

# Genetic variation in early fitness traits across European populations of silver birch (*Betula pendula*)

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## Abstract

Given that the ecological niche of tree species is typically narrower for earlier life stages, intraspecific genetic variation at early fitness traits may greatly influence the adaptive response of tree populations to changing environmental conditions. In this study, we evaluated genetic variation in early fitness traits among 12 populations of *Betula pendula* from a wide latitudinal range in Europe (41-55°N). We first conducted a chamber experiment to test for population differences in germination and the effect of prechilling treatment on seed dormancy release. We then established three common gardens spread across the species latitudinal range in order to evaluate levels of quantitative genetic variation and genotype-by-environment interaction at different early-life traits. Our results showed significant variation in chamber germination rates among populations (0-60%), with southern populations exhibiting lower germination. Prechilling treatments did not generally improve germination success. Population seedling emergence rates in the field were correlated with chamber germination rates, though being an order of magnitude lower, with an average ranging from 0 to 1.3% across gardens. Highly significant variation was found in field emergence rates among populations, and between seed-crop years within populations, but not among families within populations. Populations differed in seedling height, diameter, slenderness and budburst date, with significant among-family variation. Population latitude was positively associated with chamber germination rate and with seedling emergence rate in one of the central field sites. Overall, genetic, environmental and demographic factors seem to influence the observed high levels of variation in early fitness traits among *B. pendula* populations. Our results suggest limited regeneration capacity for the study species under drier conditions, but further field trials with sufficient replication over environments and seed crops will improve our understanding of its vulnerability to climate change.

Keywords: seed germination, seedling emergence, early mortality, regeneration, population differentiation, local adaptation, seed mass

## Introduction

Climate change models predict drier and warmer summers for Mediterranean regions in the next decades (Kovats *et al.* 2014). In Central Europe, increases in temperature and considerable contrasts in precipitation between dry and wet seasons are also predicted (EEA 2012, Collins *et al.* 2013). These conditions will result in more severe hydrological and soil moisture droughts in many areas, especially in summer months (Wong *et al.* 2011; Samaniego *et al.* 2018). As a consequence, demographic dynamics and species distribution ranges might be considerably altered (Iverson *et al.* 2004; Dyderski *et al.* 2018). In the northern edge of the range, the increase in temperature is expected to broaden the suitable habitat and distribution of many forest tree species (Eggers *et al.* 2008; Nadeau and Urban 2019). On the contrary, populations from the southern edge are expected to suffer from size reductions and increased isolation, making them particularly vulnerable to climatic changes (Jump *et al.* 2006; Peñuelas *et al.* 2007).

There is a need to assess the levels of intraspecific genetic variation in fitness-related traits, and its environmental determinants, in order to evaluate the potential and the drivers of adaptive evolution to climate change (Alberto *et al.* 2013). Adaptive evolution requires the existence of heritable genetic variation at fitness related traits within and among populations, as it increases both the response to new selective pressures and the frequency of potentially pre-adapted alleles (Jump *et al.* 2009, Kremer *et al.* 2014). Common gardens have long been used to disentangle environmental and genetic effects on phenotypic variability (Savolainen *et al.* 2007; White *et al.* 2007; Alberto *et al.* 2013). However, most common garden studies conducted under natural and semi-natural conditions have focussed on juvenile and adult trees, established from seedlings initially grown in optimal nursery conditions, thus neglecting the impact of natural selective pressures during early-life stages (Gibson *et al.* 2016). The early stages of the life cycle, and particularly the transition from seeds to seedlings, are crucial for the regeneration niche and have enormous consequences on population dynamics (Jackson *et al.* 2009; Donohue *et al.* 2010; Walck *et al.* 2011). Predictions on future species distributions based exclusively on adult tree traits could underestimate range contraction risks, given that the ecological niche is typically narrower in early life stages (Jackson *et al.* 2009). Thus, improving our understanding on intraspecific genetic variation in early fitness traits, such as dormancy, germination and seedling establishment rates, is essential to elucidate the potential ability of tree populations to regenerate and persist under climate change.

In this study, we examined the extent to which populations of *Betula pendula*, a widely-distributed tree species in Europe, differed in early-fitness traits under contrasting environmental conditions. This wind-pollinated and wind-dispersed broadleaved species has a continuous distribution across central

and northern Europe (Atkinson 1992). It is also found in the Mediterranean Basin, where it is restricted to mountain ranges (Beck, *et. al* 2016). Seeds are dispersed in late summer-autumn and stay dormant until spring, when conditions for germination and seedling establishment are more favourable (Vanhatalo *et al.* 1996). Dormancy duration and the rates of germination and seedling survival are critical parameters in the regeneration process (Donohue *et al.* 2010). The main climatic factors limiting the species recruitment are low temperatures in northern Europe (Holm 1994a,b) and summer drought in southern regions, similarly to other European temperate and boreal tree species such as *Betula alba* (Sanz *et al.* 2011) and *Pinus sylvestris* (Castro *et al.* 2004).

We expect that different selective pressures across the species range have resulted in adaptive genetic divergence across *B. pendula* populations (Savolainen *et al.* 2007). We hypothesise clinal, rather than abrupt, geographic genetic divergence among populations, given the continue distribution and high levels of gene flow in this species (Atkinson 1992, Alberto *et al.* 2013). We expect differences in early-fitness traits among seed-crop years related with the great inter-annual variation in reproduction investment in this species (Rousi *et al.* 2011, Gallego Zamorano *et al.* 2018). Differences among populations in dormancy, germination, growth and phenology traits associated with latitudinal and altitudinal clines have already been found in northern populations (e.g. Myking and Heide 1995; Viherä-Aarnio and Velling 2008, Midmore *et al.* 2015), but there is little information on range-wide genetic variation at early fitness traits assayed under natural conditions, especially for southern and mid-low latitude populations.

Our specific objectives were: (i) to explore whether *B. pendula* exhibits among-population genetic variation at early-life traits along a latitudinal gradient, (ii) to explore the potential geographic and environmental factors associated with the observed population genetic divergence, (iii) to determine the extent to which the growing environment alters patterns of population variation at early-life traits (i.e. genotype-by-environment interaction) and (iv) to examine levels of within-population genetic variation at those traits.

## Materials and methods

### Plant material

Twelve natural populations of *B. pendula* were selected for the study throughout the species distribution range in Europe (Fig. 1, Table 1). Seeds were collected in the summers of 2016 and 2017 from 11-25 open-pollinated and randomly selected trees within each population, separated at least 30 m apart. For populations ES2 and DE1, seed lots were obtained for both 2016 and 2017 crops, which were used to explore variation in early fitness traits among seed-crop years within populations (Table 1). Unless specified otherwise, ES2 and DE1 refer to the seed crop from 2016. Seeds were stored at 4°C in a dry environment until sowing.

### Chamber experiment

In winter 2018, we conducted a chamber experiment to 1) obtain benchmark germination rates, 2) assess the effect of moist-chilling on seed dormancy, germination rate and time, and 3) examine whether populations responded differently to its application. We established three treatments with different chilling duration: 15 days (T1), 30 days (T2) and 45 days (T3) and a control treatment, where seeds were not chilled (C). Chilling consisted of adjusting seeds to a moisture level of 33% at 4°C in darkness, conditions that have been reported to break dormancy while avoiding seed germination and deterioration (De Atrip and O'Reilly 2007). Seed moisturizing was achieved by adding distilled water on seeds placed in 1.5ml microtubes (Jones and Gosling 1994). After each chilling treatment, 150 seeds per population (6-9 seeds per mother tree) were placed in three different Petri dishes (i.e. 50 seeds/population/block) with moistened filter paper inside. Population GB1 was only assayed in the C treatment due to limited seed availability. Petri dishes were placed in the chamber following a randomized block design. Temperature was set at 15°C ± 2°C, to simulate approximate soil temperatures in the field, and photoperiod to 24 h light. Petri dishes were moistened periodically with distilled water. Germination was monitored three times per week until the end of the experiment (45 days after sowing).

We calculated germination percentage per petri dish, and mean germination time in days (MGT) as:

$$MGT = \frac{\sum_{i=1}^k n_i t_i}{\sum_{i=1}^k n_i}$$

where  $n_i$  is the number of seeds germinated at the  $i$ th observation date;  $t_i$ , the number of days since the beginning of the experiment to the  $i$ th date, and  $k$  the total number of dates of observation (Ranal and Santana 2006).

## Common garden trials

### *Experimental design*

In spring 2018, three common garden experiments were established across the latitudinal range of *B. pendula* in Europe in Cerezo de Arriba, Spain (41°11'49.8"N, 3°31'17.4"W), Marburg, Germany (50°48'03.6"N, 8°48'24.8"E) and Šlienava, Lithuania (54°51'18.6"N, 24°03'02.9"E) (Fig. 1). The Spanish site was located in the species southern limit, at 1,319 m.a.s.l. (metres above sea level), characterized by a temperate oceanic submediterranean climate (Rivas-Martínez *et al.* 2004) (Table 2). The other two sites were located in the centre of the species distribution range, at 325 m.a.s.l. in Germany and 70 m.a.s.l. in Lithuania, representing temperate oceanic climate and temperate continental climate, respectively (Rivas-Martínez *et al.* 2004) (Table 2).

In each garden, we established 255 experimental units distributed in three blocks (85 experimental units/block). Each experimental unit consisted of 16 cells of 30 cm<sup>2</sup> each, delimited with plastic ground-grids that were open in the bottom and lateral walls (Gutttagarden®). Grids were filled with a mixture of 50% fine sand and 50% peat to homogenize superficial soil conditions across gardens. Seeds from population DE2 were not included in the design because they did not germinate in the chamber (see Results and Table 1). The final design comprised 11 different populations, plus the second-year (2017) seed lots of populations DE1 and ES2. The family structure (i.e. the maternal identity) was maintained in the 2016 seed lots from ES2 (Spain), DE1 (Germany) and LT2 (Lithuania) (Table 1). These three populations were selected based on their proximity (geographically and climatically) to the corresponding common garden sites. Seeds from different mother trees were pooled within each of the remaining seed lots before sowing. Fifty-five out of the 85 experimental units per block corresponded to maternal families of the populations with family structure (one experimental unit/family/block). The remaining 30 units in each block corresponded to populations without family structure (three experimental units/population/block). Experimental units were randomized following a 51x5 latinized row-column design, using CycDesign software (Whitaker *et al.* 2002).

### *Seed mass, preparation and sowing*

We measured average seed mass per population and per maternal family in the populations without and with family structure, respectively. We used between 0.04 g and 0.06 g of seeds per population or maternal family for this estimation (ISTA 2007). Since chilling treatments had a negative impact on both germination rate and time (see Results), seeds used for the common gardens were not chilled. *B. pendula* typically exhibits high proportions of unviable (empty) seeds, with substantial variation among populations (e.g. 30-70% in Midmore *et al.* 2015). Reliably separating full and empty *Betula* seeds is a very time-consuming process, unfeasible for the large amounts used in this study. So, aiming to obtain a sufficient and balanced number of emerged seedlings across populations in the

field while controlling for the effect on emergence rates of potential variation in empty seed proportions, we adjusted the number of seeds sown per population or maternal family in the common gardens. Based on benchmark chamber germination rates (influenced by the presumably variable proportion of empty seeds in the lots), we estimated the number of seeds per cell needed to expect at least one germinated seed with 95% probability for each population in each cell, and multiplied this number by a safety factor of 25, considering that field emergence rates might be lower than baseline chamber rates. For maternal families from ES2, DE1, and LT2, we used the averaged population germination rate for these estimates. Seeds were sown in spring 2018 and were watered three times per week for one month.

#### *Field measurements and fitness estimates*

Emergence and survival were monitored three times per week the first month after sowing, every one-two weeks the second month, and once per month until November 2018. Each day of measurement, we counted the number of seedlings per cell and considered emergence and mortality when the number of seedlings increased or decreased since the last day of measurement, respectively.

#### *Growth and phenology traits*

At the end of the first growing season (November 2018), height and diameter of the surviving seedlings were measured. We also calculated the slenderness index, a metric of mechanical stability, as height/diameter. At the beginning of the following growing season (mid May 2019), bud burst was scored every 1-2 days in one seedling per cell when leaf tips were clearly above bud scales (Finn *et al.* 2007). Growth and phenology were only measured in the German garden, because of null emergence and null survival in the Lithuanian and Spanish sites, respectively. Consequently, garden-by-population or garden-by-family interactions were only tested for emergence rates but not for growth or for phenology traits (see also statistical analyses).

### **Statistical analyses**

#### *Chamber experiment*

Binomial mixed effects models with logit link function and lineal mixed effect models were used to test for differences in germination rate (GR) and in mean germination time (MGT) among populations and chilling treatments. Population, treatment and their interaction were included as fixed-effect factors and block as a random-effect factor. Analogous models were implemented to test for differences in GR and MGT between seed-crop years for ES1 and DE1, with seed-crop year and treatment as fixed-effect factors.



### Common gardens

Both the seedling emergence and survival rates in the common-garden sites were averaged per experimental unit (grid of sixteen cells) for the analyses. Survival was measured relative to the number of emerged seedlings. Emergence was low overall and both fitness components presented an overdispersed zero-inflated distribution, with many empty cells and most seedlings emerging (and surviving) in small clumps. We, therefore, used generalized additive models (GAMLSS) with a zero-inflated beta distribution (BEINF0) for the analyses of both the emergence and the survival rate. This distribution is a mixture of a discrete value 0 with probability  $p_0$  and a beta distribution  $f_w \sim \text{Beta}(\mu, \sigma)$  on the unit interval (0,1) with probability  $(1 - p_0)$ . The probability density function of the mixture is thus given by (Equation 1):

$$f_y(y|\mu, \sigma, \nu) = \begin{cases} p_0 & \text{if } y = 0 \\ (1 - p_0)f_w(y|\mu, \sigma) & \text{if } 0 < y < 1 \end{cases}$$

In the case of the emergence rate, the mean  $\mu$  of the beta distribution is the estimated mean emergence rate *given that* emergence is not zero (which in our experimental setting can be interpreted as the mean emergence rate across cells showing *some* emergence),  $\sigma$  is the scale parameter of the beta distribution, and  $\nu$  yields the probability  $p_0$  that the emergence rate is zero as  $p_0 = \nu / (1 + \nu)$  (which in turn can be interpreted here as the estimated proportion of experimental cells not showing any emergence at all). Analogously for the survival rate,  $\mu$  is the estimated proportion of surviving seedlings *given that* survival is not zero (which can be regarded here as the mean survival rate across cells with *some* survival),  $\sigma$  is the scale parameter and  $\nu$  yields the probability of zero survival as  $p_0 = \nu / (1 + \nu)$  (i.e. the estimated proportion of cells where all emerged seedlings died). We tested for differences among populations, gardens and their interaction in emergence and survival rates by modelling  $\mu$  and  $\nu$  parameters. We only modelled the intercept for  $\sigma$  parameter, as otherwise convergence was not reached. Models included population, garden, and population-by-garden as fixed-effect factors, and block and column both nested within garden as random effect factors. The resulting models were compared using likelihood-ratio tests. To test for differences in emergence and survival between seed-crop years of ES2 and DE1, we performed one GAMLSS for each of the two populations including seed-crop year, common garden and their interaction as fixed effect factors, and the same random structure as before. GAMLSS for emergence rate of the three populations with family structure were analysed separately, with population, garden, and population-by-garden as fixed effect factors, and block nested within garden and family nested within population as random effect factors. Linear models were used to test the associations between observed emergence rates, estimated  $\mu$  and  $p_0$  in the common gardens, and observed chamber germination rates. Linear models were also used to examine associations between observed emergence and survival rates. MGT was not analysed in field conditions since the number of emerged seedlings was very low, which affects the interpretation of this parameter (Ranal and Santana 2006).



Linear mixed models were used for the analyses of growth and phenology traits in the five populations with more than 25 seedlings alive. Population was included as a fixed-effect factor, and block, column and row as random-effect factors. To avoid the effect of competition, only cells with less than five seedlings were used for the analyses of the growth traits. Comparisons of population means were assessed using Tukey post-hoc tests. Finally, we also used linear mixed models to analyse growth and phenology traits in populations with family structure. All terms were included as random-effect factors to study their effects on the variance.

The association between trait population means and geographic and environmental variables of the seed provenances were tested using multiple regression. *B. pendula* is only present in high altitudes in the southern areas and in mid-low altitudes in northern populations. Consequently, latitude and altitude were strongly negatively correlated ( $r = -0.93$ ,  $P < 0.001$ ). Longitude and latitude were also marginally correlated ( $r = 0.55$ ,  $P = 0.054$ ). To avoid multi-collinearity, we only included latitude in the multiple regression, as it better represents the geographic structure of the sampled populations. We also included two climatic variables in the multiple regression: mean annual temperature and annual precipitation for the period 1979-2013 obtained from CHELSA (Karger *et al.* 2017). We used linear regressions to test the associations between population trait means and seed mass. A step forward-backward selection was performed. Association among traits were tested using Pearson correlations.

All analyses were conducted using R 2.3.5 (R Core Team 2014). We used the packages “lme4” (Bates *et al.* 2014) for mixed models, “emmeans” (Lenth *et al.* 2019) for post-hoc tests, “Hmisc” (Harrell and Harrell 2019) for correlation analyses, “gamlss” (Rigby and Stasinopoulos, 2005) for the analyses of traits with zero-inflated distributions (germination and survival), and “ggplot2” (Wickham *et al.* 2016) and “plotly” (Plotly Technologies Inc. 2005) for graphic representation of the results.

## Results

### Seed germination in the chamber experiment

Mixed models revealed significant differences among chilling treatments in both germination rate (GR) and mean germination time (MGT) (Table S1). On average, C and T1 treatments resulted in higher GR (mean  $17.4 \pm$  standard error  $1.50\%$  and  $16.6 \pm 1.45\%$ , respectively) than T2 ( $12.9 \pm 1.21\%$ ) and T3 ( $8.6 \pm 0.9\%$ ). In addition, C and T1 seeds had shorter MGT ( $13.3 \pm 1.23$  and  $13.7 \pm 1.27$  days, respectively) compared to T2 and T3 pre-chilled seeds ( $16.7 \pm 1.54$  and  $14.7 \pm 1.39$  days, respectively) (data not shown). There were also significant population differences in both traits, and population-by-treatment interaction in GR (Table S1). Populations LT1, LT2 and DE1, had the highest GR in all treatments, whereas most of the other populations exhibited very low values (Fig. 2).

Population DE2 did not germinate in any treatment (Fig. 2). Significant differences in GR among 2016 and 2017 seed crops were found for population DE1 (1.1% in 2017 versus 49.1% in 2016) but not for ES2 (Table S2). Population LT1 had the shortest MGT ( $10.3 \pm 1.1$  days), whereas ES1 and CH1 had the longest MGT ( $19.9 \pm 2.02$  and  $20.4 \pm 2.18$  days respectively) (data not shown). GR and MGT were not correlated ( $P = 0.201$ ).

### Seedling emergence in the field

No seedling emergence was observed in the Lithuanian site, whereas a total of 383 and 4554 seedlings emerged in the Spanish and German gardens, respectively, which represent 0.1% and 1.3% of the estimated seeds sown in each of the two sites. In the two common gardens with emergence, three populations (LT1, LT2 and DE1) showed emergence rates that were an order of magnitude higher than those of the rest (Fig. 3).

Generalized additive models (GAMLSS) showed significant differences among gardens for emergence rate in both  $\mu$  and  $\nu$  parameters (Table S3). There were also significant differences among populations in both  $\mu$  and  $\nu$  for seedling emergence, as well as significant population-by-garden interaction in  $\nu$ , but not in  $\mu$  (Table S3). The  $\mu$  parameter, which represents the estimated seedling emergence rate across experimental cells with *some* emergence, ranged between 0.82% (for ES1) and 7.15% (for LT1) in the German garden and between 0.20% (for ES1) and 0.95% (for DE1) in the Spanish garden (Table S4). In the German garden, the estimated probability of zero emergence,  $p_0$  (calculated using  $\nu$  in Equation 1) ranged between 77.8% for FR1 and CH1 and 0.0% for FR2 and LT1, indicating that all cells in the experiment for the two latter populations had some emergence (Table S4). In the Spanish garden,  $p_0$  ranged between 100% (i.e. all cells had zero emergence) for CH1, and 33% for IT2, FR1, GB1 and LT1, indicating that these four populations exhibited some emergence in two thirds of the experimental cells (Table S4). There were correlations between observed population seed germination rates in the chamber and observed population seedling emergence rates, correlations that were significant at the German garden ( $r = 0.91$ ,  $P < 0.001$ ) and marginally significant in the Spanish garden ( $r = 0.63$ ,  $P = 0.050$ ). There was no correlation between observed population emergence rates in the two common gardens ( $r = 0.43$ ,  $P = 0.213$ ).

GAMLSS revealed significant differences in emergence rates between the two different seed-crop years of the DE1 population in the German garden, both in  $\mu$  and  $\nu$  (Table S5), ranging between 3.25% and 0.01% for the 2016 and 2017 seed crops, respectively. In the Spanish garden, the null emergence of the seed crop from 2017 of DE1 contrasted with the relatively high emergence rate found for seed crop 2016 (0.95%). No significant differences in emergence were found between the seed-crops years of ES2 in any of the two gardens (Table S5). GAMLSS performed on populations with family structure for emergence rates did not reveal significant family effects for  $\nu$  or  $\mu$  (Table 3), whereas differences among populations were significant for both parameters (Table 3).

### Seedling survival in the field

All seedlings that emerged in the Spanish garden died before the end of the experiment. By contrast, 1274 seedlings (29% of emerged seedlings) survived in the German garden. Observed seedling survival rates varied from 20% to 42% for ES2 and CH1, respectively (Fig. 4). Differences among populations were significant for the  $\nu$  parameter but not for  $\mu$  (Table S6), indicating significant population variation in the estimated proportion of cells without survival ( $p_0$ , calculated from  $\nu$ ), though not in the estimated survival rate for cells exhibiting seedlings alive at the end of the experiment. Observed seedling emergence and seedling survival probability were not correlated ( $r = -0.12$ ,  $P = 0.723$ ), i.e. seeds that tended to germinate more did not tend to produce more viable seedlings. The proportion of sown seeds resulting in alive seedlings at the end of the experiment was mostly determined by the emergence rate ( $r = 0.99$ ,  $P < 0.001$ ).

The highest and lowest survival rates corresponded to both seed crops from ES2 (2016: 20% and 2017: 43.7%), although GAMLSS did not show significant differences among them in  $\mu$  or  $\nu$  (Table S5). Seedlings from the 2017 seed crop of DE1 did not survive in the German garden, precluding testing for differences between seed crops for this population.

Seedling survival rate GAMLSS performed on populations with family structure did not show a significant family effect in  $\mu$  or  $\nu$ , whereas a highly significant population effect was observed for  $\nu$  (Table 3).

### Field growth and phenology traits

There were only five populations with more than 25 seedlings alive to conduct mixed models for growth and phenology traits (see Table 1). Results showed significant differences among populations in height, diameter, slenderness and the day of bud burst (Table S7, Fig. 5). Height was strongly correlated with diameter ( $r = 0.97$ ,  $P = 0.005$ ) and slenderness ( $r = 0.94$ ,  $P = 0.015$ ). No significant correlation was found between diameter and slenderness ( $r = 0.85$ ,  $P = 0.067$ ). Correlations between bud burst and growth traits were not significant.

Analyses for growth and phenology traits at the family level were only conducted for populations LT2 and DE1, as population ES2 had a very low number of seedlings (none of the families had more than 6 seedlings alive and more than half of the families had between 0-2 seedlings). Mixed models for height and slenderness showed significant family and population effects that explained together more than 40% of the variance (Fig. 6). Mixed models for diameter showed a significant family effect that explained 26.3% of the variance, while the population effect was not significant (Fig. 6). For the date of bud burst, differences among families were significant, although this effect explained less than 10% of the variance. No significant differences among populations were found for this trait (Fig. 6).

## Climatic and geographic patterns

Multiple regression analyses showed a significant positive association between latitude and chamber germination rate, and between latitude and field seedling emergence rate at the German garden (Fig. 7 A-B). A significant positive association between latitude and  $\mu$  (i.e., the mean seedling emergence rate across cells with *some* emergence) was found in both the Spanish and German common gardens (Fig. 7 C-D). No significant associations with climatic or geographic variables were found for seed mass, emergence rate in Spain, probability of no germination ( $p_0$ ) in any of the gardens, or seedling survival in Germany (which was only measured there). Associations between growth traits and climate variables were not significant, although marginally significant negative relationships were found between latitude and height, diameter and slenderness (Fig. 7 E-G). No association was found between date to bud burst measured in the common garden and latitude. In contrast, the date to bud burst was negatively correlated with the annual mean temperature at the population of origin (Fig. 7 H). Linear regressions also showed significant positive associations between seed mass and germination rates in chamber (Fig. S1a) and between seed mass and emergence rates in both the Spanish and German gardens (Fig. S1b), but not between seed mass and growth or phenology traits (data not shown).

## Discussion

The present paper investigated genetic variation at early fitness traits among and within *Betula pendula* populations spread over a large portion of the distribution range of the species, including southern margins. By performing chamber experiments under controlled conditions and common gardens trials at three semi-natural sites across a climatic gradient, we detected strong population variation in germination and emergence rates as well as in growth and phenology traits throughout the first year of the life cycle.

### Germination rate in the chamber experiment

Germination is one of the most drastic transitions, and earliest fitness components, in plant life cycles (Donohue *et al.* 2010). In our chamber experiment, germination rates were generally low, but with substantial differences among populations (Fig. 2). Three populations exhibited germination rates above 50%, whereas nine out of 12 populations had germination rates below 10 % in all prechilling treatments (Fig. 2). Differences in germination rates among populations of *B. pendula* have been previously reported, even at smaller geographical scales (Holm *et al.* 1994a). Similar to our results, Midmore *et al.* (2015) predicted markedly high differences in germination rates among populations, ranging between 0 and 100 % among populations from 47° N to 60° N. Similar patterns of population-level variation have been found in other *Betula* species (e.g. Bevington 1986; Reyes *et al.* 1997).

Differences in germination rates among populations could have resulted from different proportions of filled seeds, which is supported by the strong positive correlation between seed mass and germination rates (Fig. S1). Midmore *et al.* (2015) showed that, on average, southern populations had a higher percentage of empty seeds (~77 %) than northern populations (~28 %). Southern *B. pendula* populations are generally more isolated, have smaller size, lower density, and lower levels of genetic variation, which could have limited ovule fertilization rates because of more irregular pollen availability and/or inbreeding depression (Holm 1994a, Palmé *et al.* 2003). In addition, resource scarcity and warm and dry conditions during seed maturation could have resulted in smaller, less vigorous embryos in southern populations. In all, lower germination rates in southern populations appear to be greatly determined by demographic and environmental factors.

Other factors could have been also important in the observed germination patterns. Specifically, *B. pendula* is a masting species with wide annual variation in the quantity and quality of seed crops (Atkinson 1992). In our study, differences in germination between seed crops from 2016 versus 2017 were strikingly high for population DE1, suggesting that investment in reproduction and environmental factors could influence the proportion of viable seeds. Finally, it is worth noting that dormancy is a typical characteristic of birch seeds (Myking and Heide 1995). Differences in germination among populations could have also resulted from differences in dormancy duration or different environmental requirements to break their dormancy. For instance, Midmore *et al.* (2015) showed that populations of *B. pendula* from higher latitudes were more sensitive to prechilling compared to populations from lower latitudes, particularly under colder temperatures. In our study, in contrast, germination rates were largely unaffected by prechilling. These results, together with the similar patterns of emergence rates observed in field conditions (see next section), suggest that potential differences in dormancy among populations are unlikely to be major determinants of observed variation in population germination rates.

### **Emergence and survival rates in the common gardens**

Three populations located in the center of the species distribution area had emergence rates an order of magnitude higher than those of the rest (Fig. 3). Demographic, environmental and genetic factors are expected to have shaped population patterns of emergence rates in the field, as discussed for germination rates. Field emergence, however, was strikingly lower than chamber germination in all populations. This is consistent with Tylkowski (2012), who found that *B. pendula* seedling emergence rates from seeds sown in containers were lower than the corresponding germination rates in chamber. We expected the Lithuanian site to be the most suitable for *B. pendula* according to climate niche models (Beck *et al.* 2016). However, null seedling emergence was found in Lithuania. Since seeds of this species require a continuously moisturized seedbed for germination (Gordon 1992), the exceptionally warm and dry 2018 spring in Lithuania probably minimized germination and precluded

any seedling emergence in this site, despite the initial watering. These results suggest that extreme climatic events can hamper natural regeneration even in the central core of the distribution of *B. pendula*, and that seedling establishment is a phase very sensitive to climatic conditions (Rousi *et al.* 2011). The very low emergence rate and the subsequent total seedling mortality observed for all provenances at the Spanish site might also have resulted from the dry conditions during spring and summer. This site is located in the southern edge of the species, in an area characterized by a mountainous Mediterranean climate where summer storms play an important role in long-term regeneration of temperate species. Rainfall in spring of 2018 was higher than average in the area, but rains were irregular, with short dry and hot intermediate periods during which soil moisture was considerably reduced. Moreover, the summer was much drier than average (Table 2). These results suggest that drought strongly limits seedling emergence and survival of *B. pendula*, and therefore that drier conditions expected in the southern edge of the species range in the next decades will hinder natural regeneration in this area (Humphries 1982, Hynynen *et al.* 2010, Beck *et al.* 2016, Pliūra *et al.* 2019).

Climatic conditions in the German site seemed more favourable, even if seedlings were exposed to low precipitation that originated some soil water deficit in mid-summer. Emergence rates in Germany did not reach the seed germination rates observed in the chamber, but still were significantly higher than those observed in Spain and Lithuania. The German garden was in addition the only of the three gardens where some seedlings survived until the end of the experiment. The comparatively high survival rate of emerged seedlings (28%) relative to the low emergence rate (1.3%) suggests that selection pressures at early-life stages in *B. pendula* are particularly strong during the seed-to-seedling transition. Furthermore, the lack of significant population differentiation in seedling survival rates among populations within experimental sites suggests, in accordance with van Andel (1998), that the effect of environmental conditions on early seedling mortality substantially exceeds the one of the genetic background. These results highlight the importance of including the germination (and not only the seedling/sapling) stage in field trials conducted to assess adaptive genetic variation and to identify the life stages that represent the major bottlenecks for recruitment (Walck *et al.* 2011).

### **Growth and phenology traits**

Our results showed population differences in growth and phenology traits. Growth in height and diameter tended to be higher in populations from lower latitudes (Fig. 7 E-G). Previous studies have also reported negative latitudinal clines in growth traits in *B. pendula* at smaller spatial scales (Velling 1979; Viherä-Aarnio and Velling 2008; Myking and Heide 1995). This latitudinal pattern could reflect local adaptation to differences in the length of the growing period. Viherä-Aarnio *et al.* (2005) found that, under a common environment, southern populations of the species were able to keep growing in days with shorter photoperiods, therefore prolonging their growing season in autumn.



Phenology in spring could also play a role in the differences in growing season length across populations. In fact, our results showed that populations of *B. pendula* differed in the timing of bud burst. However, this trait was associated with annual temperature but not with latitude. Furthermore, Li *et.al* (2002) found 40-day differences among southern and northern populations of *B. pendula* in the date of growth cessation, while differences in growth initiation in spring were of only 4-7 days. These observations suggest a minor contribution of early phenology to growth variation among populations of *B. pendula*.

Different photosynthetic capacity might also have contributed to observed growth differences among populations. Previous studies have reported gas exchange and growth potential variation across genotypes of *B. pendula* (Possen *et al.* 2011). Aspelmeier and Leuschner (2004) observed that *B. pendula* genotypes from areas with drier summers had higher gas exchange rates, including higher stomatal conductance and photosynthesis, in both well-watered and dry conditions. These authors suggested that this strategy would favor fast growth and competitiveness in drought-prone habitats.

Contrary to expectations based on previous studies, seed mass was not significantly associated with growth traits. Seed mass has been generally observed to play an important role in early growth even at intraspecific level (e.g. Oleksyn *et al.* 1998; Rose *et al.* 2009; Ramírez-Valiente and Robledo-Arnuncio 2015). One possible reason for the absence of association between seed mass and growth could be low statistical power, resulting from the small number of populations that had seedlings alive at the end of the experiment. Other reason is that seed mass was determined mostly by empty seed proportions, and not by viable seed mass differences. Finally, it could be that traits such as growth phenology and gas exchange capacity would be more important than seed mass for growth potential in this species.

### **Within-population variation at early fitness traits**

Most forest trees show high within-population genetic variation (Hamrick 2004), particularly widely-distributed species (e.g. Ramírez-Valiente *et al.* 2014, 2015). Studies in *B. pendula* have shown significant intrapopulation genetic variation in growth and phenology traits in adult and juvenile trees (Baliuckienė and Baliuckas 2006; Baliuckas and Pliūra, 2008, Rousi and Heinonen 2007). We also found differences among families within populations in early growth traits and in the timing of bud burst, with the family effect explaining between nine and 25% of the total phenotypic variance for these traits. The variance explained by the family effect was indeed larger than that of the population effect for diameter and bud burst. These results align with those of previous studies suggesting that intrapopulation genetic variance in *B. pendula* growth and phenology traits is an important component of the intraspecific genetic diversity in this species. However, the substantial levels of genetic variance in bud burst reported within *B. pendula* populations might be insufficient to enable fast



enough adaptation to the expected rate of change in temperature (Billington and Pelham 1991; Possen *et al.* 2014). On the other hand, we failed to detect significant variation in the rates of seedling emergence and survival among families within populations (Table 4). One possible reason for this negative result is that the low germination rates resulted in low statistical power for the analysis of intra-population genetic variation. Another possible explanation is that the environmental conditions might have imposed a very strong selective pressure (reflected by the low emergence rates), thus reducing the expression of intra-population genetic variability.

In conclusion, our study revealed extremely low germination and seedling survival of *B. pendula*, particularly under dry conditions, suggesting limited future regeneration capacity if drought conditions increase, especially in the southern margins. Populations greatly differed in early fitness components, with significant intrapopulation variation detected for growth and phenology traits but not for emergence and survival rates. Genetic differences and other non-genetic factors, such as demography, climate and maternal effects, could have shaped the observed population variation patterns. These results, along with high among-year variation in seed production and germination, highlight the difficulty of achieving general conclusions concerning tree early-fitness traits. Further studies under sufficiently replicated environmental conditions will be necessary to test for plastic responses, ideally using seeds collected in different years, to improve our understanding on the potential regeneration niche and vulnerability of forest trees species such as *B. pendula* to climate change.

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## Data and supporting information

Data and supporting information are available in the online version of this article.

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## Literature cited

- Alberto FJ, Aitken SN, Alía R, González-Martínez SC, Hänninen H, Kremer A, Lefèvre F, Lenormand T, Yeaman S, Whetten R, Savolainen O. 2013. Potential for evolutionary responses to climate change - evidence from tree populations. *Global Change Biology* **19**:1645–1661.
- Aspelmeier S, Leuschner C. 2004. Genotypic variation in drought response of silver birch (*Betula pendula*): leaf water status and carbon gain. *Tree physiology* **24**:517–528.
- Atkinson MD. 1992. *Betula pendula* Roth (*B. verrucosa* Ehrh.) and *B. pubescens* Ehrh. *The Journal of Ecology* **80**:837.
- Baliuckas V, Pliūra A. 2008. Phenogenetic variation pattern in adaptive traits of *Betula pendula*, *Alnus glutinosa* and *Quercus robur* in Lithuania. *Biologija* **2**.
- Baliuckienė A., Baliuckas V. 2006. Genetic Variability of Silver Birch (*Betula pendula* L.) Wood Hardness in Progeny Testing at Juvenile Age. *Baltic Forestry* **12**: 134–140.
- Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* **67**:1–48.
- Beck P, Caudullo G, de Rigo D, Tinner W. 2016. *Betula pendula*, *Betula pubescens* and other birches in Europe: distribution, habitat, usage and threats. In: San-Miguel-Ayán J, de Rigo D, Caudullo G, Houston Durrant, Mauri A. (Eds.), *European Atlas of Forest Tree Species*. Publ. Off. EU, Luxembourg, pp. e010226
- Bevington J. 1986. Geographic differences in the seed germination of paper birch (*Betula papyrifera*). *American Journal of Botany* **73**:564–573.
- Billington HL, Pelham J. 1991. Genetic variation in the date of budburst in Scottish birch populations: implications for climate change. *Functional Ecology*, **5**:403–409.
- Castro J, Zamora R, Hódar JA, Gómez JM. 2004. Seedling establishment of a boreal tree species (*Pinus sylvestris*) at its southernmost distribution limit: consequences of being in a marginal Mediterranean habitat. *Journal of Ecology* **92**:266–277.
- Collins M, Knutti R, Arblaster J, Dufresne JL, Fichetef T, Friedlingstein P, Gao X, Gutowski WJ, Johns T, Krinner G, Shongwe M, Tebaldi C, Weaver AJ, Wehner MF, Allen MR, Andrews T, Beyerle U, Bitz CM, Bony S and Booth, BBB. 2013. Long-term Climate Change: Projections, Commitments and Irreversibility. in TF Stocker, D Qin, G-K Plattner, MMB Tignor, SK Allen, J Boschung, A Nauels, Y Xia, V Bex & PM Midgley (eds), *Climate Change 2013 - The Physical Science Basis: Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. Intergovernmental Panel on Climate Change, Cambridge University Press, New York NY USA, pp. 1029–1136.
- De Atrip N, O'Reilly C. 2007. Germination response of alder and birch seeds to applied gibberellic acid and priming treatments in combination with chilling. *Annals of Forest Science* **64**:385–394.
- Donohue K, Rubio de Casas R, Burghardt L, Kovach K, Willis CG. 2010. Germination, Postgermination Adaptation, and Species Ecological Ranges. *Annual Review of Ecology, Evolution, and Systematics* **41**:293–319.
- Dyderski MK, Paź S, Frelich LE, Jagodziński AM. 2018. How much does climate change threaten European forest tree species distributions? *Global Change Biology* **24**: 1150–1163.
- EEA. 2012. *Climate Change, Impacts and Vulnerability in Europe 2012, an Indicator-Based Report*. EEA Report No. 12/2012, European Environment Agency (EEA), Copenhagen, Denmark, 304 pp.
- Eggers J, Lindner M, Zudin S, Zaehle S, and Liski J. 2008. Impact of changing wood demand, climate and land use on European forest resources and carbon stocks during the 21st century. *Global Change Biology*, **14**: 2288–2303.
- Finn GA, Straszewski AE, Peterson V. 2007. A general growth stage key for describing trees and woody plants. *Annals of Applied Biology* **151**:127–131.
- Gallego Zamorano J, Hokkanen T, and Lehtikoinen A. 2018. Climate-driven synchrony in seed production of masting deciduous and conifer tree species. *Journal of Plant Ecology*, **11**: 180–188.
- Gibson AL, Espeland EK, Wagner V, Nelson CR. 2016. Can local adaptation research in plants

- inform selection of native plant materials? An analysis of experimental methodologies. *Evolutionary Applications* **9**:1219–1228.
- Gordon AG, 1992. Seed Manual for Forest Trees. *Forestry Commission Bull.* 83, HMSO, London, 132 pp.
- Hamrick JL. 2004. Response of forest trees to global environmental changes. *Forest Ecology and Management* **197**:323–335.
- Harrell Jr FE and Harrell Jr MFE. 2019. Package ‘Hmisc’. CRAN2018, 235-6.
- Hijmans RJ, Cameron S, Parra J, Jones P, Jarvis A, and Richardson K. 2005. WorldClim, version 1.3. University of California, Berkeley.
- Holm SO. 1994 a. Reproductive patterns of *Betula pendula*, and *B. pubescens* coll. along a regional altitudinal gradient in northern Sweden. *Ecography* **17**:60–72.
- Holm SO. 1994 b. Reproductive variability and pollen limitation in three *Betula* taxa in northern Sweden. *Ecography* **17**:73-81.
- Humphries RN, Jordan MA, Guarino L. 1982. The effect of water stress on the mortality of *Betula pendula* Roth. and *Buddleia davidii* Franch. Seedlings. *Plant and Soil* **64**:273-276.
- Hynynen J, Niemisto P, Viherä-Aarnio A, Brunner A, Hein S, Velling P. 2010. Silviculture of birch (*Betula pendula* Roth and *Betula pubescens* Ehrh.) in northern Europe. *Forestry* **83**:103–119.
- ISTA. 2007. International Rules for Seed Testing. The International Seed Testing Association, Basserdorf, Switzerland.
- Iverson LR, Schwartz MW, Prasad AM. 2004. How fast and far might tree species migrate in the eastern United States due to climate change? *Global Ecology and Biogeography* **13**:209–219.
- Jackson ST, Betancourt JL, Booth RK, Gray ST. 2009. Ecology and the ratchet of events: Climate variability, niche dimensions, and species distributions. *PNAS* **106**:19685–19692.
- Jones SK, Gosling PG. 1994. “Target moisture content” prechill overcomes the dormancy of temperate conifer seeds. *New Forests* **8**:309-321.
- Jump AS, Hunt JM, Peñuelas J. 2006. Rapid climate change-related growth decline at the southern range edge of *Fagus sylvatica*. *Global Change Biology* **12**:2163–2174.
- Jump AS, Marchant R, Peñuelas J. 2009. Environmental change and the option value of genetic diversity. *Trends in Plant Science* **14**:51–58.
- Karger DN, Conrad O, Böhrer J, Kawohl T, Kreft H, Soria-Auza RW, Zimmermann NE, Linder HP, Kessler M. 2017. Climatologies at high resolution for the earth’s land surface areas. *Scientific Data* **4**:170122.
- Kovats RS, Valentini R, Bouwer LM, Georgopoulou E, Jacob D, Martin E, Rounsevell M, Soussana JF. 2014. Part B: Regional Aspects Europe. In: Climate Change 2014: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Barros V., Field CB, Dokken DJ, Mastrandrea MD, Mach KJ, Bilir TE, Chatterjee M, Ebi KL, Estrada YO, Genova RC, Girma B, Kissel ES, Levy AN, MacCracken S, Mastrandrea PR, White LL (eds.)]. *Cambridge University Press*, Cambridge, United Kingdom and New York, NY, USA, pp. 1267-1326.
- Kremer A, Potts BM, Delzon S. 2014. Genetic divergence in forest trees: understanding the consequences of climate change. *Functional Ecology* **28**:22–36.
- Lenth R, Singmann H, Love J, Buerkner P, Herve M. 2019. Package “emmeans”. <https://cran.r-project.org/web/packages/emmeans/>
- Li C, Puhakainen T, Welling A, Viherä-Aarnio A, Ernstsén A, Junttila O, Heino P, Palva T. 2002. Cold acclimation in silver birch (*Betula pendula*). Development of freezing tolerance in different tissues and climatic ecotypes. *Physiologia Plantarum* **116**:478–488.
- Midmore EK, McCartan SA, Jinks RL, Cahalan CM. 2015. Using thermal time models to predict germination of five provenances of silver birch (*Betula pendula* Roth) in southern England. *Silva Fennica* **49**:1266.
- Myking T, Heide OM. 1995. Dormancy release and chilling requirement of buds of latitudinal ecotypes of *Betula pendula* and *B. pubescens*. *Tree Physiology* **15**:697–704.
- Nadeau CP, and Urban MC. 2019. Eco- evolution on the edge during climate change. *Ecography* **42**:1280-1297.
- Oleksyn J, Modrzyński J, Tjoelker MG, Zytkowskiak, R, Reich, PB, Karolewski P. 1998. Growth and physiology of *Picea abies* populations from elevational transects: common garden evidence for

- altitudinal ecotypes and cold adaptation. *Functional Ecology* **12**:573-590.
- Palmé AE, Su Q, Rautenberg A, Manni F and Lascoux M. 2003. Postglacial recolonization and cpDNA variation of silver birch, *Betula pendula*. *Molecular ecology*, **12**: 201-212.
- Peñuelas J, Ogaya R, Boada M, S. Jump A. 2007. Migration, invasion and decline: changes in recruitment and forest structure in a warming-linked shift of European beech forest in Catalonia (NE Spain). *Ecography* **30**:829–837.
- Pliūra A, Jankauskienė J, Bajerkevičienė G, Lygis V, Suchockas V, Labokas J, and Verbylaitė R. 2019. Response of juveniles of seven forest tree species and their populations to different combinations of simulated climate change-related stressors: spring-frost, heat, drought, increased UV radiation and ozone concentration under elevated CO<sub>2</sub> level. *Journal of Plant Research*, **132**: 789-811.
- Plotly Technologies Inc. 2015. Collaborative data science. *Plotly Technologies Inc.* Montréal. <https://plot.ly>
- Possen BJ, Oksanen E, Rousi M, Ruhanen H, Ahonen V, Tervahauta A, Heinonen J, Kärelampi S, Vapaavuori, E. 2011. Adaptability of birch (*Betula pendula* Roth) and aspen (*Populus tremula* L.) genotypes to different soil moisture conditions. *Forest Ecology and Management* **262**:1387-1399.
- Possen B J, Rousi M, Silfver T, Anttonen MJ, Ruotsalainen S, Oksanen E and Vapaavuori E. 2014. Within-stand variation in silver birch (*Betula pendula* Roth) phenology. *Trees*, **28**: 1801-1812.
- R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, <http://www.R-project.org/>.
- Ramírez-Valiente JA, Valladares F, Aranda I. 2014. Exploring the impact of neutral evolution on intrapopulation genetic differentiation in functional traits in a long-lived plant. *Tree genetics & genomes* **10**:1181-1190.
- Ramírez-Valiente JA, Valladares F, Delgado A, Nicotra AB, Aranda I. 2015. Understanding the importance of intrapopulation functional variability and phenotypic plasticity in *Quercus suber*. *Tree Genetics & Genomes* **11**:35.
- Ramírez-Valiente JA, and Robledo-Arnuncio JJ. 2015. Exotic gene flow affects fitness trait values but not levels of heritable trait variation in the southernmost population of Scots pine (*Pinus sylvestris* L. var. *nevadensis*). *Biological Conservation* **192**:331-342.
- Ranal MA, Santana DG. 2006. How and why to measure the germination process? *Revista Brasileira de Botânica* **29**:1–11.
- Reyes O, Casal M, Trabaud L. 1997. The influence of population, fire and time of dissemination on the germination of *Betula pendula* seeds. *Plant Ecology* **133**:201–208
- Rigby RA, Stasinopoulos DM. 2005. Generalized Additive Models for Location Scale and Shape. *Applied Statistics* **54**:507–554.
- Rivas-Martínez S, Penas A, Díaz TE. 2004. Cartographic Service, University of León, Spain.
- Rose L, Leuschner C, Köckemann B, Buschmann H. 2009. Are marginal beech (*Fagus sylvatica* L.) provenances a source for drought tolerant ecotypes?. *European Journal of Forest Research* **128**:335-343.
- Rousi M, Heinonen J. 2007. Temperature sum accumulation effects on within-population variation and long-term trends in date of bud burst of European white birch (*Betula pendula*). *Tree Physiology* **27**:1019–1025.
- Rousi M, Heinonen J, Neuvonen S. 2011. Intrapopulation variation in flowering phenology and fecundity of silver birch, implications for adaptability to changing climate. *Forest Ecology and Management* **262**:2378–2385.
- Samaniego L, Thober S, Kumar R, Wanders N, Rakovec O, Pan M, Zink M, Sheffield J, Wood EF, Marx A. 2018. Anthropogenic warming exacerbates European soil moisture droughts. *Nature Climate Change* **8**:421–426.
- Sanz R, Pulido F, Camarero JJ. 2011. Boreal trees in the Mediterranean: recruitment of downy birch (*Betula alba*) at its southern range limit. *Annals of Forest Science* **68**: 793–802.
- Savolainen O, Pyhäjärvi T, Knürr T. 2007. Gene Flow and Local Adaptation in Trees. *Annual Review of Ecology, Evolution, and Systematics* **38**: 595–619.
- Tylkowski T. 2012. *Betula pendula* seed storage and sowing pre-treatment: effect on germination and seedling emergence in container cultivation. *Dendrobiology* **67**:49–58



- van Andel J. 1998. Intraspecific variability in the context of ecological restoration projects. Perspectives in Plant Ecology. *Evolution and Systematics*, **1**: 221-237.
- Vanhatalo V, Leinonen K, Rita H, Nygren M. 1996. Effect of prechilling on the dormancy of *Betula pendula* seeds. *Canadian Journal of Forest Research* **26**:1203–1208
- Velling, P. 1979. Initial development of different *Betula pendula* Roth provenances in the seedling nursery and in field trials. *Folia Forestalia* 379:1–14. In Finnish with English summary.
- Viherä-Aarnio A, Hakkinen R, Partanen J, Luomajoki A, Koski V. 2005. Effects of seed origin and sowing time on timing of height growth cessation of *Betula pendula* seedlings. *Tree Physiology* **25**:101–108.
- Viherä-Aarnio A, Velling P. 2008. Seed transfers of silver birch (*Betula pendula*) from the Baltic to Finland – effect on growth and stem quality. *Silva Fennica* **42**:735-751.
- Walck JL, Hidayati SN, Dixon KW, Thompson K, Poschlod P. 2011. Climate change and plant regeneration from seed: climate change and plant regeneration. *Global Change Biology* **17**:2145–2161.
- Whitaker D, Williams ER, John JA. 2002. CycDesign version 2: A package for the computer generation of experimental designs. CSIRO, Canberra, Australia
- White TL, Adams WT, and Neale DB. 2007. Forest genetics. Cabi.
- Wickham H. 2016. ggplot2: Elegant Graphics for Data Analysis. *Springer-Verlag New York*. ISBN 978-3-319-24277-4,
- Wong WK, Beldring S, Engen-Skaugen T, Haddeland I, Hisdal H. 2011. Climate Change Effects on Spatiotemporal Patterns of Hydroclimatological Summer Droughts in Norway. *Journal of Hydrometeorology* **12**:1205–1220

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Figure 1. A) Location of sampled *Betula pendula* populations with available seeds (black circles) and the sites of the common garden trials (orange triangles). The green area indicates the species distribution range (EUFORGEN, 2011). B) Natural *B. pendula* populations DE1 (top) and IT1 (bottom). C) German garden and zoom-in of a cell with seedlings.

Figure 2. Germination rates for 14 *Betula pendula* populations in controlled chamber conditions under four treatments: a control without prechilling (C) and pre-chilling treatments with a duration of 15 days (T1), 30 days (T2) and 45 days (T3). Population GB1 was only tested in the control treatment (C) due to low seed availability. Means that do not share letters indicate significant differences among populations within treatments ( $P < 0.05$ , Tukey test). Boxes show the median (inside line), the interquartile range (hinges are the 25<sup>th</sup> and 75<sup>th</sup> percentiles) and 1.5 times the interquartile range (whiskers). Populations are ordered by latitude (codes as in Table 1).

Figure 3. Population means (dots) and reaction norms (lines) for seedling emergence rates of the studied populations of *Betula pendula* in two common gardens in Germany and Spain. Panel A) shows all studied populations in both common gardens. Panel B) displays the zoom-in of populations with emergence rates below 1%. Population codes as in Table 1. Standard errors are not shown for clarity.

Figure 4. Observed seedling survival rate during the first growing season for *Betula pendula* populations in a common garden experiment in Germany. Generalized additive models showed differences among populations in  $\nu$  ( $P = 0.010$ ) but not in  $\mu$  ( $P = 0.171$ ), indicating that populations differed in the estimated proportion of cells without survival ( $p_0$ ), but not in the estimated proportion of cells with at least one seedling alive at the end of the experiment (Table S6). Boxes show the median (inside solid line), the mean (inside dashed line), the interquartile range (hinges are the 25<sup>th</sup> and 75<sup>th</sup> percentiles), 1.5 times the interquartile range (whiskers) and outliers (dots). Population codes as in Table 1. Populations are ordered by latitude, colours as in Figure 3.

Figure 5. Means and standard errors for height, diameter, slenderness and bud burst measured in seedlings of *B. pendula* populations growing in the German common garden experiment. Only populations with more than 25 seedlings still alive in spring 2019 were used. Populations are ordered by latitude. Values that do not share letters are significantly different ( $P < 0.05$ , Tukey test). Population codes as in Table 1. Population colours as in Figure 3 and Figure 4.



Figure 6. Percentage of phenotypic variance of early growth and phenology traits (height, diameter, slenderness index: S.I. and bud burst) explained by mixed model factors block, population, and maternal family within populations in *Betula pendula* common gardens. The numbers inside the bars are *P*-values of the effects, ns indicates a non-significant effect. There was no block variance for bud burst.

Figure 7. Linear regressions between the latitude of *Betula pendula* populations and (A) population chamber germination rate, GR; (B) seedling emergence rate, ER, in the German site; (C) the estimated proportion of emerged seedlings in cells with *some* emergence ( $\mu$ ) in the Spanish site; (D)  $\mu$  in the German site; (E) seedling height; (F) seedling diameter; and (G) seedling slenderness. Panel (H) shows the linear regression between annual mean temperature of the populations and bud burst date. Dots represent population means. Grey shaded areas indicate 95% confidence intervals. Emergence rate in Spain was not significantly associated with any geographic or climatic variable (not shown). Growth and phenology traits (height, diameter, slenderness and bud burst) were only tested in the German garden. Standard errors of population trait means are not shown for clarity.

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Table 1. Population code, country, latitude, longitude, altitude, annual mean temperature (AMT) and annual precipitation (AP) of the studied *Betula pendula* populations. Next columns represents the number of experimental units used for the analyses of germination rate in chamber (GR), emergence rate (ER) in Spain and Germany and survival, as well as the number of seedlings for the analysis of growth and phenology traits. See Material and Methods for more details. Climatic data for the period 1979 - 2013 obtained from CHELSA (Karger *et al.* 2017).

Population	Country	Latitude	Longitude	Altitude (m)	AMT (°C)	AP (mm)	GR	ER	Survival	Growth	Phenology
ES1	Spain	41° 58' N	2° 37' W	1271	8.8	531	12	18	5		
ES2	Spain	42° 40' N	0° 19' W	988	9.1	1001	12 <sup>a</sup>	120 <sup>ab</sup>	26 <sup>ab</sup>	29	29
IT1	Italy	42° 09' N	13° 37' E	1498	6.9	963	12	18	7		
IT2	Italy	43° 36' N	11° 42' E	1084	8.9	1134	12	18	8		
FR1	France	44° 11' N	7° 04' E	1128	6.8	799	12	18	2		
FR2	France	44° 12' N	7° 05' E	1519	5.1	920	12	18	9	20	18
CH1	Switzerland	46° 08' N	8° 59' E	1089	8	1815	12	18	2		
DE1	Germany	51° 50' N	14° 26' E	72	9.6	573	12 <sup>a</sup>	90 <sup>b</sup>	33 <sup>b</sup>	127 <sup>b</sup>	135 <sup>b</sup>
DE2	Germany	52° 32' N	14° 3' E	55	9.3	532	12				
GB1	U.K.	54° 13' N	3° 01' W	31	9.8	1105	3 <sup>c</sup>	18	8		
LT1	Lithuania	54° 37' N	24° 13' E	118	7	661	12	18	9	53	60
LT2	Lithuania	55° 01' N	23° 0' E	56	7.4	669	12 <sup>b</sup>	120 <sup>b</sup>	52 <sup>b</sup>	212 <sup>b</sup>	231 <sup>b</sup>

a: Populations with two seed crops available (2016 and 2017) for the analysis of temporal variation. b: Populations with maternal family structure for the analysis of intrapopulation genetic variation. c: populations assayed only in control treatment in the chamber experiment.

Table 2. Monthly precipitation (Pp, in mm) and monthly mean, maximum and minimum temperatures ( $T_{\text{mean}}$ ,  $T_{\text{max}}$ ,  $T_{\text{min}}$ , in °C) during three months after sowing at the three common garden sites. Values correspond to averages for the reference period 1970-2000 obtained from Worldclim (Hijmans *et al.* 2005), and to records for the study year (2018).

		May		June		July	
		Reference	2018	Reference	2018	Reference	2018
Lithuania	Pp	49	19	68	58	79	138
	$T_{\text{mean}}$	12.4	16.9	15.7	17.3	17.1	20.7
	$T_{\text{max}}$	18.0	23.1	21.3	23.4	19.4	25.4
	$T_{\text{min}}$	6.8	10.8	10.1	11.3	14.9	13.8
Germany	Pp	67	36.2*	69	19.6	67	243
	$T_{\text{mean}}$	12.4	17.5*	15.4	17.9	17.2	21.3
	$T_{\text{max}}$	17.1	23.8*	20.1	23.8	19.7	28.6
	$T_{\text{min}}$	7.7	12.2*	10.6	12.8	14.7	14.2
Spain	Pp	68	NA	45	65	21	2
	$T_{\text{mean}}$	10.3	NA	15.1	16.0	18.9	18.8
	$T_{\text{max}}$	16.4	NA	21.2	19.9	23.3	23.9
	$T_{\text{min}}$	4.2	NA	9.0	12.0	14.6	13.7

NA: no available data. \*Data available from May 15<sup>th</sup>.

Table 3. Results of the generalized additive models (GAMLSS) for *Betula pendula* populations with family structure (ES2, DE1, LT2) for seedling emergence rate in the Spanish and German gardens and for seedling survival rate in the German garden.  $\mu_1$  is the estimated proportion of emerged seedlings in cells with some emergence and  $\nu_1$  yields the probability of no emergence ( $p_0$ ), as  $p_0 = \nu / (1 + \nu)$  (i.e. the estimated proportion of cells without any emergence);  $\mu_2$  is the estimated proportion of surviving seedlings in cells where survival is not zero and  $\nu_2$  yields the probability of no survival ( $p_0$ ), as  $p_0 = \nu / (1 + \nu)$  (i.e. the estimated proportion of cells where all emerged seedlings died). LRT: likelihood ratio test, Df: degrees of freedom. Significant values are in bold type ( $P < 0.05$ ).

Trait	Parameter	Factor	Df	LRT	P
Emergence rate	$\mu_1$	Residual	313		
		Garden	<b>1</b>	<b>11.02</b>	<b>&lt; 0.001</b>
		Block (Garden)	1	0.72	0.396
		Population	<b>2</b>	<b>40.52</b>	<b>&lt; 0.001</b>
		Family (Population)	1	2.22	0.136
		Pop x Garden	2	2.68	0.261
	$\nu_1$	Garden	1	2.68	0.102
		Block (Garden)	<b>1</b>	<b>4.53</b>	<b>0.033</b>
		Population	<b>2</b>	<b>18.33</b>	<b>&lt; 0.001</b>
		Family (Population)	1	1.22	0.268
		Pop x Garden	<b>2</b>	<b>10.95</b>	<b>0.004</b>
		Seedling survival	$\mu_2$	Residual	100
Block	1			3.15	0.076
Population	2			5.19	0.075
Family (Population)	1			2.44	0.119
$\nu_2$	Block		1	0.72	0.397
	Population		<b>2</b>	<b>14.59</b>	<b>&lt; 0.001</b>
	Family (Population)		1	1.93	0.164

Figure 1

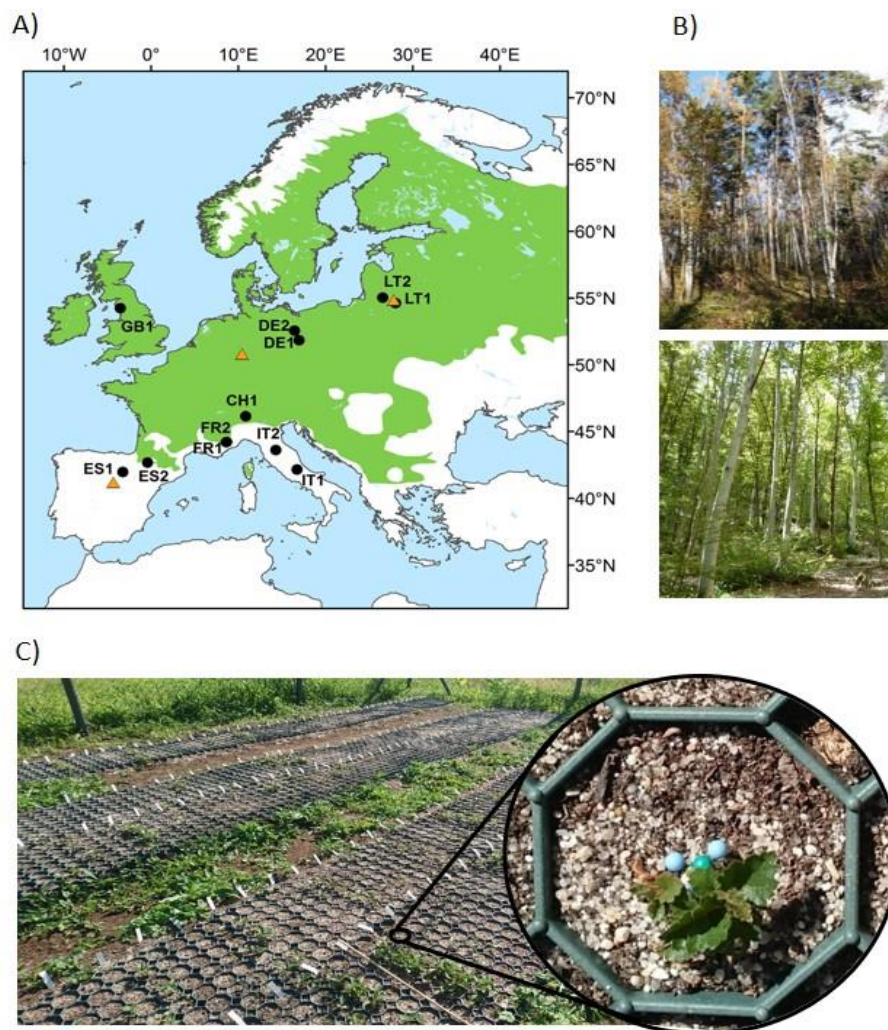
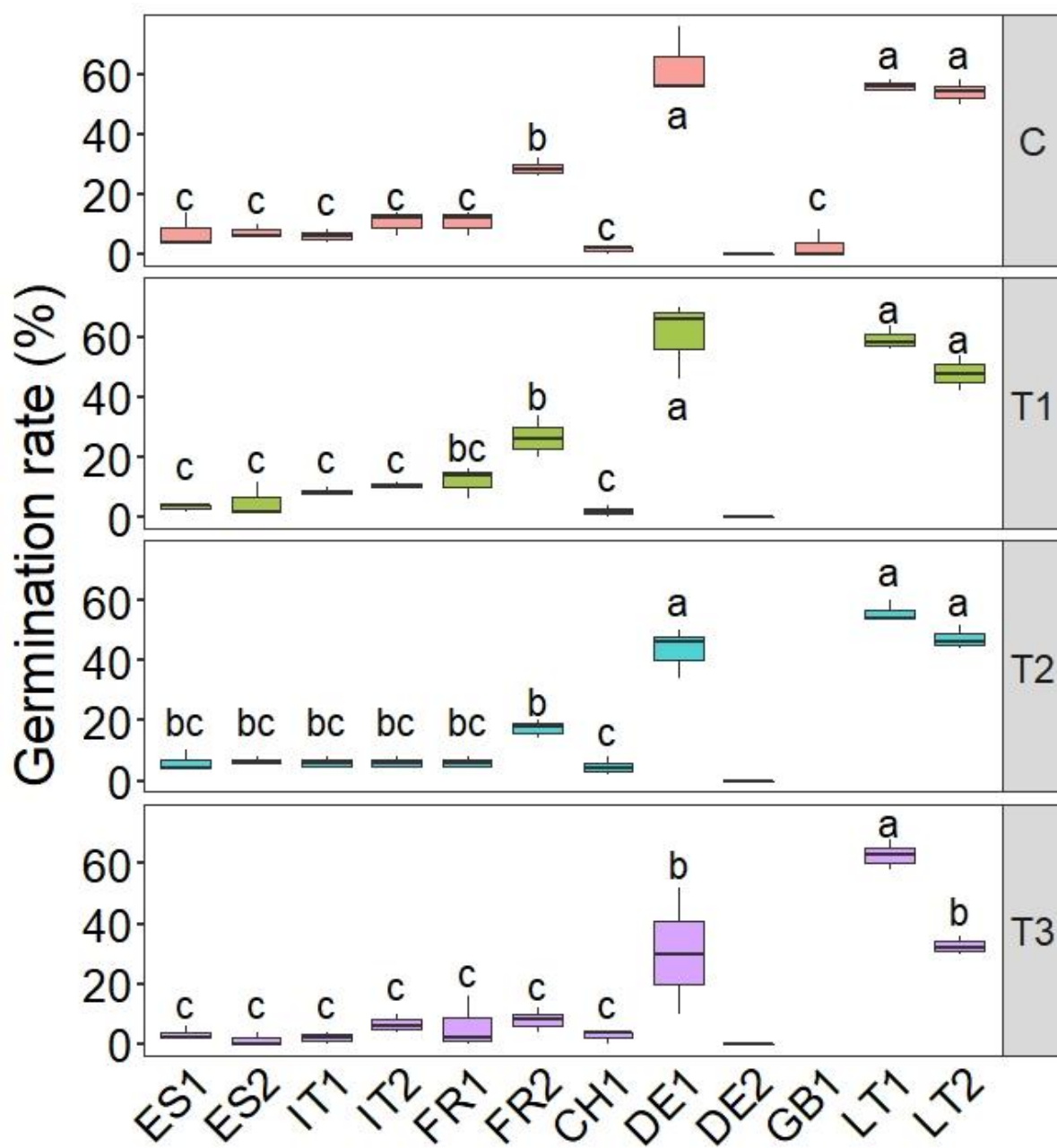


Figure 2



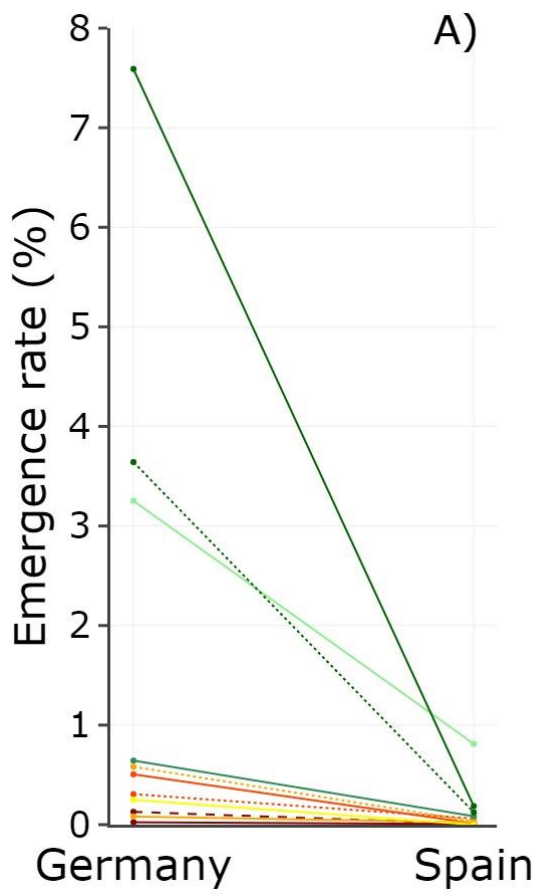
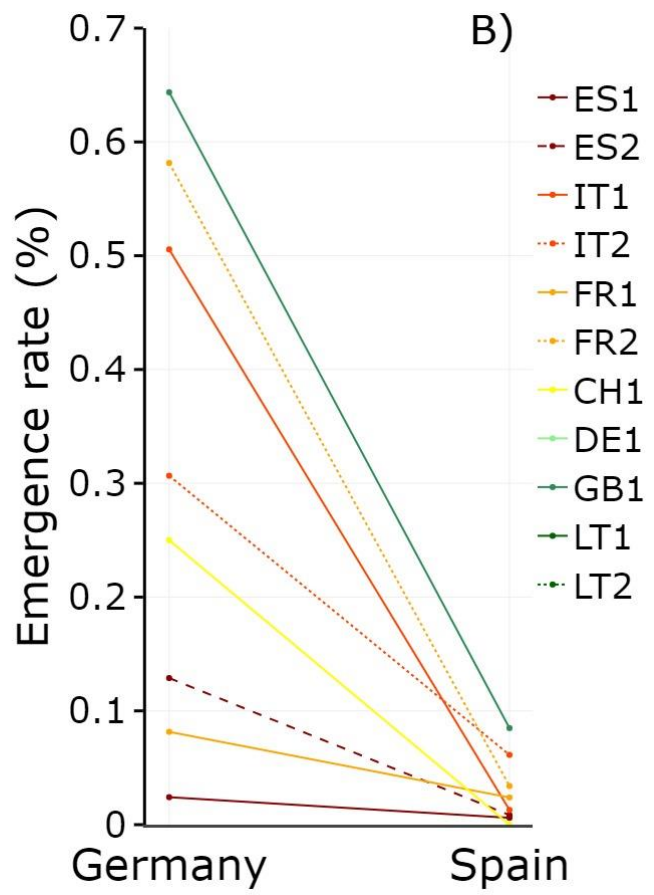


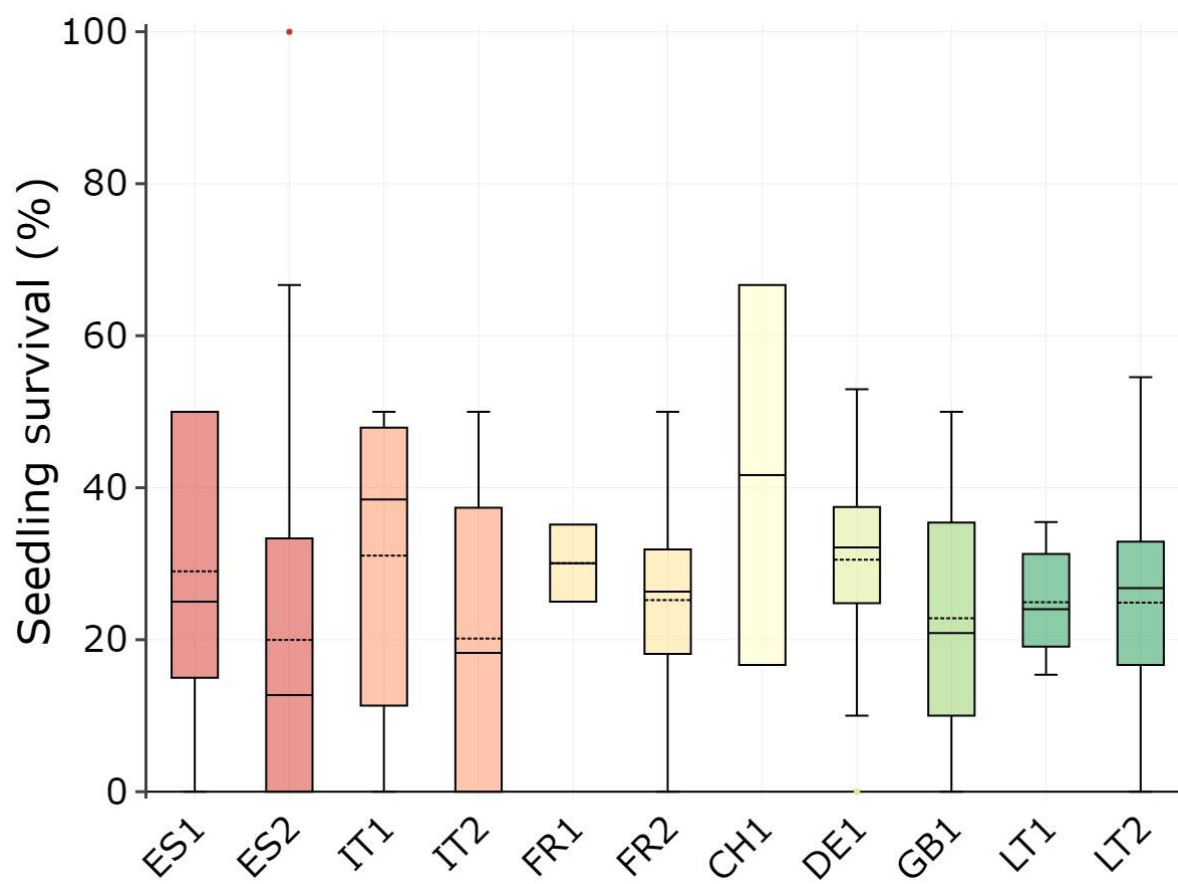
Figure 3



Accepted

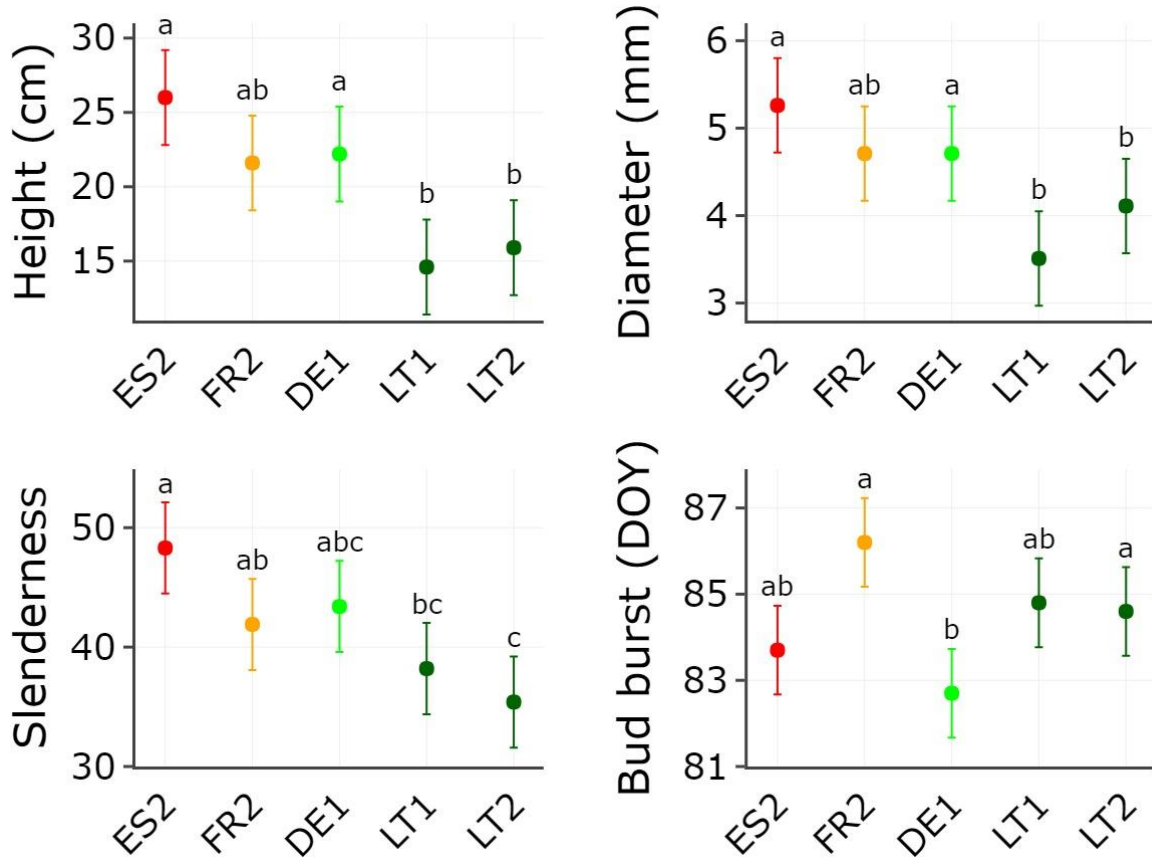


Figure 4



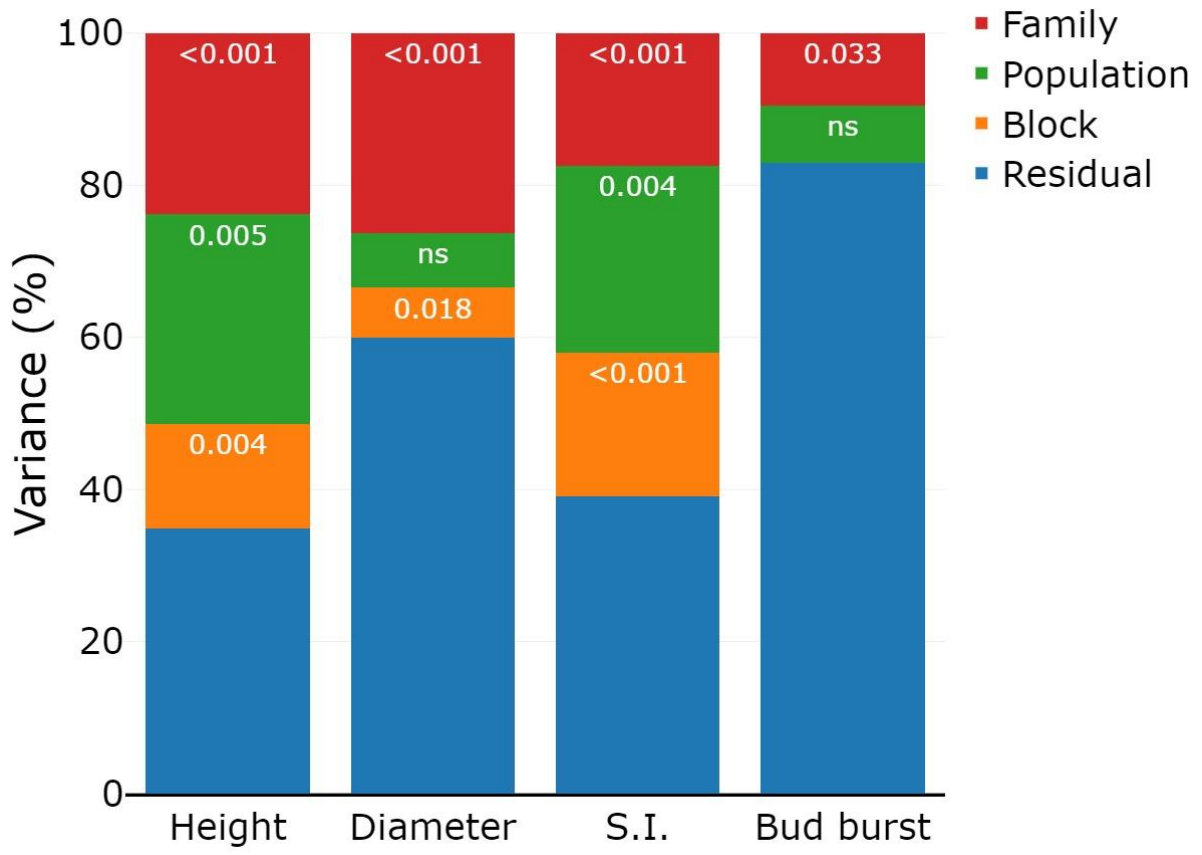
Accepted

Figure 5



Accepted

Figure 6



Accepted

Figure 7

