variables climáticas propias de las zonas de origen se comprobó mediante correlaciones de Pearson. Como variable integradora de la condiciones ambientales, se eligió el índice de continentalidad o rango anual de temperaturas (temperaturas máximas – temperaturas mínimas). Un índice de continentalidad alto está asociado a un periodo de crecimiento vegetativo corto y viceversa (SANTOS-DEL-BLANCO et al. 2012).

En aquellos ensayos con representación de progenies (familias), se cuantificó la existencia de varianza genética aditiva dentro de procedencias incluyendo en modelos mixtos el factor familiar además de corregir por las posibles diferencias medias entre procedencias. Para los caracteres de crecimiento se incluyó en la parte fija la estructura de bloques del diseño para reducir el ruido ambiental. Este factor fue eliminado para el cálculo de parámetros genéticos para tener estimaciones más cercanas a las que serían observables en la naturaleza. Para caracteres reproductivos se incluyó como covariable la biomasa de los árboles. De esta forma los modelos reflejan la variación en reproducción entre familias y procedencias debidas a causas diferentes a la variación en tamaño. A partir de los componentes de varianza de modelos mixtos aplicados a datos de procedencias-progenies se calculó la heredabilidad en sentido estricto (h^2), diferenciación entre procedencias (Q_{ST}) y coeficiente de variación de varianza genética aditiva (CV_A) (FALCONER 1989).

La compensación a nivel fisiológico y genético entre caracteres de crecimiento vegetativo y reproductivos (*tradeoffs*) se calculó a tres niveles: individual dentro de procedencias y familias (correlaciones fenotípicas), familiar dentro de procedencias (correlaciones genéticas) y a nivel de procedencia mediante modelos mixtos y correlaciones de Pearson.

4. Resultados

4.1. Diferenciación entre procedencias

Tanto los caracteres de crecimiento como los reproductivos mostraron diferencias generalizadas entre procedencias para ambas especies, aunque estas diferencias fueron algo menores para caracteres de crecimiento en *P. halepensis* (Tabla 2).

4.2. Correlaciones ambientales

Los caracteres reproductivos, en especial el tamaño umbral de reproducción femenino, mostraron correlaciones positivas con el índice de continentalidad mientras que en los caracteres de crecimiento vegetativo las correlaciones fueron negativas en *P. pinaster* y en el ensayo de *P. halepensis* con los árboles más desarrollados (P24MON). En el resto de ensayos de *P. halepensis*, las correlaciones no fueron significativas. En resumen, valores crecientes del índice de continentalidad se relacionaron con menor crecimiento vegetativo y mayor inversión en reproducción femenina (Tabla 3).

4.3. Parámetros genéticos

Se hallaron valores altos de heredabilidad para caracteres de crecimiento y reproductivos en el ensayo de *P. pinaster*, mientras que para el ensayo menos desarrollado de procedencias-progenies de *P. halepensis* (F24MEG), los valores fueron moderados. En el ensayo más desarrollado (F24MON), la variación sólo fue significativa entre procedencias y no entre familias de una misma procedencia (Tabla 4).

4.4 Compensación entre funciones (*tradeoffs*)

Las correlaciones entre crecimiento vegetativo y reproducción femenina en ambas especies fueron positivas a nivel fenotípico individual (r_{ph}) y negativas a nivel genético (r_a y r_{pr}). En éste último caso, la correlación a nivel de procedencia fue de mayor magnitud.

Tabla 2. Valores de significatividad del efecto de la procedencia sobre diferentes variables relacionadas con el crecimiento vegetativo y la reproducción masculina y femenina para Pinus pinaster y P.halepensis en varios sitios de ensayo. H, altura total; DBH, diámetro normal; biom, biomasa; TSRf, tamaño umbral de reproducción femenino; TSRm, tamaño umbral de reproducción masculino; Rep.f., reproducción femenina cuantitativa; Rep.m. reproducción masculina cuantitativa. n.d., dato no disponible

Especie	**Ensayo**	**H**	**DBH**	**biom**	**TSRf**	**TSRm**	**Rep.f.**
P. pinaster	F26MER	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
P.halepensis	F24MEG	<0.001	0.318	0.048	<0.001	0.004	<0.001
P.halepensis	F24MON	0.013	0.002	0.012	n.d.	n.d.	<0.001
P.halepensis	P24OLM	<0.001	<0.001	<0.001	<0.001	n.d.	<0.001
P.halepensis	P24ADE	<0.001	<0.001	<0.001	<0.001	n.d.	<0.001

Tabla 3. Coeficientes de correlación ente valores del índice de continentalidad y diferentes variables relacionadas con el crecimiento vegetativo y la reproducción masculina y femenina para Pinus pinaster y P. halepensis en varios sitios de ensayo. Valores en negrita indican correlaciones significativamente ($P < 0.05$) diferentes de 0. Ver abreviaturas en Tabla 2.n.d., dato no disponible

Especie	**Ensayo**	**H**	**DBH**	**biom**	**TSRf**	**TSRm**	**Repf**	**Repm**
P. pinaster	F26MER	**-0.80**	**-0.65**	**-0.70**	**-0.71**	0.00	0.40	**-0.50**
P.halepensis	F24MEG	0.09	**-0.40**	0.33	**-0.51**	-0.16	**0.48**	0.10
P.halepensis	F24MON	**-0.37**	**-0.43**	**-0.41**	n.d.	n.d.	**0.45**	n.d.
P.halepensis	P24OLM	0.03	0.01	-0.04	**-0.53**	n.d.	**0.53**	n.d.
P.halepensis	P24ADE	-0.02	0.02	0.06	**-0.40**	n.d.	**0.33**	n.d.

Tabla 4. Parámetros genéticos (h^2, heredabilidad, Q_{ST}, diferenciación ente procedencias CV_A, coeficiente de varianza genética aditiva) para altura (H), biomasa (biom) y reproducción femenina (TSRf) para Pinus pinaster y P. halepensis en varios sitios de ensayo. Ver abreviaturas en Tabla 2. n.d., dato no disponible

Especie	**Ensayo**	**H**			**biom**			**TSRf**	
		h^2	Q_{ST}	CV_A	h^2	Q_{ST}	CV_A	h^2	Q_{ST}
P. pinaster	F26MER	0.60	0.08	3.56	0.61	0.05	0.43	0.69	0.13
P.halepensis	F24MEG	0.12	0.15	1.05	0.11	0.12	0.89	0.14	0.26
P.halepensis	F24MON	n.s.	n.s.	n.s.	0.05	0.19	0.05	n.d.	n.d.

Tabla 5. Correlaciones fenotípicas a nivel individual (r_{ph}) y genéticas a nivel familiar (r_a) y a nivel de procedencia (r_{pr}) entre valores de biomasa y reproducción femenina después de descontar el efecto del tamaño individual. n.d., dato no disponible

Especie	Ensayo	r_{ph}	p.valor	r_a	p.valor	r_{pr}	p.valor
P. pinaster	F26MER	**0.23**	<0.001	**-0.14**	0.047	**-0.45**	0.031
P.halepensis	F24MEG	**0.46**	<0.001	**-0.15**	0.073	0.14	0.468
P.halepensis	F24MON	**0.31**	<0.001	0.01	0.946	**-0.46**	0.011
P.halepensis	P24OLM	**0.33**	<0.001	n.d.	n.d.	**-0.39**	0.004
P.halepensis	P24ADE	**0.36**	<0.001	n.d.	n.d.	-0.12	0.375

5. Discusión

Los resultados basados en ensayos de campo para procedencias de todo el rango de distribución de *P. pinaster* (un ensayo) y *P. halepensis* (cuatro ensayos) demuestran una estrecha relación entre los caracteres reproductivos y el ambiente de origen de las procedencia así como ente caracteres reproductivos y de crecimiento entre sí.

La existencia de variaciones para caracteres adaptativos a nivel de procedencia dentro de especies es un hecho admitido para aquellas especies en las que se reconocen subespecies (e.g. *Pinus nigra*) o para las que presentan amplia diferenciación genética neutral ente procedencias (*P. pinaster*), pero es menos conocido cuando se trata de especies que no cumplen estas condiciones (*P. halepensis*). Sin embargo, incluso en la Península Ibérica, donde la variación genética de *P. halepensis* es menor debido a la historia de migración de la especie (GRIVET et al. 2009), la diferenciación entre procedencias también está presente (Tabla 2). Así, salvo casos excepcionales como *P. pinea* (MUTKE et al. 2010), la variación intraespecífica es un hallazgo común. Este hecho pone de manifiesto la relevancia de los datos obtenidos en ensayos de ambiente común para caracterizar los materiales de base de cara a la gestión forestal sostenible.

La mencionada variación intraespecífica descrita tanto para caracteres de crecimiento como reproductivos en *P. pinaster* y *P. halepensis* no es aleatoria sino que está relacionada con las condiciones ambientales en las que las procedencias han evolucionado, sugiriendo que los caracteres estudiados tienen valor adaptativo y que se han producido (y/o están produciendo) fenómenos de adaptación local. Los resultados obtenidos concuerdan plenamente con las predicciones derivadas de la teoría de historia vital, que postulan un mayor tamaño umbral de reproducción en aquellos ambientes favorables para el crecimiento (ROFF 1992).

Sin embargo, a pesar de que exista abundante evidencia de la influencia del ambiente local, por un lado, éste no es estático (LINDNER et al. 2010) y por otro, los mecanismos de adaptación de las especies suelen estar desfasados respecto a los cambios ambientales (REHFELDT et al. 1999). La selección natural actúa sobre fenotipos, que son el resultado de la interacción del genotipo y el ambiente. La influencia del ambiente es variable para diferentes caracteres, estando el crecimiento vegetativo más influenciado por la heterogeneidad espacial a pequeña escala (ZAS 2006) que los caracteres alométricos de reproducción (SANTOS-DEL-BLANCO et al. 2012). Dado que sólo las diferencias genéticas pueden promover el cambio evolutivo, una mayor correlación entre genotipo y fenotipo implica una mayor eficiencia de los fenómenos de selección en la naturaleza. En el presente caso de estudio, las heredabilidades ligeramente superiores para el tamaño umbral de reproducción femenino que para caracteres de crecimiento (altura, biomasa) hacen pensar en una selección más eficiente para caracteres reproductivos que de crecimiento, aunque el criterio de selección natural sea el número de descendientes de un individuo que llegan a edad adulta (*fitness*). La mayor diferenciación entre poblaciones encontrada para caracteres reproductivos encaja con esta visión.

Sin embargo, ambos conjuntos de caracteres no son independientes entre sí sino que su expresión en los organismos depende de unos recursos finitos por los que las funciones compiten. Estas restricciones se conocen como compensaciones o tradeoffs. Para un nivel de recursos fijo, un aumento en la inversión en reproducción suele implicar una menor inversión en crecimiento (ROFF 2000). A nivel individual, las compensaciones pueden no ser evidentes puesto que no todos los árboles tienen acceso al mismo nivel de recursos. Así dentro de una población, un árbol con acceso a más recursos puede alcanzar un mayor tamaño y al mismo tiempo producir un mayor número de conos que otro. Esto a su vez, produce una correlación positiva entre crecimiento y reproducción para el conjunto de la población (DE JONG & KLINKHAMER 2005). De hecho, los tratamientos encaminados a aumentar la producción de piñas en una masa (claras, podas, fertilización), explotan esta relación entre mayor tamaño y mayor producción de piñas (DE LAS HERAS et al. 2007, GONZALEZ-OCHOA et al. 2004, ORTIZ et al. 2011). Por el contrario, a nivel familiar o de poblaciones, una correlación negativa indica incompatibilidades más o menos fuertes entre funciones a nivel genético. De esta forma, una selección o mejora genética para crecimiento implica una menor inversión en reproducción; aunque, dado que la correlación no es perfecta (menor que |1|), sería posible la selección para el aumento en crecimiento y reproducción simultáneamente.

6. Conclusiones

Los datos sobre caracteres reproductivos en el inicio de la reproducción en poblaciones españolas y del resto del rango de distribución de *P. pinaster* y *P. halepensis* obtenidos en ensayos de ambiente común han permitido describir un importante grado de diferenciación entre poblaciones, normalmente ligada a las condiciones ambientales de origen y que ilustra la acción de procesos de selección natural. Bajo un nuevo paradigma de gestión enfocado a aumentar la resiliencia de las masas (DE LAS HERAS et al. 2007) tienen también cabida actuaciones por parte de la genética forestal, informando sobre las cualidades de los materiales de base, describiendo patrones ecotípicos y posibilitando la mejora genética para el aumento de la resiliencia (*breeding for resilience*).

7. Agradecimientos

Agradecemos la asistencia técnica de Fernando del Caño, Diana Barba, David Lafuente, Eduardo Ballesteros, Isabel Rodríguez, Abel Blanco, Elena Alamillo. Este trabajo ha sido financiado por los proyectos VAMPIRO (CGL2008-05289-C02-02), MITIGENFOR (RTA 2011-00016), TREESNIPS (QLK3-CT2002-01973), AT07-002 y AT10-007. Los datos usados en este trabajo forman parte de la red nacional de ensayos genéticos forestales GENFORED (www.genfored.es).

8. Bibliografía

ALÍA, R.; GIL, L.; PARDOS, J.A.; 1995. Performance of 43 *Pinus pinaster* Ait. provenances on 5 locations in Central Spain. Silvae Genet. 44 75-81.

ARANDA, I.; ALÍA, R.; ORTEGA, U.; DANTAS, Â.K.; MAJADA, J.; 2010. Intra-specific variability in biomass partitioning and carbon isotopic discrimination under moderate drought stress in seedlings from four *Pinus pinaster* populations. Tree Genet. Genomes. 6 169-178.

CHAMBEL, M.R.; CLIMENT, J.; ALÍA, R.; 2007. Divergence among species and populations of Mediterranean pines in biomass allocation of seedlings grown under two watering regimes. Ann For. Sci. 64 87-97.

CLIMENT, J.; COSTA E SILVA, F.; CHAMBEL, M.R.; PARDOS, M.; ALMEIDA, M.H.; 2009. Freezing injury in primary and secondary needles of Mediterranean pine species of contrasting ecological niches. Ann For. Sci. 66 407-407.

CLIMENT, J.; PRADA, M.A.; CALAMA, R.; CHAMBEL, M.R.; DE RON, D.S.; ALÍA, R.; 2008. To grow or to seed: Ecotypic variation in reproductive allocation and cone production by young female Aleppo pine (*Pinus halepensis*, *Pinaceae*). Am. J. Bot. 95 833-842.

CODESIDO, V.; FERNANDEZ-LOPEZ, J.; 2009. Genetic variation in seasonal growth patterns in radiata pine in Galicia (northern Spain). Forest Ecol. Manag. 257 518-526.

DE JONG, T.J.; KLINKHAMER, P.G.L.; 2005. Evolutionary ecology of plant reproductive strategies. Cambridge University Press. Cambridge.

DE LAS HERAS, J.; MOYA, D.; LÓPEZ-SERRANO, F.R.; CONDÉS, S.; 2007. Reproduction of postfire *Pinus halepensis* Mill. stands six years after silvicultural treatments. Ann For. Sci. 64 59-66.

ESPELTA, J.M.; VERKAIK, I.; MÀRCIA, E.; LLORET, F.; 2008. Recurrent wildfires constrain long-term reproduction ability in *Pinus halepensis* Mill. Int. J. Wildland Fire 17 579-585.

FADY, B.; 2012. Biogeography of neutral genes and recent evolutionary history of pines in the Mediterranean Basin. Ann For. Sci. 421-428.

FALCONER, D.; 1989. Introduction to quantitative genetics. New York. Longman.

GIL, L.; LÓPEZ, R.; GARCÍA-MATEOS, A.A.; GONZÁLEZ-DONCEL, I.; 2009. Seed provenance and fire-related reproductive traits of *Pinus pinaster* in central Spain. Int. J. Wildland Fire 18 1003-1009.

GONZALEZ-OCHOA, A.I.; LÓPEZ-SERRANO, F.R.; DE LAS HERAS, J.; 2004. Does post-fire forest management increase tree growth and cone production in *Pinus halepensis*? Forest Ecol. Manag. 188 235-247.

GONZALO-JIMÉNEZ, J.; 2010. Diagnosis fitoclimática de la España Peninsular: hacia un modelo de clasificación funcional de la vegetación y de los ecosistemas peninsulares españoles. Organismo Autónomo de Parques Nacionales.

GRIVET, D.; SEBASTIANI, F.; GONZALÉZ-MARTÍNEZ, S.C.; VENDRAMIN, G.G.; 2009. Patterns of polymorphism resulting from long-range colonization in the Mediterranean conifer Aleppo pine. New Phytol. 184 1016-1028.

HIJMANS, R.J.; CAMERON, S.E.; PARRA, J.L.; JONES, P.G.; JARVIS, A.; 2005. Very high resolution interpolated climate surfaces for global land areas. Int. J. Climatol. 25 1965-1978.

IRAIMA, V.; ESPELTA, J.M.; 2004. Effect of thinning and post-fire regeneration age on the reproductive characteristics of *Pinus halepensis* Mill. forests. J. Ecol. 2 1-7.

KEELEY, J.E.; 2012. Ecology and evolution of pine life histories. Ann For. Sci. 445-453.
KNOPS, J.M.H.; KOENIG, W.D.; 2012. Sex allocation in california oaks: trade-offs or resource tracking? PloS one 7 e43492.

LEV-YADUN, S.; SEDEROFF, R.; 2000. Pines as model gymnosperms to study evolution, wood formation, and perennial growth. J. Plant Growth Regul. 19 290-305.

LINDNER, M.; MAROSCHEK, M.; NETHERER, S.; KREMER, A.; BARBATI, A.; GARCIA-GONZALO, J.; SEIDL, R.; DELZON, S.; CORONA, P.; KOLSTRÖM, M.; LEXER, M.J.; MARCHETTI, M.; 2010. Climate change impacts, adaptive capacity, and vulnerability of European forest ecosystems. Forest Ecol. Manag. 259 698-709.

MATYAS, C.; VARGA, G.; 2000. Effect of intra-specific competition on tree architecture and aboveground dry matter allocation in Scots pine. Forest Syst. 9 111-119.

MONTERO, G.; RUIZ-PEINADO, R.; MUÑOZ, M.; 2005. Producción de biomasa y fijación de CO2 por los bosques españoles. Instituto Nacional de Investigacion y Tecnología Agraria y Alimentaria.

MOYA, D.; ESPELTA, J.M.; M VERKAIK, I.; LÓPEZ-SERRANO, F.R.; DE LAS HERAS, J.; 2007. Tree density and site quality influence on *Pinus halepensis* Mill. reproductive characteristics after large fires. Ann For. Sci. 64 649-656.

MUTKE, S.; GORDO, J.; CHAMBEL, M.R.; PRADA, M.A.; ÁLVAREZ, D.; IGLESIAS, S.; GIL, L.; 2010. Phenotypic plasticity is stronger than adaptative differentiation among Mediterranean stone pine provenances. Forest Syst. 19 354-366.

NE'EMAN, G.; GIDI GOUBITZ, S.; SHIRRINKA NATHAN, R.; 2004. Reproductive traits of *Pinus halepensis* in the light of fire–a critical review. Plant Ecol. 171 69-79.

NE'EMAN, G.; GOUBITZ, S.; WERGER, M.J.A.; SHMIDA, A.; 2011. Relationships between tree size, crown shape, gender segregation and sex allocation in *Pinus halepensis*, a Mediterranean pine tree. Ann. Bot. 108 197-206.

ORTIZ, O.; OJEDA, G.; ESPELTA, J.M.; ALCANIZ, J.M.; 2011. Improving substrate fertility to enhance growth and reproductive ability of a *Pinus halepensis* Mill. afforestation in a restored limestone quarry. New Forest. 43 365-381.

REHFELDT, G.E.; E.; YING, C.C.; C.; SPITTLEHOUSE, D.L.; L.; HAMILTON, D.A.; HAMILTON JR, D.A.; A; 1999. Genetic responses to climate in *Pinus contorta*: niche breadth, climate change, and reforestation. Ecol. Monogr. 69 375-407.

ROFF, D.A.; 1992. The evolution of life histories: theory and analysis. Chapman and Hall. New York.

ROFF, D.A.; 2000. Trade-offs between growth and reproduction: an analysis of the quantitative genetic evidence. J. Evolution. Biol. 13 434-445.

SANTOS-DEL-BLANCO, L.; CLIMENT, J.; GONZÁLEZ-MARTÍNEZ, S.C.; PANNELL, J.R.; 2012. Genetic differentiation for size at first reproduction through male versus female functions in the widespread Mediterranean tree *Pinus pinaster*. Ann. Bot. 110 1449-1460.

SANTOS-DEL-BLANCO, L.; NOTIVOL, E.; ZAS, R.; CHAMBEL, M.R.; MAJADA, J.; CLIMENT, J.; 2010. Variation of early reproductive allocation in multi-site genetic trials of Maritime pine and Aleppo pine. Forest Syst. 19 381-392.

STEARNS, S.C.; 1992. The evolution of life histories. Oxford University Press.

TAPIAS, R.; CLIMENT, J.; PARDOS, J.A.; GIL, L.; 2004. Life histories of Mediterranean pines. Plant Ecol. 171 53-68.

THOMAS, S.C.; 2011. Age-related changes in tree growth and functional biology: the role of reproduction. *En* F.C. Meinzer, B. Lachenbruch & T.E. Dawson, eds.Size-and Age-Related Changes in Tree Structure and Function. p.33-64. Springer Netherlands.

VERKAIK, I.; ESPELTA, J.M.; 2006. Post-fire regeneration thinning, cone production, serotiny and regeneration age in *Pinus halepensis*. Forest Ecol. Manag. 231 155-163.

VOLTAS, J.; CHAMBEL, M.R.; PRADA, M.A.; FERRIO, J.P.; 2008. Climate-related variability in carbon and oxygen stable isotopes among populations of Aleppo pine grown in common-garden tests. Trees-Struct. Funct. 22 759-769.

ZAS, R.; 2006. Iterative kriging for removing spatial autocorrelation in analysis of forest genetic trials. Tree Genet. Genomes. 2 177-185.