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

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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 population of Pinus pinaster to test whether maternal age and growth and 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.

Results: Seed mass was related to maternal age and growth at the time of seed production; i.e., slow-growing, older mothers had smaller seeds, and fast-growing, young mothers had larger seeds, which could be interpreted either as a proxy of senescence or as a maternal strategy. Seed mass had a positive effect on germination success, but aside from differences in seed mass, maternal age had a negative effect and diameter had a positive effect on germination timing and subsequent survival.

Conclusions: The results 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

Traditionally, plant breeding strategies have focused on the genetic factors that influence phenotypes, overlooking 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 unique 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 realization 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). Because maternal effects have long been studied for a variety of species and contexts (Bernardo, 1996; Russell and Lummaa, 2009; Pick et al., 2019), we can find different definitions of these effects in the literature (for a discussion of maternal effects in plants, see Roach and Wulff, 1987). Here we use the common definition of a maternal effect in the quantitative genetic sense 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 and Etterson, 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), the embryo and the endosperm are derived from a fertilization event, 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 and Peters, 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 cotyle-
dons start photosynthesizing (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 species (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 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 prospectively (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 in 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 that seed mass will vary with respect to the maternal developmental stage and the environmental conditions when the seeds are forming and that this variation mediates the variation in offspring performance (i.e., germination success, timing of germination, and survival). Therefore, we hypothesized that (1) 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 favorable conditions trees produce heavier seeds), (2) that maternal age, growth, and environmental conditions at the time of seed development shape germination success and timing, as well as survival, and (3) 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 population of maritime pine (Pinus pinaster Aiton, Pinaceae), where more than 58% of the trees were found to be serotinous (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 than for 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 and 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.

MATERIALS 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 (Figure 1), where serotiny is common (Tapias et al., 2001; Calvo et al., 2016). This pine forest is located at an altitude of 900 m a.s.l. In November 2017, we randomly sampled 20 trees that were at least 20 m 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 4°C during the lab processing.

**Cone and seed age characterization**

To infer the year in which a cone was produced in the field, we used the stem node counting method (Lamont, 1985; Appendix S1). We then counted 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 10-yr-old seeds in 2017. We discarded four trees due to indistinguishable and/or missing branch wood rings; therefore, we kept 16 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 center (Norton et al., 1987), then 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 as described by 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...
MATERNAL EFFECTS IN AN AERIAL SEED BANK

further statistical analysis. Using 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.

Climatic 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; Vicente-Serrano et al., 2010), a multiscalar drought index based on climatic data that can be compared with other SPEI values over time and space. 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 5 months (SPEI 5July) 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 60°C for 2.5 h, 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, 10 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 40°27′24.77″N, longitude 3°45′06.32″W, 597 m a.s.l.) once the lab processing was completed, i.e., 6 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 × 37 × 32 cm) filled with natural pine forest soil (eutric cambisol, based on 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 before the natural substrate to ensure water drainage and aeration of the roots. See Appendix S2 for an overview of the study design. We sowed 4620 seeds using a plastic grid to facilitate seed location and further measurements and to prevent any confusion with the eventual germination of seeds that might have been present already within the soil (although seed soil banks in pines are negligible). In short, with 10 seeds per cone, three cones per cohort and tree, and at least six 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 3 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 SPEI 5July 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 did we find 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, 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, germination timing, 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 ± SD was 55.2 ± 12.5 mg and varied significantly among trees (32% of variance explained; $\chi^2 = 1966.2, P < 0.001$) and cohorts (15% of variance explained; $\chi^2 = 844.54, P < 0.001$).

By comparing within- and between-mother tree effects prior to fitting the final model, we found a nonsignificant effect of age within mothers ($t_{144} = -0.5, P = 0.6$), and a negative between-mother-tree effect of age ($t_{11.8} = -2.56, P = 0.025$); that is, 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) (Figure 2A). The effects of ring width were nonsignificant within ($t_{14.4} = 0.3, P = 0.77$) and between ($t_{11.8} = 0.5, P = 0.65$) mother trees (Figure 2B). The effect of tree diameter on seed mass was positive and significant between individuals ($t_{12.1} = 2.2, P = 0.045$); that is, bigger trees produced heavier seeds (Figure 2C).

If we ignore the distinction between within- and among-individual variation, since the differentiation of their effect is not significant, and analyze raw seed mass rather than individual means and deviations from these means (see Methods), we find that although basal diameter increases with age ($t_{10.02} = 63.90, P < 0.001$, Figure 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, Figure 2). The effects of ring width and SPEI were nonsignificant. See Table 1 for parameter estimates and statistical details.

Forty 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 are not considered as failures because they might germinate later.

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 germinated later, whereas the effect of basal diameter tended to be negative, with larger mothers producing seeds that germinated 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 analytical method (Figure 4). The vertical distance between 0 and 1 represents the change in cumulative probability of not germinating as the curve advances. We found differences between trees.

| Variable      | Estimate | SE  | t    | 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 1 Determinants of seed mass (measured in milligrams). Fixed effect estimates, standard error, 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)
FIGURE 2 Within- and between-mother trees effects of (A) maternal age, (B) centered ring width, and (C) tree diameter on seed mass for the 16 sampled mother trees. The y-axis represents residual seed mass after accounting for calendar year effects (cohort). Thin black lines show individual linear regressions for each mother tree. The thick black line in each panel was drawn from the estimated parameters in the mixed model when that predictor was significant (Table 1).

TABLE 2 Predictors of germination probability based on a binomial generalized linear mixed model. P-values are based on likelihood ratio tests. Estimates are on a logit scale. SPEI: standardised precipitation-evapotranspiration index (Vicente-Serrano et al., 2010)

| Variable     | Estimate | SE   | $\chi^2$ | Pr ($\chi^2$) |
|--------------|----------|------|----------|---------------|
| Seed mass    | 0.04     | 0.01 | 93.23    | <0.001        |
| Maternal age | -0.04    | 0.14 | 0.10     | 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)

| Variable     | Estimate | SE   | t   | df   | Pr(|t|) |
|--------------|----------|------|-----|------|-------|
| Seed mass    | -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 |
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 (e.g., 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; Kolodziejek, 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 (Blade 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 2- to 4-fold, even within individuals (Michaels et al., 1988). Noteworthy, we found up to 5-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 in P. sylvestris, see Castro, 1999).

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, large, 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 not as strong as 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 growth trajectories across the individual trees (Figure 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 result is 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

**FIGURE 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.
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 SPEI 5July, Vicente-Serrano et al., 2010) on seed mass. The fact that our mother trees were of different age and size, and microenvironmental 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 effect 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 we accounted 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 analyzed) had a significant delay in germination, in line with other studies (Leishman et al., 2000; Alvarez et al., 2005), whereas seeds from larger trees germinated earlier. Age effects could be suggestive of senescence or could indicate that seeds from older plants have greater physical or mechanical dormancy; that is, seeds are more impermeable or have a harder endosperm (Alvarez et al., 2005). Importantly, germination timing has been postulated to be more determinant for the success of post-fire regeneration than germination percentage per se because seedlings have to compete 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 cause of seedling mortality (Gómez-Aparicio et al., 2005). Drought-related mortality due mainly to midsummer stress 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 in an unpredictable Mediterranean climate. Nevertheless, beyond the high influence of germination timing, seed mass also had a positive effect on reducing mortality risk, favoring 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 nongenetic 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 to environmental changes (Herman and Sultan, 2011; Yakovlev et al., 2012; Vivas et al., 2013). For instance, the temperature during seed formation by the mother tree has been found to shape the phenological responses of the seedlings (Johnsen et al., 2005). Our study provides 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 contributes to a foundation for further research on maternal effects as an adaptive strategy. Our study also opens a new path in our understanding of the transgenerational plasticity of long-lived plants as climate change is aggravated and can have implications for the adaptive management of natural forests.

ACKNOWLEDGMENTS

We thank E. Ballesteros, F. Del Caño, S. San Segundo, and E. Pistola for assistance in fieldwork. We are also grateful to C. Guadaño and D. León and everyone associated with establishing and maintaining the common garden experiment at C.N.R.G.F. "Puerta de Hierro". We thank the anonymous reviewers for their constructive comments and especially the Associate Editor Dylan Schwilk for his thorough and helpful suggestions. Climate data used in this research was provided by the Spanish National Research Council (CSIC). This research was supported by the Spanish Government via the Ministry of Science, Innovation and Universities (MICIU) grants AGL2015-68274-C03-1-R and RTI2018-094691-B-C32 and by a predoctoral research fellowship from the Spanish MICIU (BES-2016-077347).

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 did the fieldwork. 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. wrote the manuscript. E.P., G.G.I., and J.M.C. supervised the draft manuscript. J.M.C. secured funding.
DATA AVAILABILITY STATEMENT
The R code (https://doi.org/10.6084/m9.figshare.17158469) and primary data (https://doi.org/10.6084/m9.figshare.15097185) are available in Figshare.

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**SUPPORTING INFORMATION**
Additional supporting information may be found in the online version of the article at the publisher’s website.

**Appendix S1.** Node-counting method to estimate cone age.

**Appendix S2.** Experimental design for cone and seed sampling from cohorts and mother trees and for experimental blocks containers. Representative pictures of the common garden experiment.

**Appendix S3.** Contour plot showing the relationship between seed mass, tree age at the embryo development and diameter.

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**How to cite this article:** Callejas-Díaz, M., M. R. Chambel, J. San-Martín-Lorén, G. Gea-Izquierdo, L. Santos-Del-Blanco, E. Postma, and J. M. Climent. 2022. The role of maternal age, growth, and environment in shaping offspring performance in an aerial conifer seed bank. *American Journal of Botany* 109(3): 366–376. [https://doi.org/10.1002/ajb2.1811](https://doi.org/10.1002/ajb2.1811)