The role of maternal age, growth and environment in shaping offspring performance in an aerial conifer seed bank

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Running head: Maternal effects in an aerial seed bank

Abstract

PREMISE: Maternal effects have been demonstrated to affect offspring performance in many organisms and, in plants, seeds are important mediators of these effects. Some woody plant species maintain long-lasting canopy seed banks as an adaptation to wildfires. Importantly, these seeds stored in serotinous cones are produced by the mother plant under varying ontogenetic and physiological conditions.

METHODS: We sampled the canopy seed bank of a highly serotinous *Pinus pinaster* population to test if maternal age and growth, as well as the environmental conditions during each crop year, affected seed mass and ultimately germination and early survival. After determining retrospectively the year of each seed cohort, we followed germination and early survival in a semi-natural common garden.

KEY RESULTS: We found that seed mass was related to maternal age and growth at the time of seed production, i.e. slow growth-older mothers had smaller seeds and fast growth-young mothers had bigger seeds, which could be interpreted either as a proxy of senescence or as a maternal strategy. We also confirmed that seed mass had a positive effect on germination success, but beyond differences in seed mass, maternal age had a

negative effect and diameter had a positive effect on germination timing and subsequent survival.

CONCLUSIONS: Thereby we highlight the importance of maternal conditions combined with seed mass in shaping seedling establishment. Our findings open new insights in the offspring performance deriving from long-term canopy seed banks, which may have high relevance for plant adaptation.

Keywords: Canopy seed bank, germination, maternal effects, *Pinus pinaster*, recruitment, seed mass, serotiny, survival, transgenerational plasticity.

INTRODUCTION

Traditionally, plant breeding strategies have focused on the genetic factors that influence phenotypes, overlooking the potential maternal effects and their adaptive significance (Russell and Lummaa, 2009; Vivas *et al.,* 2020). However, given the substantial evolutionary and ecological implications that maternal effects can have, it is essential to investigate the performance of plant offspring derived from heterogeneous maternal conditions, in terms of age, growth and environment. To this end, aerial seed banks provide a privileged experimental system compared to standard germplasm banks (Levin, 1990; Barrett *et al.,* 2005).

The phenotypes of all living beings are determined by their genotype, their environment, and the interaction between the two, and there is a growing realisation that an important part of this environment may be provided by the mother during early life stages, such as the embryonic development stage (Mousseau and Fox, 1998; Diggle *et al.,* 2010). As maternal effects have long been studied in a variety of taxa and contexts (Bernardo, 1996; Russell and Lummaa, 2009, Pick *et al.,* 2019), we can find different definitions in the literature (for a discussion of maternal effects in plants, see Roach and Wulff, 1987). Here we use

the common (quantitative genetic) definition of a maternal effect as the contribution of the maternal parent to the phenotype of its offspring beyond the equal chromosomal contribution expected from each parent (Roach and Wulff, 1987; Kirkpatrick and Lande, 1989). In other words, maternal effects occur when the environment or physiological state of a mother changes the offspring phenotype without a corresponding change in the genotype (Bock *et al.,* 2019). Importantly, this does not mean that maternal effects cannot have a genetic basis (Wolf and Wade, 2016).

Transgenerational maternal effects provide a flexible mechanism by which sedentary or sessile organisms can cope with heterogeneous environments (Galloway, 2007). In plants, seed mass, which is determined by the seed coat, the megagametophyte and the embryo, may be an important mediator of maternal effects (Bischoff and Mueller-Schaerer, 2010). In flowering plants (angiosperms), both the embryo and the endosperm are derived from individual fertilization events, and only the seed coat that encloses these tissues is purely of maternal origin (Westoby and Rice, 1982; Baroux *et al.,* 2002). By contrast, in gymnosperms only the diploid embryo contains genes of both parents, while all other tissues, including the conspicuous megagametophytic tissue surrounding the embryo, are maternal in origin (Linkies *et al.,* 2010). Hence, in gymnosperms there is a potentially wider role for maternal effects mediated by seed provisioning, together with other aspects of the external and internal maternal environment (Herman and Sultan, 2011).

Maternal effects that affect germination and early stages of plant development are among the best-documented examples (Donohue *et al.,* 2005; Bischoff and Mueller-Schaerer, 2010; Cendán *et al.,* 2013). Therefore, seed mass is a

cornerstone trait that links the evolutionary ecology of reproduction with seedling establishment and the ecology of vegetative growth (Shipley *et al.*, 1990; Leishman *et al.,* 2000). In conifers, the haploid megagametophyte is the main storage tissue providing reserves to the embryo for germination, before the needle-like cotyledons start photosynthesising (Burrows *et al.,* 2017). So, it is not surprising that maternal effects related to seed mass and seedling performance (survival and growth) are particularly well-documented in conifers (Sorensen and Campbell, 1993; Zas and Sampedro, 2015).

In addition to seed mass, maternal age has been shown to have a negative effect on offspring performance in other taxa (Lansing, 1954; Priest *et al.,* 2002; Bock *et al.,* 2019). Many organisms exhibit age-related declines in offspring quality and, while the ultimate causes of such decline are still largely unknown, a decrease in parental care or provisioning due to maternal senescence is generally invoked to explain such trends (Barks and Laird, 2020), as well as the transmission of epigenetic factors from aging parents to their offspring (Schroeder *et al.,* 2015).

Long-lasting seed banks are particularly valuable when it comes to assessing the importance of maternal effects because they contain viable seeds that have been formed across a range of maternal developmental stages and environmental conditions (Lamont *et al.,* 1991; Barrett *et al.,* 2005). Unlike soil seed banks, aerial seed banks of woody plants consist of serotinous fruits or cones (i.e. that delay dehiscence after ripening) that can be individually dated retrospectively (Tapias *et al.,* 2001, Martín Sanz *et al.,* 2017). Moreover, fruits or cones formed early in the mother plant life coexist with those formed at more developed stages, and this ontogenetic gradient may interact with the environment under which each seed cohort was formed (Leslie and Losada, 2019). In addition to providing valuable

general insights, serotiny provides a valuable model for the study of the causes and consequences of maternal effects in general.

Considering that seed mass-mediated maternal effects are costly in terms of resources (Martín Sanz *et al.,* 2017), we expect seed mass to vary with respect to the maternal developmental stage and the environmental conditions at time of seed formation, and this variation to mediate variation in offspring performance (i.e. germination success, timing of germination and survival). Therefore, we hypothesized that (a) the aerial seed bank will show variation in seed mass between and within mother trees that depends on maternal age (i.e. older trees produce lighter seeds), growth and environmental conditions at the time of seed development (i.e. under favourable conditions trees produce heavier seeds), (b) that maternal age, growth and environmental conditions experienced at the time of seed development shape germination success and timing, as well as survival, and finally c) that these effects on offspring performance are at least partly mediated by variation in seed mass. To test these hypotheses, we sampled the canopy seed bank of a highly serotinous maritime pine (*Pinus pinaster* Aiton, Pinaceae) population, where more than 58% of serotinous trees can be found (Tapias *et al.,* 2004; Calvo *et al.,* 2016), ensuring a representative level of serotinous cone ages. Furthermore, retrospective cone dating in this species is easier in comparison with other serotinous pines in our area such as *Pinus halepensis*, which usually develop several consecutive growth units per year (high polycyclism) and false xylem growth rings (Buissart *et al.,* 2015). We retrospectively determined each seed's crop year, as well as the mother tree's age, growth, and the abiotic environment at the time of seed formation, i.e. at each of those crop years. Subsequently, we followed germination and early survival in a semi-natural nursery common garden.

MATERIAL AND METHODS

Study site and sampling regime. We collected serotinous cones in a natural population of *Pinus pinaster* located in Tabuyo del Monte (latitude 42°18′46″N, longitude 6°12′12″W) in northwestern Spain (Fig. 1), where serotiny is common (Tapias *et al*., 2001; Calvo *et al.,* 2016). This pine forest is located at an altitude of 900 m above sea level. In November 2017, we randomly sampled 20 trees that were at least twenty meters apart over an area of 55 ha with at least six age cohorts of serotinous cones per branch. Cones were collected from three branches per tree and stored at 4ºC during the lab processing.

Cone and seed age characterisation. To infer the year in which a cone was produced in the field, we used the stem node counting method (Lamont, 1985. **Appendix S1**, **see the Supplementary Data with this article**). These estimates were followed-up by counting the number of rings in the branch at the insertion point in the laboratory (Tapias *et al.* 2001, Martín-Sanz *et al*., 2017). From the estimated cone crop year, we deduced the age of each cone and thereby of the seeds therein. For example, a cone produced in 2007 contained ten years old seeds in 2017. We discarded four trees due to indistinguishable and/or missing branch wood rings, therefore we kept sixteen mother trees for the next steps of the experiment.

Mother tree characterization. We measured the basal diameter of all sampled trees, and we extracted two basal cores with a Pressler increment borer to determine tree age and measure annual radial growth in the laboratory (Stokes and Smiley 1968). Each core was mounted and cut with the help of a microtome until tree rings were clearly visible. Tree ring series were visually cross-dated to identify locally absent rings and to check for errors (Fritts, 1976). Crossdating was not

statistically verified because most trees were very young and individual time series were shorter than 25 years in several cases. Ring width was measured at 0.01 mm accuracy using the LINTAB system and TSAP-Win (Rinntech, Heidelberg, Germany). If cores failed to reach the centre (Norton *et al.,* 1987) the number of missing rings was estimated by dividing the length of the missing radius by the mean growth rate of the rings adjacent to the largest visible arc on the core following Rozas (2003). To better compare the interannual variability in radial growth among different trees, raw ring-width measures were normalized, i.e. transformed to have a mean of 0 and a standard deviation of 1 before further statistical analysis. Based on the basal diameter as measured in the field and the ring-width data collected in the laboratory, we retrospectively inferred the age and diameter of each mother tree in the year a cone was produced.

Climate data. To complement our temporal data on the environment provided by the mother over time, we used the Standardised Precipitation-Evapotranspiration Index (SPEI; https://spei.csic.es), a multiscalar drought index based on climatic data that can be compared with other SPEI values over time and space (Vicente-Serrano *et al.,* 2010). The SPEI database offers long-time robust information about drought conditions at the global scale across a range of timescales. We used the SPEI calculated for five months (SPEI5July) of each embryo development year. Positive values are associated with above-average wet conditions, and negative values with above-average dry conditions. Here we used SPEI data based on monthly precipitation and potential evapotranspiration as collected by the Climatic Research Unit of the University of East Anglia.

Seed extraction and measurement. Cones were introduced in a chamber at 60ºC for 2.5 hours, after which they were opened manually to obtain all seeds. Further

heating could have negative effects on germination success (Escudero *et al.,* 2002). We weighed the total seed mass from each cone, and after using a float test to remove empty seeds (Serrano Antolín and Calderón Guerrero, 2009) and to ensure the same conditions for all trees and cohorts, ten seeds per cone were selected at random and weighed individually. Note that various tests can be used to ensure that only viable seeds are planted (e.g. float test such as in our case or x-ray), but nevertheless germination cannot be guaranteed (Davis *et al.,* 2004). No dormancy-break treatment was applied to the *Pinus pinaster* seeds, since the objective was to mimic the natural germination conditions as much as possible.

Common garden experiment. The common garden experiment was conducted under semi-natural conditions in a nursery (latitude 40º27'24.77" N, longitude 3º45'06.32" W, 597 m above sea level) once the lab processing was completed, i.e. six months after the field sampling. Although the environmental conditions at the nursery did not match those at the sampling site, they were similar to the warmer and drier continental range of the species.

We used plastic containers (57 x 37 x 32 cm) filled with natural pine forest soil (*eutric cambisol* after FAO taxonomy) collected in a natural *Pinus pinaster* stand located in the Central Range west of Madrid to ensure early seedling mycorrhization (Trappe, 1977; Pera and Álvarez, 1995; Buscardo *et al.,* 2009). A bottom layer of 10 cm of expanded clay was added prior to the natural substrate to ensure water drainage and aeration of the roots.

See appendix S2 for an overview of the study design. We sowed a total of 4620 seeds, using a plastic grid to facilitate seed location and further measurements, and also to prevent any confusion with the eventual germination of seeds coming with the soil (although seed soil banks in pines are negligible). In short, with 10 seeds

per cone, 3 cones per cohort and tree, and at least 6 cohorts with a maximum of 12 per tree, all for a total of 16 trees. The 30 seeds per cohort per tree were divided into six groups of five seeds each. This resulted in six batches of 770 seeds each. By hand, seeds from each batch were randomly planted across 10 containers (referred to here as a block), resulting in a total of 60 containers. The location of each seed within a container was recorded to allow for individual-level monitoring. Containers were covered with a mesh to protect seeds from predation. Pots were kept well-watered to ensure sufficient hydration for germination until the end of July. Germination and survival were recorded every three days from April to October, after which the monitoring frequency was reduced to every 10 days.

Statistical analyses. First, we used a linear mixed model (LMM) to quantify the percentage of variance in seed mass explained by mother tree ID and cone age cohort by fitting each as a random effect. Their statistical significance was assessed using likelihood ratio tests. We subsequently included mother tree age, diameter growth, ring-width and SPEI5July for the year at embryo development/cone production as fixed covariates to quantify their roles in shaping variation in seed mass among mother trees, cohorts and blocks. To account for variation in the effect of age, diameter and ring width among individuals, all models included a random slope term for these covariates (Schielzeth and Forstmeier 2009). Note that because all seeds were collected in the same year, seed age and cohort are perfectly correlated (i.e. all seeds that are 10 years old were produced in 2007) whatever a mother tree's age. However, we expected the random cohort effect to mostly capture random variation in the environmental conditions during cone and seed formation, whereas we expect the fixed maternal age covariate to capture systematic age-related changes.

Initially we used within-subject centering (Van de Pol and Wright, 2009) to separate within- versus between-individual effects of maternal age, diameter and ring width. To this end, we aggregated all measurements of each predictor for the same individual into an average value, and subsequently subtracted this individual mean from each measurement within an individual. We then fitted both the mean and the deviation of the mean as predictors in the model. While we acknowledge that statistical power was relatively low for this comparison, for none of these predictors we found a significant difference between the within- and the among-individual effect (see Results). Hence, we subsequently fitted a similar model, but this time with the original measurements as fixed covariates. In addition, in order to complement the results and explanations, we fitted a mixed model to show the general relationship between maternal age and basal diameter, including tree and cohort as random effects.

Second, we quantified the importance of maternal condition and seed mass on three aspects of offspring performance: germination probability, timing of germination, and seedling survival. Germination and survival probability were modelled as binary traits (germination/survival after germination until 224 days) with a binomial generalized linear mixed model (GLMM). Germination timing was modelled using a linear mixed model with days needed to germinate (i.e. germination day) as the response variable. All models included mother tree ID, cohort and experimental block as random effects, and seed mass, maternal age, diameter, ring width and SPEI as fixed covariates. These analyses were complemented by time-to-event analyses (also known as survival analyses) to show the effect of mother tree ID on seed germination. We used the Kaplan-Meier

method to estimate both the probability of germination in a given length of time (measured in days).

All mixed models were fitted using the lme4 package (Bates *et al.,* 2015) in R version 3.5.3 (R Core Team, 2019). Significance was inferred via Satterthwaite's degrees of freedom method as implemented in the lmerTest package (Kuznetsova *et al.,* 2017). Figures were displayed using ggplot2 (Wickham, 2016), patchwork (Pedersen, 2020) and dplyr packages (Wickham *et al.,* 2021). Kaplan-Meier survival curves were estimated using the survival package (Therneau, 2015).

RESULTS

Sampled trees ranged from 20 to 69 years of age with a basal diameter between 16.3 and 34.2 cm. The first seeds germinated 17 days after sowing, and the last seeds recorded germinated as late as the second spring, 55 weeks after sowing.

Mean seed mass ± standard deviation was 55.2 ± 12.5 mg, and varied significantly among trees (32% of variance explained; χ^2_1=1962.8, p<0.001) and cohorts (15% of variance explained; χ^2_1=844.54, p<0.001).

By comparing within- and between- mother tree effects prior to fitting the final model, we found a non-significant effect of age within mothers ($t_{14.4}$=-0.5, p=0.6), and a negative between- mother tree effect of age ($t_{11.8}$=-2.56, p=0.025), i.e. older trees produced smaller seeds, but seed mass did not change with cone age within a tree. Despite a large amount of among-individual variation in within-individual slopes, overall seed mass decreased with maternal age ($t_{13.9}$=-3.2, p<0.006, Table 1) (Fig. 2A). The effects of ring width were non-significant both within- ($t_{14.4}$=0.3, p=0.77) and between- ($t_{11.8}$=0.5, p=0.65) mother trees (Fig. 2B). The effect of tree

diameter on seed mass was positive and significant between individuals ($t_{12.1}$=2.2, p=0.045), i.e bigger trees produced heavier seeds (Fig. 2C).

If we ignore the distinction between within- and among-individual variation, since the differentiation of their effect is not significant, and analyse raw seed mass rather than individual means and deviations from these means (see Methods), we find that although basal diameter increases with age ($t_{10.02}$=63.90, p<0.001, Fig. 3), a model including both maternal age and basal diameter again reveals a negative relationship between mother tree age and seed mass (i.e. older trees produce lighter seeds), whereas the effect of basal diameter tends to be positive (i.e. larger trees produce heavier seeds, Fig. 2). The effects of ring width and SPEI were non-significant. See Table 1 for parameter estimates and statistical details.

40 days after sowing, 50.8% of the seeds had germinated. Seedling survival was very high over this period, with 98.9% of the seedlings surviving until that date. Up until the onset of winter and the cessation of germination for that year, 224 days after sowing, 89% of the seeds had germinated and 83.3% of all seedlings were still alive. Note that the 11% of seeds that did not germinate until this time point cannot not be considered as failures as they may germinate at a later time point.

Seed mass predicted whether a seed germinated or not as a binary response, with larger seeds being more likely to germinate (Table 2). None of the other predictors were statistically significant.

The timing of germination on the other hand was unaffected by seed mass, but there was a significantly positive effect of maternal age, with older mothers producing seeds that germinate later, whereas the effect of basal diameter tends to be negative, with larger mothers producing seeds that germinate earlier (Table 3). The timing of germination was unaffected by ring width and SPEI.

To illustrate the effect of the mother trees on germination timing, we used the time-to-event analysis method (Fig. 4). The vertical distance between 0 and 1 represents the change in cumulative probability of not germinating as the curve advances. We can see differences between trees.

Finally, we found that both seed mass and germination timing showed a significant effect (χ^2_1=7.273, p<0.01; χ^2_1=127.426, p<0.001 respectively) on mortality, such that higher seed mass significantly reduced seedling mortality risk.

DISCUSSION

In this work we quantified the role of seed mass as a mediator of maternal effects on offspring performance in a long-lasting aerial seed bank of a conifer species, and the importance of maternal age and conditions in shaping these effects. With the seed, the independence of the next generation of plants begins (Bewley, 1997), therefore it is essential to disentangle the factors that will determine its future, including maternal condition and the environmental conditions during seed formation. Maternal age (Cooper *et al.,* 2020) and its sensitivity to the environment can lead to variation in its growth, condition and physiological state (Schmid and Dolt, 1994; Galloway, 2005) that could have important consequences for the next generation, like shown in animals (see, for example Mousseau and Fox, 1998).

We found significant differences in seed mass both between trees and among cohorts of the same mother tree, with 13.6% of the variation in seed mass being attributable to variation among trees versus 9.3% being attributable to cohort effects. This is in line with research on other species, where most variation in seed mass was also observed between individuals (Thompson, 1984; Kołodziejek, 2017; Wang and Ives, 2017). The variation in seed mass, both within and between mother

trees, is largely due to effects associated with the maternal tree and environmental conditions during seed development (Bladé and Vallejo, 2008).

The first prediction of the Smith-Fretwell model (1974) is that plants should produce seeds of equal size. However, large seeds are costly (i.e. cost of reproduction) and we therefore expect seed mass to depend on the resource status of the mother plant (Geritz, 1995), which may vary over time and with age (Plaistow et al., 2007). Such variation will cause variation in seed mass between yearly crops of the same individual (Wulff, 1986). In line with this expectation, we found a substantial and statistically significant amount of variation among cohorts within individuals. High within-individual variation is a constant in many organisms, and in particular seed mass typically varies two to fourfold, even within individuals (Michaels *et al.,* 1988). Noteworthy, we found up to five-fold variation between cohorts of some individuals, which is similar to results reported in other species of pine (*Pinus aristata* and *P. flexilis*, Borgman *et al.,* 2014; *P. nigra*, Tiscar and Lucas-Borja, 2010, but see Castro, 1999 in *P. sylvestris*).

We confirmed that the age of the mother tree had a negative effect on seed mass, suggestive of maternal senescence or perhaps of a maternal strategy, resulting in the production of smaller cones containing fewer and heavier seeds at younger age and larger cones with more though smaller seeds at older ages (Cruz *et al.,* 2019). In other studies, maternal age has been shown to have a negative effect on offspring performance either directly or mediated by, for example, an effect of maternal age on seed size (Lembicz *et al.,* 2011) or germination (Alonso-Crespo *et al.,* 2020). At the same time, despite an increase in size with age, mother size (assessed by its diameter), had a significant positive relationship with seed mass; in other words, big young mothers produced larger seeds. This result confirms that

mother size and age have independent effects on seed mass: even when there is a positive correlation between age and diameter, the correlation is less strong than often believed (Fritts 1976; Pederson, 2010) and very often older trees are not the larger ones (see Appendix S3), particularly in natural and natural managed forests. In line with this, we found two or even three different growth trajectories across the individual trees (Fig. 3). However, we cannot associate these patterns with differences in the microenvironment, in terms of light or soil conditions. The history of silvicultural practices in this population could help explain these trajectories since they can directly or indirectly influence tree growth (Long *et al.,* 2004).

Various studies that have examined the relationship between growth, measured as tree ring width, and reproductive output have provided evidence that reproduction reduces tree performance (Thomas, 2011; Lucas-Borja and Vacchiano, 2018). However, we found that annual ring-width was unrelated with seed mass and hence found no evidence for a trade-off between growth and reproduction. This is in fact in line with other studies examining maternal effects in other pine species, which found that inter-annual variability in mother twig growth during seed provisioning was not significantly related to differences in seed mass (Borgman *et al.,* 2014). However, seed mass is only one aspect of reproductive investment, and more work using a more comprehensive measure of reproductive investment is needed.

Climatic fluctuations, such as changes in precipitation and temperature patterns associated with climate change, can be an important determinant of reproductive performance (Pérez-Ramos *et al.,* 2010, Basto *et al.,* 2018, Hatzig *et al.,* 2018). However, contrary to other works (Lacey *et al.,* 1997, Murray *et al.,* 2004), we found no effect of the climate experienced by the mothers during embryo

development (measured through the SPEI5July, Vicente-Serrano *et al.,* 2010) on seed mass. The fact that our mother trees were of different age and size, and micro-environmental differences in the natural stand could explain a more variable reaction to the same climatic factors at a given year.

We found a significant positive effect of seed mass on germination success in line with a well-supported trend (Castro, 1999, Linkies *et al.,* 2010, Cendán *et al.,* 2013). This could be a result of better-provisioned offspring from higher seed reserves having greater establishment success (Leishman *et al.,* 2000; Herman and Sultan, 2011). However, when accounting for maternal traits, there was no effect of seed mass on the timing of germination. In our study, the age and diameter of the mother were the main effects, such that seeds from older trees (within the age range analysed) had a significant delay in germination, in line with other studies (Leishman *et al.,* 2000; Alvarez *et al.,* 2005), whereas seeds from larger trees germinate earlier. Age effects could be suggestive of senescence or could indicate that seeds from older plants have a greater physical or mechanical dormancy, i.e. seeds are more impermeable or have a harder endosperm (Alvarez *et al.,* 2005). Importantly, germination timing has been postulated to be more determinant to the success of post-fire regeneration than germination percentage *per se* since seedlings will have to compete efficiently for light and water (Cruz *et al.,* 2017). Confirming this assertion, in our experiment early-germinating seeds (before June) had much lower mortality risk (9%) compared to those germinating in midsummer (28% by the end of this season). Early seedling emergence in the growing season confers a greater rate of survival or better growth if early emergence provides advantages with respect to an environmental cue (Verdú and Traveset, 2005; Castro, 2006).

This advantage of early germination is more evident in milder Mediterranean climates where late frosts are less frequent or intense and summer drought is the main source of seedling mortality (Gómez-Aparicio et al., 2005). Drought-related mortality due mainly to midsummer has the potential to act as a filter during early life stages (Warwell and Shaw, 2019). In addition, seed germination timing may influence subsequent seedling phenology and developmental changes by determining the seasonal conditions experienced by seedlings, as studied in annual plants (Donohue, 2009), in which the timing of germination determined whether an annual or biennial life cycle was expressed. However, ensuring a wide range of dormancy due to age effects may be an advantage under an unpredictable Mediterranean climate. Nevertheless, beyond the high influence of germination timing, seed mass had also a positive effect on reducing mortality risk, favouring the survival of the better-provisioned seedlings (Simons and Johnston, 2000).

Seed banks provide an exciting and challenging model for studying the evolutionary implications of genetic and non-genetic transgenerational effects. Even in long-lived plants like forest trees, maternal effects related to seed resource allocation and epigenetic mechanisms linked to embryogenesis and seed maturation may contribute to the rapid adaptation of these long-lived organisms for coping with environmental changes (Herman and Sultan, 2011; Yakovlev *et al.,* 2012; Vivas *et al.,* 2013). For instance, temperature experienced by the mother tree during seed formation has been found to shape phenological responses of seedlings (Johnsen *et al.,* 2005). This paper provides a new insight into the role of the maternal environment and age in shaping the performance of its offspring, complementing past and future studies into genetic effects, transgenerational plasticity -known as epigenetic memory- (Henderson and Jacobsen, 2007;

Yakovlev *et al.,* 2012; Correia *et al.,* 2013; Vivas *et al.,* 2013) and into other forms of ecological inheritance such as associations between plants and the microbiome (Vivas *et al.,* 2015). In addition, it could lay the foundation for further research into the importance of maternal effects as an adaptive strategy.

Our study opens a new path in our understanding of the trans-generational plasticity of long-lived plants under a changing environment. This is highly relevant as climate change is aggravated and can have implications for the adaptive management of natural forests.

Conflict of Interest

We have no competing interests.

Author contributions

M.C.D., M.R.C. and J.M.C. conceived and designed the experiment. M.C.D., J.S.M.L., M.R.C., and many field assistants carried out the field work. M.C.D., J.S.M.L, G.G.I., and M.R.C. prepared the data. M.C.D., E.P., J.M.C. and L.S.D.B. discussed the statistical approach. M.C.D. conducted all analyses and E.P supervised the analyses. M.C.D. drafted the manuscript. E.P., G.G.I. and J.M.C. supervised the draft manuscript. J.M.C. secured funding.

Data Availability
The R code (doi: 10.6084/m9.figshare.17158469) and primary data (doi: 10.6084/m9.figshare.15097185) are available in *Figshare*.
Supporting Information
Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Node-counting method.
Appendix S2. Experimental design.
Appendix S3. Supplementary Figure.

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Tables

Table 1. Determinants of seed mass (measured in milligrams). Fixed effect estimates, standard errors, t-values, degrees of freedom using Satterthwaite approximation (as implemented in the lmerTest package, Kuznetsova et al., 2017) and p-values. SPEI: Standardised Precipitation-Evapotranspiration Index (Vicente-Serrano *et al.,* 2010).

	Estimate	**Std. Error**	**t value**	**df**	**Pr(>|t|)**
Maternal age	-5.24	1.63	-3.2	13.9	0.006
Tree Diameter	4.37	1.88	2.3	13.1	0.037

Ring width	1.14	2.54	0.5	19.3	0.659
SPEI	-0.18	1.87	-0.1	8.9	0.928

Table 2. Predictors of germination probability based on a binomial generalised linear mixed model. P-values are based on likelihood ratio tests (LRT). Estimates are on a logit scale. SPEI: Standardised Precipitation-Evapotranspiration Index (Vicente-Serrano *et al.,* 2010).

|	Estimate	Std. Error	Chi2	Pr (Chi)
Seed Weight	0.04	0.01	93.23	<0.001
Maternal Age	-0.04	0.14	0.101	0.751
Tree Diameter	-0.01	0.13	0.006	0.937
Ring Width	0.08	0.08	0.976	0.323
SPEI	0.07	0.13	0.065	0.799

Table 3. Determinants of the timing of germination. Estimated regression parameters, standard errors, *t*-values, degrees of freedom using Satterthwaite approximation (as implemented in the lmerTest package, Kuznetsova et al., 2017) and P-values for the LMM about germination timing. SPEI: Standardised Precipitation-Evapotranspiration Index (Vicente-Serrano *et al.,* 2010).

	Estimate	Std. Error	t value	df	Pr(>|t|)
Seed weight	-0.07	0.07	-1.1	2451.1	0.286
Maternal age	7.78	2.84	2.7	20.1	0.013
Diameter	-5.51	2.03	-2.7	69.10	0.009
Ring width	0.86	0.86	1.0	255.39	0.314

|					
SPEI	-1.0	1.75	-0.6	9.01	0.580

Figure Legends
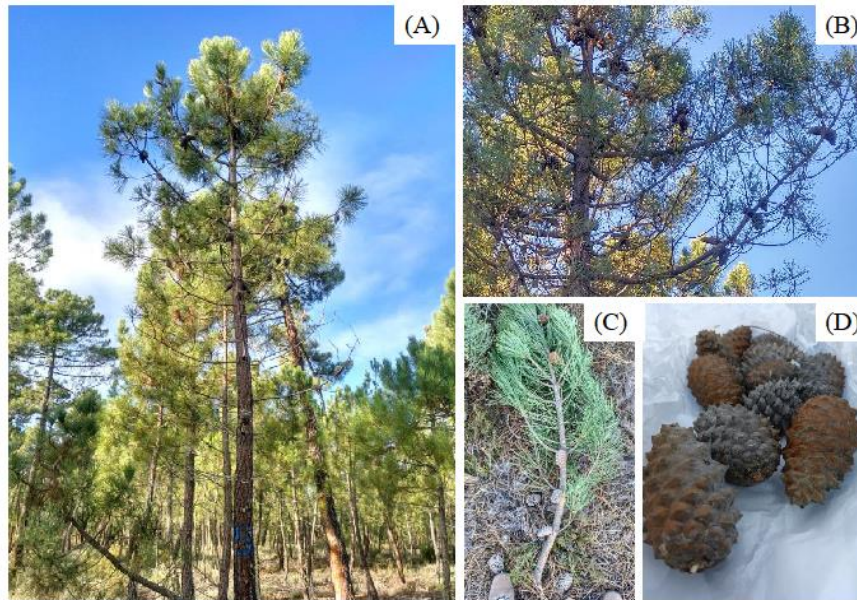
Fig. 1. Representative pictures of the natural population of *Pinus pinaster* Ait. located in Tabuyo del Monte (northwestern Spain): (A) One of the mother trees sampled as an example, (B) an aerial seed bank with serotinous cones from different cohorts, (C) a detail of a branch with serotinous cones (see Appendix 1 to complement figures 1B and 1C) and (D) a subsample of serotinous cones.
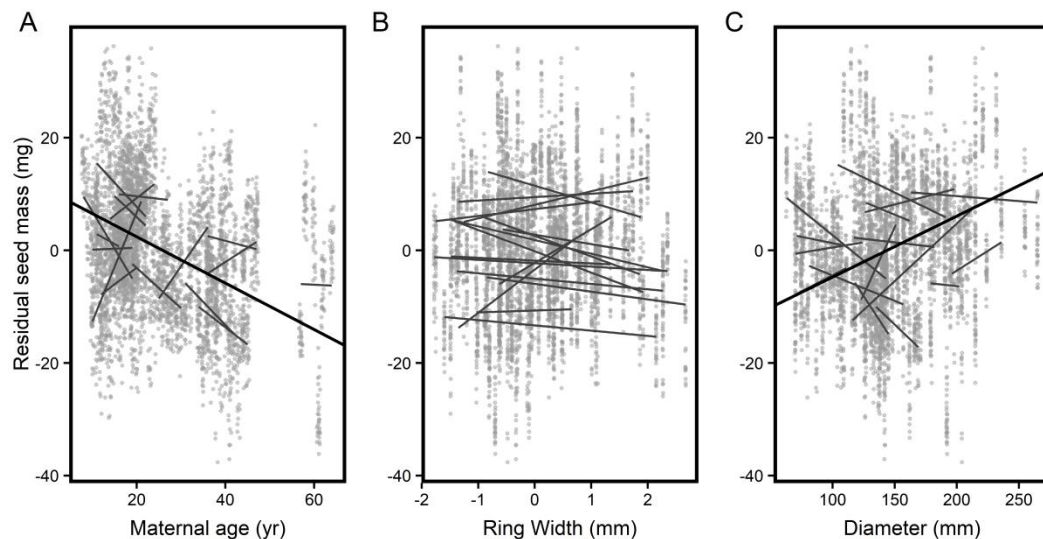

Fig. 2. Within- and between- mother trees effects in seed mass for our sixteen sampled mother trees. The thinner black lines show individual linear regressions for each mother tree. The thicker black line in each panel was drawn from the estimated parameters in the mixed model when that predictor was significant (Table 1). Y-axis represents residual seed mass after accounting for calendar year

effects (cohort). In the x-axis, the effect of (A) maternal age; (B) centered ring width and (C) tree diameter.
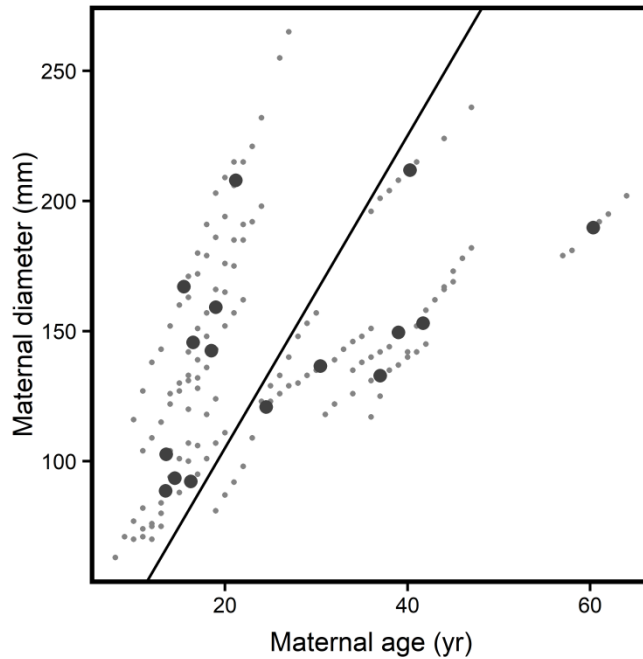
Fig. 3. Relationship between age and diameter of mother trees. Large points correspond to the mean age of each mother tree and small points represent values for each cohort within trees. The black line was drawn from the estimated parameters of the mixed model fitted ($t_{10.02}$=63.90, p<0.001).
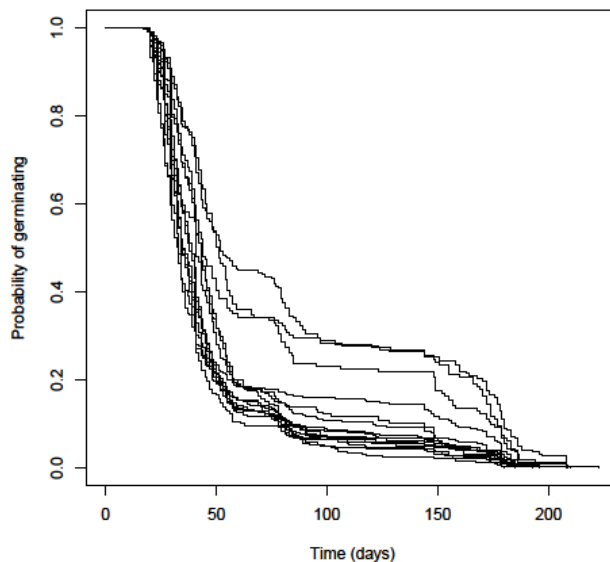
Fig. 4. Kaplan–Meier estimates of germination probability curves for all trees, illustrating the differences in the germination time between individuals. The vertical distance between 0 and 1 represents the change in cumulative probability of germination as the curve advances.