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

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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-

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:

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

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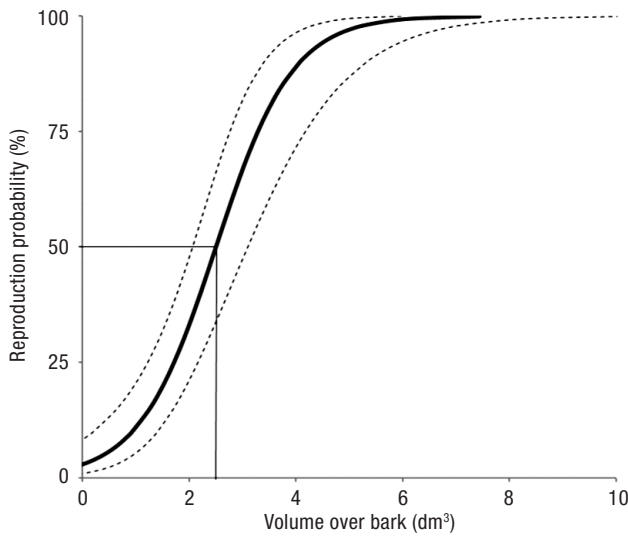
Figure 1. Example of a logistic curve representig probability of reproduction as a function of size (stem volume over bark) in Pinus halepensis from Hijar, NE Spain. Dashed lines indicate the 75% confidence intervals. The vertical line denotes the volume V_{50} at which the probability for a tree to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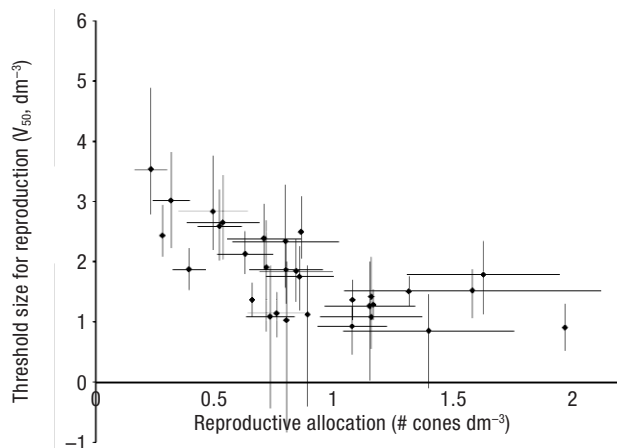

Figure 2. Relationship across populations between reproductive allocation (RA) in 2005 in AMEG and the corresponding Volume over bark at which the reproduction probability was 50% (V_{50}). Each point represents a population. Vertical lines indicate 75% confidence intervals for V_{50} and horizontal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

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.

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 contrast 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)

AMEG05 (218 ± 1.8 cm)

Pinus halepensis

219 ± 13.1 cm

164 ± 2.3 cm

243 ± 2.0 cm

Pinus pinaster

306 ± 4.0 cm

PMER (161 ± 1.0 cm)

200 ± 2.0 cm

165 ± 1.3 cm

164 ± 2.3 cm

199 ± 2.5 cm

PCAV (182 ± 1.2 cm)
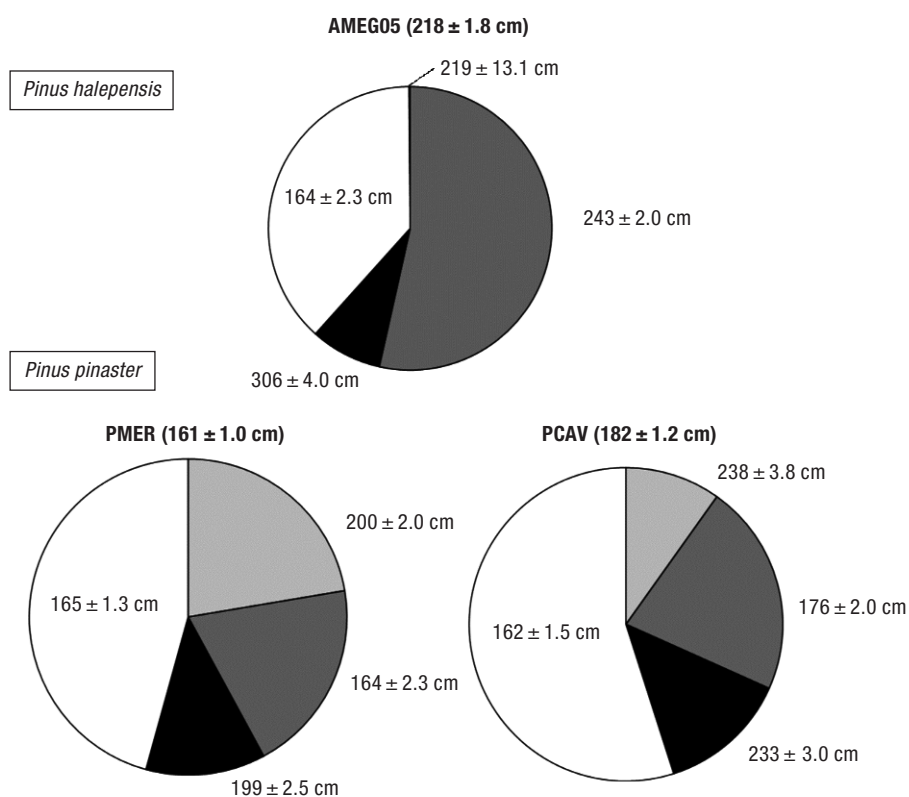
238 ± 3.8 cm

176 ± 2.0 cm

162 ± 1.5 cm

233 ± 3.0 cm

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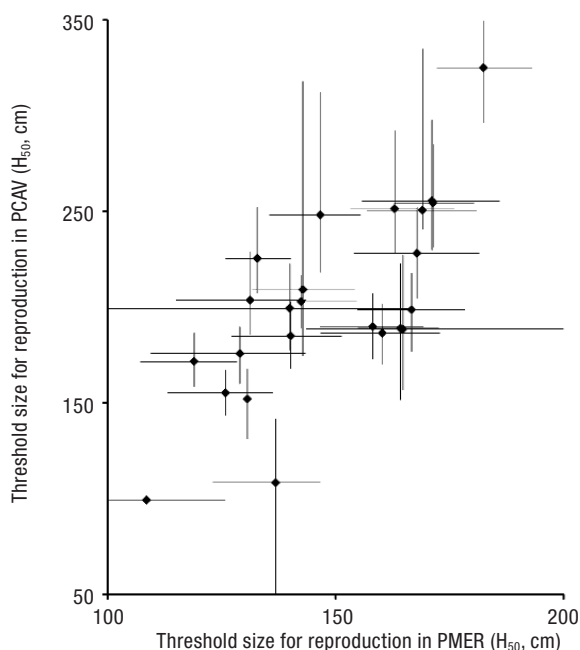
Figure 4. Relationship across populations between threshold size for reproduction (H_{50}, height at which reproduction probability is 50% for a given population) in two Maritime pine trial sites, PMER and PCAV. Vertical and horizontal lines indicate 75% confidence intervals for H_{50}. Lines were not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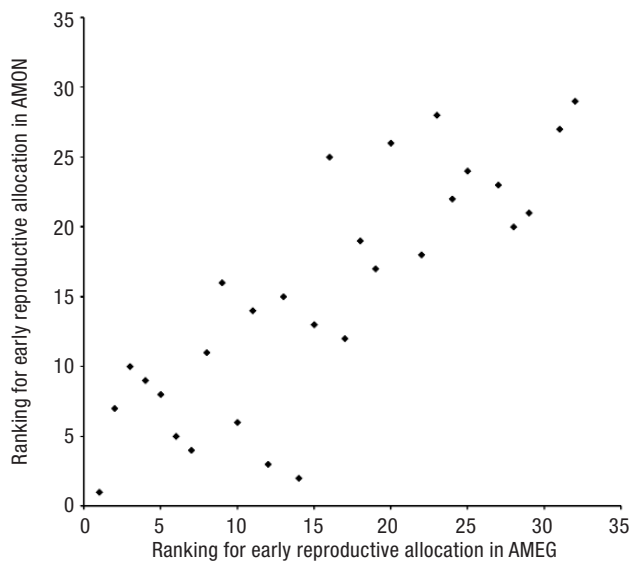

Figure 5. Relationship between the population ranking for early reproductive allocation (RA) estimated in two contrasting *P. halepensis* sites in 2009, AMEG and AMON. Each point represents a population.
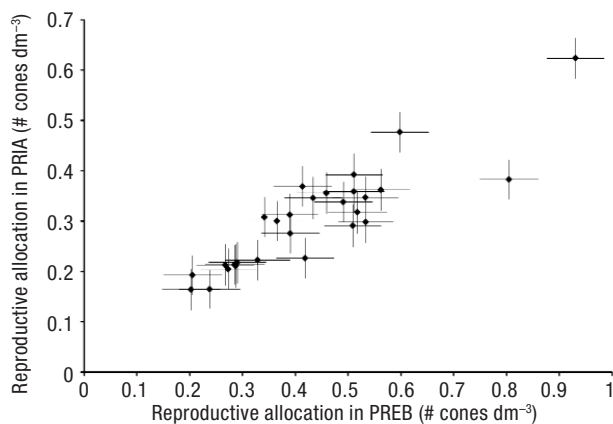

Figure 6. Family relationship in early reproductive allocation between two *P. pinaster* progeny trials, PREB and PRIA. Each point represents an open-pollinated familiy. Vertical and horizontal lines denote standard errors.

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 herbivory 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 repeatedly-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 varia-

bles, 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

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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