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DR JOSE ALBERTO RAMIREZ-VALIENTE (Orcid ID : 0000-0002-5951-2938)

DR LARS OPGENOORTH (Orcid ID : 0000-0003-0737-047X)

DR RAQUEL BENAVIDES (Orcid ID : 0000-0003-2328-5371)

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Selection patterns on early-life phenotypic traits in *Pinus sylvestris* are associated with precipitation and temperature along a climatic gradient in Europe

José Alberto Ramírez-Valiente[1,*], Aida Solé-Medina[1,2], Tanja Pyhäjärvi[3,4], Outi Savolainen[3,4], Sandra Cervantes[3,4], Robert Kesälahti[3], Sonja T. Kujala[3,5], Timo Kumpula[3], Katrin Heer[6], Lars Opgenoorth[7,8], Jan Siebertz[7], Darius Danusevicius[9], Eduardo Notivol[10], Raquel Benavides[11], Juan José Robledo-Arnuncio[1]

[1] Department of Forest Ecology & Genetics, INIA-CIFOR, Ctra. de la Coruña km 7.5, 28040 Madrid, Spain

[2] Escuela Internacional de Doctorado, Universidad Rey Juan Carlos, C/ Tulipán s/n, 28933 Móstoles, Spain

[3] Department of Ecology and Genetics, FIN-90014, University of Oulu, Oulu, Finland

[4] Biocenter Oulu, FIN-90014, University of Oulu, Oulu, Finland

[5] Natural Resources Institute Finland (Luke), 90570, Jokioinen, Finland

[6] Conservation Biology, Philipps Universität Marburg, Karl-von-Frisch Strasse 8, 35043 Marburg, Germany.

[7] Plant Ecology and Geobotany, Philipps Universität Marburg, Karl-von-Frisch Strasse 8, 35043 Marburg, Germany

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[8] Swiss Federal Research Institute WSL, Zürcherstrasse 111, 8903 Birmensdorf, Switzerland
[9] Faculty of Forest Science and Ecology, Vytautas Magnus University, Studentų str. 11, LT-53361 Akademija, Kaunas, Lithuania
[10] Unidad de Recursos Forestales, CITA, Avda. Montañana 930, 50059 Zaragoza, Spain
[11] LINCGlobal, Department of Biogeography and Global Change, Museo Nacional de Ciencias Naturales, CSIC, C/ José Gutiérrez Abascal 2, 28006, Madrid, Spain
*Corresponding autor: josealberto.ramirezvaliente@gmail.com

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ORCID:
José Alberto Ramírez-Valiente: 0000-0002-5951-2938
Tanja Pyhäjärvi: 0000-0001-6958-5172
Outi Savolainen: 0000-0001-9851-7945
Sonja T. Kujala: 0000-0003-0949-6156
Katrin Heer: 0000-0002-1036-599X
Lars Opgenoorth: 0000-0003-0737-047X
Darius Danusevicius: 0000-0002-1196-9293
Eduardo Notivol: 0000-0003-4272-4536
Raquel Benavides: 0000-0003-2328-5371
Juan José Robledo-Arnuncio: 0000-0002-3909-8928
Timo Kumpula: 0000-0002-6124-8929

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Abstract

- Rationale: Understanding the dynamics of selection is key to predict the response of tree species to new environmental conditions in the current context of climate change. However, selection patterns acting on early recruitment stages and its climatic drivers remain largely unknown in most tree species, despite being a critical period of their life cycle.
- We measured phenotypic selection on *Pinus sylvestris* seed mass, emergence time and early growth rate over two years in four common garden experiments established along the latitudinal gradient of the species in Europe.
- Significant phenotypic plasticity and among-population genetic variation were found for all measured phenotypic traits. Heat and drought negatively affected fitness in the southern sites, but heavy rainfalls also decreased early survival in middle latitudes. Climate-driven directional selection was found for higher seed mass and earlier emergence time, while the form of selection on seedling growth rates was contrasting among sites and populations. Evidence of adaptive and maladaptive phenotypic plasticity was found for emergence time and early growth rate, respectively.
- Seed mass, emergence time and early growth rate have an adaptive role at the early stages of *P. sylvestris* and climate strongly influences the patterns of selection on these fitness-related traits.

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Introduction

Widespread dieback events, mainly driven by drought and heat waves, have been increasingly documented in forest ecosystems in recent years (Van Mantgem *et al*., 2009; Adams *et al*., 2009; Peng *et al*., 2011; Anderegg *et al*., 2013; Williams *et al*., 2013; Allen *et al*., 2015). Other abiotic and biotic factors such as pest outbreaks, low temperatures and heavy rainfall are also responsible for causing extensive tree mortality and forest decline in many regions of the planet (Kramer *et al*., 2008; Allen *et al*., 2010; Niu *et al*., 2014). However, the negative impacts of increased climate-induced mortality on early-life stages of forest tree species are comparatively less studied, perhaps because they are less visible. Whereas the interest in the causes of global tree mortality is burgeoning, current knowledge on large-scale patterns of recruitment in forest trees and their potential climatic drivers is more limited (Walck *et al*., 2011; Benavides *et al*., 2013).

Tree species are particularly vulnerable to climatic conditions during the crucial transition from seed to seedling and the critical first months after emergence (e.g. Cavender-Bares & Bazzaz, 2000; Mediavilla & Escudero, 2004). The percentage of viable seeds that reaches the seedling stage and survives up to the first year is usually less than 10% in natural conditions and even less than 1% in harsh environments (e.g. Castro *et al*., 2005; Vizcaíno-Palomar *et al*., 2014). These mortality rates, which are much higher than those recorded in the aforementioned adult tree dieback events, might translate into strong early selective processes that influence adaptation across the entire life cycle (Donohue, 2005). Measuring the direction and magnitude of selection on early-stage phenotypic traits is thus key to predict the ability of tree species to respond to new environmental conditions (Kingsolver & Pfennig, 2007; Hoffmann & Sgrò, 2011).

There is substantial information on the form and magnitude of natural selection on functional, life-history and phenology traits in short-lived species of plants (e.g. Etterson & Shaw, 2001; Etterson, 2004; Caruso *et al*., 2005; Davis *et al*., 2005; Donovan *et al*., 2007). There has been also increasing interest in quantifying trait selection and its natural drivers in forest trees (Alía *et al*., 2014; Ramírez-Valiente *et al*., 2015; De la Mata *et al*., 2017; Warwell & Shaw, 2018, 2019). Although changes in the direction and magnitude of selection with climatic and environmental conditions are well-documented in short-lived species (e.g. Etterson, 2004; Donovan *et al*., 2009), very few studies have shown them in tree seedlings. For example, Warwell & Shaw (2019) found that the selection on both higher seed density and early emergence date increased under dry conditions in seedlings of ponderosa pine (*Pinus*

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ponderosa). Ramírez-Valiente *et al*. (2015) found that selection on the specific leaf area of cork oak (*Quercus suber*) saplings shifted from negative (favoring more sclerophyllous leaves) in a dry year to null in a mesic year, whereas selection on leaf size showed the opposite pattern. These studies provide some evidence of climate-driven selection on phenotypic traits in seedlings of forest trees, but our understanding remains limited to single populations or narrow geographical scales.

Ongoing climate change is likely to alter selection patterns in tree populations. In Europe, predictions based on recent models suggest an increase of 2-4°C in annual temperature by the end of the century (Jacob *et al*., 2014). Precipitation is expected to increase in winter and to decrease in summer, with substantial regional differences (IPCC, 2013). More frequent extreme weather events such as heat waves, severe droughts and heavy precipitation phenomena are also predicted (Jacob *et al*., 2014; Stott, 2016; Lhotka *et al*., 2018). Under new selective pressures, the persistence of populations in their current locations will depend on the phenotypic plasticity and adaptive evolution of traits important for fitness (i.e. adaptive traits) (Davis & Shaw, 2001; Etterson & Shaw, 2001; Shaw & Etterson, 2012). Plastic responses might involve functional, phenological and developmental adjustments. If such responses are beneficial for fitness, phenotypic plasticity is considered to be adaptive (Matesanz *et al*., 2010; Gianoli & Valladares, 2011). However, phenotypic plasticity can be also maladaptive or adaptively neutral if it has negative or null effects on fitness (Van Kleunen & Fischer, 2005).

In this study, our general goal was to determine the patterns and climatic correlates of selection on seed mass, emergence time and early growth rate across the broad European latitudinal distribution of Scots pine (*Pinus sylvestris*; Fig S1). Extensive population genetic differentiation has been reported for a high variety of Scots pine traits such as growth, survival, phenology, allometric patterns, gas exchange and ^{13}C isotope discrimination (e.g. Cregg & Zhang, 2001; Notivol *et al*., 2007; Reich & Oleksyn, 2008; Salmela *et al*., 2013; Matías & Jump, 2014; Seidel & Menzel, 2016; Semerci *et al*., 2017; Kujala *et al*., 2017). Such intraspecific genetic variation seems the result of adaptation to contrasting temperature and precipitation regimes, light, photoperiod and other biotic and abiotic factors (Savolainen *et al*., 2007). Phenotypic plasticity under different watering and temperature treatments has been also reported in many traits (Matías & Jump, 2014; Ramírez-Valiente & Robledo-Arnuncio, 2015; Seidel & Menzel, 2016; Semerci *et al*., 2017). Despite this apparent ability to adapt to heterogeneous environments, increasing mortality has been reported in some parts of the species range (Allen *et al*., 2010, Anderegg *et al.*, 2019). Assessing the response of seedlings of *P. sylvestris* to a warmer and changing climate will be crucial to

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understand the population dynamics and long-term persistence of *P. sylvestris* forests (Rehfeldt *et al.*, 2002; Savolainen *et al.*, 2004; Reich & Oleksyn, 2008; Kuparinen *et al.*, 2010).

Seed mass, emergence rate and seedling growth rates have been suggested to play important roles in *P. sylvestris* early fitness in dry Mediterranean regions (Castro, 1999; Castro *et al.*, 2005; Castro, 2006). Genetic differentiation and phenotypic plasticity for these early-stage phenotypic traits have been also previously reported (Notivol *et al.*, 2007; Salmela *et al.*, 2013). However, as with many other trees, how selection acts on early life stages and how climate variation affects patterns of selection are largely unknown in this species. Our specific objectives in this study were: i) to examine the extent to which climate affects fitness and alters the direction and magnitude of selection on *P. sylvestris* seed mass, emergence time and early seedling growth rates, ii) to test whether selection patterns vary across populations and iii) to examine the adaptive role of phenotypic plasticity in emergence time and growth rate.

We hypothesized that drought and high summer temperatures would decrease survival, particularly in southern latitudes, whereas low winter temperatures would be critical for early survival in northern areas (Rehfeldt et al., 2002; Reich & Oleksyn, 2008; Persson *et al.*, 2010). We also hypothesized that selection on early life traits would differ among sites with different climate, favoring heavier seeds, earlier emergence and faster development in southern environments, as adaptations to cope with harsh summers (Castro *et al.*, 1999; Castro *et al.*, 2005). As *P. sylvestris* is geographically structured (Rehfeldt *et al.*, 2002), we also expected phenotypic divergence resulting in unequal selection among populations growing in the same environment. Finally, we hypothesized that populations growing in climatic conditions similar to their local environment would exhibit higher fitness than non-local populations.

Material and methods

Cone sampling and seed extraction

In early 2017, teams from the EU GenTree project consortium sampled mature cones from 20-25 open-pollinated maternal trees randomly selected within eighteen natural *Pinus sylvestris* L. populations spanning the latitudinal gradient of the species in Europe. Mother trees were separated at least 50 m. Cones were air-dried for several days and, once opened, empty and filled seeds were separated by

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decantation in water. The maternal family structure was maintained in four of the populations, while seeds from different mothers were pooled within each of the other populations. Seeds were stored at 4°C until sowing.

Experimental design and measurements

In spring 2017, four common garden experiments were established across the latitudinal gradient of the species in Spain, Germany, Lithuania and Finland, in open areas with flat topography and full sun exposure, since *P. sylvestris* is a pioneer light-demanding species that recruits under forest gaps or after disturbances (de Chantal *et al*., 2003). The main physical and chemical soil properties of the provenances and experimental sites were obtained from https://www.isric.org/explore/soilgrids (Hengl *et al*., 2017) and are summarized in Table S1.

The trials followed a latinized row-column design (see e.g. Piepho *et al*., 2015) with 42 rows and 9 columns organized in three replications (i.e. blocks). The eighteen sampled populations were represented in the common gardens, but seeds were individually weighed only for the four populations closest to the experimental sites, on which this study is focused (Table 1, Fig. S1). At each replication, we established one experimental unit per maternal family per each of those four populations. The experimental units consisted in sixteen seeds that were individually sown in adjacent 30 cm^2 octagonal cells of plastic grids (Guttagarden®). Before sowing, around 5 cm depth of soil was removed to place the grids in the field, which were then filled with the sieved soil. Grid cells were open in the bottom and lateral walls so roots were allowed to grow into the soil. Sowing was carried out in 1-4 days between June 14th and June 22nd 2017, depending on the site. A total of 23,191 seeds were sown with 15,327 seeds corresponding to the four populations used for this study (4 sites × 4 populations × 18-20 families/population × 3 replications × 16 seeds/experimental unit). The experimental design was constructed with the software CycDesigN (Whitaker *et al*., 2002).

Watering was applied after sowing in all experimental sites. In the southernmost site, due to extremely dry conditions in summer 2017, the trial was watered regularly (every two-four days) for six weeks to simulate an average dry season in the area. We monitored emergence, survival and time needed to get fully-expanded juvenile needles (‘developmental time’ hereafter) every two or three days until November 2017. In spring 2018, when snow had melted, overwinter survival was recorded. Then, we monitored survival every 10-14 days till the end of the summer in the Finnish and Spanish sites. In

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the German and Lithuanian sites survival in 2018 was only recorded at the end of the summer. Common gardens were weeded frequently to reduce vegetative competition, and insecticide and fungicide were applied to diminish herbivory by insects and fungal diseases.

We measured five early fitness variables: rate of emergence in 2017 (number of seeds producing visible shoot or cotyledons on the ground surface during 2017/total number of seeds sown), survival in 2017 (number of seedlings alive at the end of summer 2017/number of emerged seedlings in 2017), survival in 2018 (number of seedlings alive at the end of summer 2018/number of seedlings alive at the end of 2017), cumulative survival (number of seedlings alive at the end of the experiment/number of emerged seedlings), and cumulative fitness (number of alive individuals at the end of the experiment/total number of seeds sown). Our cumulative fitness definition integrates both emergence and survival rates. As phenotypic traits, we used seed mass, emergence time (days since sowing until emergence), and developmental time (days since sowing until juvenile needles are fully expanded). Developmental time was used as a surrogate of initial growth rate, due to the logistic challenge of measuring growth accurately in large numbers of emerging seedlings (see Chambel *et al*., 2011).

Climate characterization of sites and populations

Climatic variables in the experimental sites were obtained from weather stations established at the sites, national meteorological services and KNMI Climate Explorer weather stations (https://climexp.knmi.nl/selectdailyseries.cgi). Climate data of the seed sources were obtained using WorldClim for the reference period 1970-2000 (Fick & Hijmans, 2017).

Differences in fitness variables and phenotypic traits among sites and populations

Binomial mixed models with logit link functions were used to test for differences among populations and sites in the five binary-outcome variables of fitness: emergence, survival 2017, survival 2018, cumulative survival and cumulative fitness. Population, Site and their interaction were considered fixed-effect factors. Family nested within Population, Row, Column and Replication and Experimental unit nested within Site were treated as random-effect factors. Linear mixed models were implemented for emergence time and developmental time, using the same fixed-effect and random-effect structure. A linear mixed model was also run to test for differences among populations in seed mass. Since seeds

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were sampled in field conditions, this mixed model only included population as a fixed-effect factor and family nested within population as a random-effect factor. Generalized and linear mixed models were fitted using 'lmerTest' (Kuznetsova *et al*., 2017) and 'car' packages (Fox & Weisberg, 2019) in R 3.6.1 (R Development Team, 2018).

Temporal patterns of mortality and climatic drivers

We used Cox-proportional hazard-mixed effect models (Cox, 1972) to explore temporal patterns of seedling mortality, and potential climatic correlations. We used the 'coxme' package (Therneau *et al*., 2020) in R 3.6.1 (see Methods S1 for equation details). We performed these analyses for all sites in 2017 and for Spain in 2018. Although we regularly monitored survival in Finland in 2018, only 24 out of 1,351 seedlings died so analyses were not conducted for that year. Models were performed using only summer mortality as response variable because overwinter mortality 2017/2018 was very low in all gardens. We first performed one hazard model per site including Population as a fixed-effect predictor variable and Family nested within population, Row, Column, Replication and Experimental unit as random-effect predictor variables. To estimate the effect of climate, we performed additional hazard models where we also included as fixed-effect predictor variables the average maximum temperatures and precipitation over the week preceding the census day. The interactions Population-by-Temperature and Population-by-Precipitation were also included as fixed-effect factors. Temperature and precipitation were standardized within sites to make the coefficients comparable across sites. In the Spanish site in 2018, coefficients for precipitation and temperature were obtained from separate models due to multicollinearity between these two variables tested using variance inflation factor (VIF) (Chatterjee & Hadi, 2015). The quantity of supplemental water added during the first six weeks of the experiment in 2017 was not accurately estimated in the Spanish site, so the coefficients for temperature and precipitation obtained from fitted hazard models were shown after that period (see results).

Phenotypic selection

Phenotypic selection on traits was estimated following Janzen & Stern (1998), who define $W_j(z)$ = $P(W_j=1|z)$ as the probability to survive (or emerge) for an individual during the time period

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corresponding to the *j*th considered fitness component (e.g. survival 2017), depending on the individual's vector of phenotypic trait values *z*, expressed as:

$$W_j(z) = \frac{e^{\alpha_0 + \alpha^T z}}{1 + e^{\alpha_0 + \alpha^T z}}$$

where α_0 is the intercept and $\alpha = (\alpha_1, \alpha_2, \ldots, \alpha_k)^T$ are the logistic regression coefficients associated with the vector of the *k* measured phenotypic traits $z = (z_1, z_2, \ldots, z_k)^T$. Quadratic (e.g. stabilizing, disruptive) selection can be estimated by adding quadratic terms to the logistic regression model.

Trait values were standardized to a mean of zero and variance of one. The logistic regression coefficient α for a given trait was transformed into an approximated selection differential (*S*) multiplying it by the average of $[W_j z\ (1- W_j z\ )]$ across individuals. Estimated *S* values are then multiplied by $1/W_j z$ to convert them to a relative fitness scale. In this study, we estimated selection differentials, which integrate direct selection on the trait and indirect selection mediated by phenotypic correlations with other traits. Selection gradients were not estimated because not all traits affected the same fitness components (e.g. developmental time did not affect emergence rate, because it can only be measured in emerged seedlings).

Following Janzen & Stern (1998), we used generalized linear mixed models with fixed- and random-effect factors. We performed the analyses at two levels (see Etterson, 2004 for a similar procedure). First, we estimated phenotypic selection across all populations within each site, by running one model per site that included the trait of interest as a fixed-effect factor (covariate) and the random-effect structure (i.e. Family, Column, Row, Replication, Experimental unit). Population was also included as a fixed-effect factor, trying to account for potential differences among populations in fitness and genetic correlations with unmeasured fitness traits. Second, we estimated phenotypic selection for each combination of site and population by running models that contained the trait of interest (covariate) as a fixed effect factor and the random-effect structure as in previous models (Etterson, 2004). We estimated quadratic selection adding quadratic terms to the models, with transformation and standardization of the corresponding logistic coefficients, which were multiplied by a factor of two to obtain final quadratic differentials (see Lande & Arnold, 1983; Stinchcombe *et al.*, 2008). We followed the notation by Phillips & Arnold (1989) for linear (*S*) and quadratic (*C*) selection differentials. Significant *S* estimates were considered as evidence of directional selection, while the simultaneous observation of a significant *C*

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estimate *and* an intermediate maximum (or minimum) of the fitness function was considered as evidence of stabilizing (or disruptive) selection (Mitchell-Olds & Shaw, 1987; Schluter, 1988).

Differences in selection among sites were tested using mixed models that included Trait, Site and Trait-by-Site interactions as fixed effect factors. We also included Population as a fixed-effect factor in these models. Then, in order to test for differences in selection among populations within sites, we additionally ran one model per site that included Trait and Trait-by-Population interactions as fixed effect factors. See Etterson (2004) for a similar approach. Phenotypic selection analyses were performed for each of the five fitness components separately (Wade & Kalisz, 1989; Fairbairn & Reeve, 2001). We used 'lmerTest' (Kuznetsova *et al*., 2017) and 'car' (Fox & Weisberg, 2019) packages in R 3.6.1 (R Development Team, 2018) for the phenotypic selection analyses. We used ggplot2 (Wickham, 2016), interactions (Long, 2019) and survival (Therneau, 2020) packages in R 3.6.1 for figures.

Following Caruso *et al*. (2006), we investigated if plasticity in emergence and developmental time was adaptive by (i) assessing if selection on a trait within a site was concordant with the direction of plastic differences in that trait between sites; and (ii) conducting across-environment phenotypic selection analyses on plasticity for each pair of sites, regressing the relativized mean fitness across sites against standardized family trait means and a standardized measure of plasticity across sites for each trait (see Methods S1 for details).

Results

Climate

Average temperatures during the reference period (1970-2000) followed a latitudinal cline, with more southern populations presenting higher mean summer temperatures (Table 2). In the experimental sites, temperatures also showed a negative trend with latitude for some but not all months, and the differences between the German and Lithuanian sites were generally minimal (Table 2). In general, the summer temperatures recorded in the experimental sites in 2017, and particularly in 2018, were warmer than average values at the geographically closest populations (Table 2).

Summer precipitation during the reference period (1997-2000) was lower in southern populations, particularly in Spain during July and August (Table 2). In the experimental sites, precipitation was higher in Germany in 2017 and Lithuania in 2018, and lowest in Spain both years

(Table 2). Differences between the precipitation registered in the common garden sites during the study period and in the population source locations during the reference period were considerable, but they did not exhibit a consistent pattern across the four sites (Table 2).

Differences among sites and populations in fitness and phenotypic traits

Generalized mixed models revealed significant differences among sites and populations and strong Population-by-Site interaction in all five considered fitness components (Table 3). The German population was the one with the highest emergence rates in Spain (0.83), Lithuania (0.53) and Finland (0.55), but not in Germany , where the Spanish population was the one with the highest emergence rate (0.44, Fig. 1a). The highest survival rates were observed at the Finnish site in both study years, 2017 (0.89-0.92) and 2018 (0.98-1.00) (Fig. 1b, 1c), and the lowest in Lithuania in 2017 (Fig 1b) and Spain in 2018 (Fig 1c), with large differences among populations at the latter site (0.97 and 0.03 for the Spanish and Finnish populations, respectively). In general, populations did not have higher cumulative survival or fitness in the sites that were climatically and geographically closest to them (Fig. 1d, 1e).

The linear mixed model for seed mass showed significant differences among populations that followed a negative latitudinal pattern, that is, populations from lower latitudes had larger seeds (Table 3, Fig. 1f). Both emergence time and developmental time showed significant differences among sites and populations, with significant Population-by-Site interaction in linear mixed models (Table 3). Overall, seedlings emerged earlier in Spain and later in Germany, but with differences among populations (Fig. 1g). The Spanish (15.5-17.2 days) and German (14.4-18.5 days) populations had the earliest emergence across gardens, whereas the Lithuanian population (20.1-24.0 days) had the latest emergence in all gardens (Fig. 1g). Overall, the developmental time was longer in Finland and Spain indicating slower early growth rates in these sites, but with differences among populations (Fig. 1h). On average, the German (15.1-26.4 days) and Spanish (13.5-26.5 days) populations had the most rapid development across sites (i.e. faster growth rates), whereas the Finnish population (20.7-28.0 days) had the slowest development in all sites (Fig. 1h).

Temporal patterns of mortality and climatic drivers

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Cox-proportional hazard mixed models revealed contrasting patterns of temporal mortality across sites and populations (Fig. 2). In 2017, around 70% of the mortality occurred within the first fifteen days after emergence in Germany, Lithuania and Finland (Fig. 2). In contrast, in the Spanish site, survival decreased progressively over time (Fig. 2). In 2018, high mortality rates occurred mainly in the second half of the dry season in Spain, with significant differences among populations (Fig. 2). Temperature and precipitation presented highly significant coefficients in all sites (Fig. 3). In Spain, higher temperatures were significantly associated with increased mortality rates (i.e. positive coefficient) throughout the summer in both 2017 and 2018 (Fig. 3). Lower precipitation was also significantly associated with increased mortality rates in both years in this garden (i.e. negative coefficient), particularly in 2018 (Fig. 3). In Germany, mortality rates were mainly associated with lower precipitation during the summer of 2017 (Fig. 3). In Lithuania, higher mortality was associated with higher precipitation and lower temperatures at the beginning of the summer, when mortality rates were highest (Fig. 3). In the second half of the summer, in contrast, higher mortality rates were associated with lower precipitation in this garden (Fig. 3). In Finland, increased mortality rates were significantly associated with higher temperatures and lower precipitation during the first month after emergence, and only weakly associated with temperature for a short period after the first month (Fig. 3). Hazard models also revealed Population-by-Precipitation interaction in Lithuania and Spain in both 2017 and 2018, indicating that the association between precipitation and mortality rates was population-dependent in these two gardens (Fig. S2).

Phenotypic selection

Phenotypic selection analyses combining populations within sites showed directional selection for larger seed mass (significant positive S estimates) in all sites when considering emergence rate as fitness component (Fig. 4, Table S2). Quadratic coefficients (C) of the regression of emergence rate on seed mass were significant in Germany and Finland (indicating a non-linear relationship), but there was no evidence of stabilizing selection, since the shape of the estimated fitness function did not exhibit a clear intermediate maximum within the range of observed phenotypes (Fig. 4). Seed mass was also under positive directional selection when considering either survival 2017 or survival 2018 as fitness component, but mainly in the Spanish site, indicating that heavier seeds were particularly important for seedling survival in the warmest and driest site (Fig. 4, Table S2). Regression of cumulative seedling

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fitness on seed mass further supported positive directional (and not stabilizing) selection on the latter (Fig. 4).

Phenotypic selection analyses also revealed that seedlings that emerged earlier had a higher probability of survival (as indicated by significant negative linear selection differentials for emergence time) during the first year (2017) in Spain and Lithuania (Fig. 5, Table S2). However, we also detected a combination of significantly positive quadratic selection estimates (*C*) and a clear intermediate fitness minimum within the range of observed emergence times in Germany (Fig. 5, Table S2), indicating disruptive selection on emergence time at this site in 2017, where early but also late emergence favored seedling survival. Considering cumulative survival as fitness component, all sites showed a negative *S* for emergence time, indicating that overall selection favored early emergence (Fig. 5, Table S2). Germany and Finland had also positive *C*, but without a clear intermediate fitness minimum (Fig. 5, Table S2).

Estimates of the form and intensity of selection on seedling developmental time were different across sites, particularly in 2017 (Fig. 5, Table S2). In that year, there was significant negative linear directional selection on developmental time (i.e. increasing survival with faster early growth rates) in Spain (negative significant *S*) (Fig. 5, Table S2). In contrast, the seedlings with the fastest growth onsets were the ones with the lowest survival probabilities in Lithuania (positive significant *S*). In addition, seedlings with the lowest early-growing rates also exhibited decreased survival at both Lithuania and Germany (negative significant *C*), a stabilizing selection pattern evidenced by the intermediate fitness maximum and significant quadratic coefficients (Fig. 5, Table S2). The results were similar considering cumulative survival as fitness component, although we observed also a weak but significant negative *S* in Finland.

Phenotypic selection analyses conducted for individual populations within sites revealed significant among-population differences in linear selection on seed mass at the German and Finnish sites, where heavier seeds were significantly more strongly selected (larger positive *S*) for populations with lighter seeds (Fig. 6a-d; see also Fig. 1c). This selective difference was mediated by both decreased seedling emergence and survival rates for smaller seeds in the latter populations. Differences among populations in the magnitude of selection on seedling emergence time were non-significant in most sites, except for stronger selection on earlier emergence for the Spanish versus German population observed at the Lithuanian site (Fig. 6e-h). Similarly, differences among populations in selection differentials for developmental time was not significant, except for stronger selection on faster early growth for

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Lithuania versus Finland in the Lithuanian site (Fig. 6i-l). Differences among populations in quadratic differentials (C) for seed mass, emergence time and developmental time were generally not significant either (Table S3).

Using survival 2017 as a fitness component, we detected significant positive linear selection on plasticity of emergence time between Lithuanian and German gardens and between Lithuanian and Finish gardens (Table S4, Fig. 7). In contrast, we detected significant negative linear selection on plasticity of developmental time between the German and Spanish and between Lithuanian and Spanish gardens (Table S4, Fig. 7). Selection using survival 2018 and cumulative survival and quadratic selection using any fitness component were generally non-significant (Table S4).

Discussion

Differences in selection patterns among environments and the role of temperatures and precipitation

Phenotypic selection analyses revealed that selection on seed mass, emergence time and developmental time differed among study sites for most fitness components. Considering survival as fitness component, selection for higher seed mass was mainly observed in the driest and warmest site, near the southern limit of the distribution of *P. sylvestris* (Table 1, Fig. 4). In addition, in the Spanish site, hazard models revealed that mortality was positively associated with temperature and negatively associated with precipitation in both 2017 and 2018 (Fig. 3). In a recent review, Leslie *et al*. (2017) found a positive association between annual mean temperature and seed volume in *Pinaceae*. Other authors have also suggested that lower seed size/mass is favored in species from more seasonal, colder environments (Rueda *et al*., 2017; Rubio de Casas *et al*., 2017; Salazar-Tortosa *et al*., 2020). In addition, positive associations between seed mass and survival have been reported in species inhabiting regions with marked dry seasons (e.g. Moles & Westoby, 2004; Gómez 2004; Ramírez-Valiente *et al*., 2009; Larson *et al*., 2015; Lebrija-Trejos *et al*., 2016), including pine species (Parker *et al*., 2006; Cendán *et al*., 2013; Zas *et al*., 2013). In fact, Salazar-Tortosa *et al*. (2020) found consistent positive associations between seed mass and aridity both within and among *Pinus* subgenera. These authors suggested independent or recurrent events of positive selection on heavier seeds in arid environments. The positive association between seed mass and survival under dry conditions has been usually explained by the fact that larger seeds have more reserves to produce seedlings with larger growths and/or deeper roots (Surles *et al*.,

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1993; Castro, 1999; Leishman *et al*., 2000; Westoby *et al*.,1992, 2002; Wennström *et al*., 2002, Bladé & Vallejo, 2008; Parker *et al*., 2006; Tíscar & Lucas, 2010; Zas *et al*., 2013). Moreover, larger seeds may be associated with allometric patterns (Lloret *et al*., 1999), for instance, Ramírez-Valiente & Robledo-Arnuncio (2015) found a positive association between seed mass and seedling survival rate under dry conditions in *P. sylvestris* that was explained by lower needle-to-root ratio in seedlings from heavier seeds. Similarly, Matías & Jump (2014) observed higher *P. sylvestris* seedling survival for southern provenances with larger seeds than for northern populations with smaller seeds, associated with higher root investment in the former.

There were also differences among sites in the magnitude of selection on seed mass considering emergence as fitness component, the association being significantly positive in all sites (Fig. 4, Table S2). In general, the higher amount of reserves in heavier seeds increases the chances of embryo development and germination (Castro *et al*., 2006). However, some authors suggest that germination and emergence can be also triggered by the composition of the seeds (e.g. content in starch and proteins) independently of their mass (Vleeshouwers *et al*., 1995; Dyer, 2004; Van Mölken *et al*., 2005). Reported associations between seed mass and emergence rate across different *Pinus* species highly vary in sign and magnitude (e.g. Parker *et al*., 2006; Bladé & Vallejo, 2008, Calvo *et al*., 2016; Wahid & Bounoua, 2013; Larson *et al*., 2015, Suárez-Vidal *et al*., 2017). Within species, the associations between seed mass and germination/emergence seem to be population- and/or environment-specific. In *P. sylvestris*, either positive or no associations between seed mass and germination/emergence have been reported for different populations (Reich *et al*., 1994; Castro, 1999; Ramírez-Valiente & Robledo-Arnuncio, 2015). Multiple genetic- and environment-dependent mechanisms probably underlie the potential effect of seed mass on germination and seedling emergence success, precluding consistent associations (e.g. Easton & Kleindorfer, 2008).

Results for emergence time and developmental time also showed contrasting selection among sites, but the patterns were complex. Mainly, seedlings with earliest emergence dates showed increased survival across all study sites, but especially in the first summer in Spain and Lithuania (Fig. 4), the two sites with the lowest survival rates. The association between emergence time and survival has been extensively studied in species inhabiting seasonal climates (e.g. Stratton, 1992; Seiwa & Kikuzawa, 1996; Seiwa, 2000; Simons & Johnston, 2000; Donohue, 2002; Shimono & Kudo, 2003; Castro, 2006; Benard & Toft, 2007; Warwell & Shaw, 2019). Generally, selection is observed to favor early germination and emergence (see Verdu & Traveset, 2005; Donohue *et al*., 2010 for reviews). Early emerged seedlings

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usually have a longer time to develop, a potential advantage to resist unfavorable conditions later in the growing season. In our study, access to ephemerally available water could explain the higher survival of early-emerged seedlings observed in the warmest and driest site in Spain (see Warwell & Shaw, 2019 for further discussion). Higher resistance to the extreme rainy period that was associated with most of the mortality in Lithuania could explain the survival benefits of early-emerged seedlings in this site. In contrast, some studies have also reported higher survival of late emerged seedlings (e.g. Shimono & Kudo, 2003). In our German study site, a strong positive quadratic selection differential combined with a clear intermediate minimum in the fitness function indicated that early but also late emergence favored survival in the first summer in this site. However, we could not identify any particular local climatic event whose avoidance could have benefited late emergence.

Developmental time in our study represents the time needed to produce juvenile needles in seedlings and is a measure of early growth rate in *Pinus* sp. (Chambel *et al*., 2007). The main results for this trait showed that in the Spanish site, developmental time was under strong negative directional selection (i.e. faster initial growth rates monotonically increased survival), whereas in Lithuania, and to a lesser extent in Germany, there was significant evidence of stabilizing selection on this trait, with both the fastest and slowest early growth rates decreasing survival rates (Fig. 5). In Spain, where conditions were warm and dry, growing faster at early stages might be beneficial by enhancing access to deeper soil water. In fact, as mentioned before, root investment is highly associated with survival under dry conditions in *P. sylvestris* (Matías & Jump, 2012; Ramírez-Valiente & Robledo-Arnuncio, 2015). Conversely, high investment in above-growth might reduce the resources available to tolerate some stress and might explain the strong selection on slower or intermediate growth rates in Lithuania and Germany.

Differences in selection among populations

Considering the cumulative fitness measure over the two-year period, we found that the magnitude of estimated linear selection differentials on seed mass varied significantly among populations within two sites, Germany and Finland, such that phenotypic selection on seed mass was stronger in populations with lighter seeds (Fig. 6). This result is consistent with the steeper slope of the fitness functions for lower seed mass values found when combining populations within sites (Fig. 4). The analyses also revealed that most populations exhibited strong positive directional selection on seed mass in their local

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environment during the study years (Fig. 6), in spite of which we observed substantial variance in seed size within populations. Multiple reasons could explain the maintenance of the phenotypic variance in seed size within populations under apparently strong directional selection. For example, warmer conditions in the study years inducing different selective regimes than average climatic values at the seed provenances; genetic correlations among traits (Assis *et al.*, 2016); maternal fitness trade-offs between seed size and number, resulting in mother-offspring conflicts over the allocation of resources to individual seeds (Shaanker *et al.*, 1988); phenotypic variation in seed mass due to differences in environmental and maternal effects during maturation; and temporally fluctuating selection.

Population differences in phenotypic selection on emergence time were only observed in the Lithuanian site (Fig. 6). Observed differences in selection among populations in that site could not be simply explained by the mean trait values of the populations. For instance, the Spanish and German populations had virtually the same emergence time (16.21 vs. 16.26 days, respectively), but differed significantly in linear selection differentials for this trait (-0.57 vs. -0.27) indicating stronger selection on the former (Fig. 6). Similar patterns were observed for developmental time. For instance, the Lithuanian and Finish populations did not show significant differences in mean values in the Lithuanian site (18.14 vs. 20.28 days) but did differ in linear selection differentials (0.81 vs. 0.09). These apparently incongruent patterns between population trait means and phenotypic selection might be the result of the influence of non-measured correlated fitness traits (Etterson & Shaw, 2001). An additional explanation could lie in the variation among populations in the distribution of trait values around the (similar) phenotypic mean, as the fitness function was non-linear for both emergence and developmental time in the Lithuanian site.

The adaptive role of phenotypic plasticity

All populations of *P. sylvestris* showed plastic responses of emergence time and developmental time to the site environment. Earlier emergence time was observed in Spain and Lithuania (Fig. 1g), a potentially adaptive plastic response, as these were the two sites where selection towards shorter emergence time was stronger (Fig. 5). Phenotypic selection analyses on plasticity of emergence time between pairs of sites indicated that families with greater plastic shifts towards earlier emergence in Lithuania, relative to Germany or Finland, had significantly greater average fitness across the two sites, supporting a potential adaptive role of plasticity for that trait for these two population pairs (Fig. 7, Table S4). A contrasting

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(maladaptive) plastic response was observed for developmental time. On average, populations produced juvenile needles later (i.e. slower growth rate) in the Spanish site (Fig. 1h), within which selection favored shorter development (faster growth rates) (Fig. 5), and earlier in the Lithuanian site (Fig. 1h), within which selection largely favored longer developmental times (slower growth rates) (Fig. 5). Our selection analyses demonstrated that greater plasticity towards earlier development was under negative selection across Spain-Germany and Spain-Lithuania site pairs, providing evidence of a maladaptive role of phenotypic plasticity in those cases (Fig. 7). Maladaptive plastic responses have been found for a variety of traits in plants such as photosynthetic rates (Caruso *et al*., 2006), dormancy (Morin *et al*., 2008) and specific leaf area (Liu *et al*., 2016). Both adaptive and maladaptive responses of plasticity for the study traits, however, were blurred after the first year (Table S4). Further analyses should be conducted to better understand the role of phenotypic plasticity of early-life traits for life-time fitness.

Local adaptation of Pinus sylvestris at early stages?

Our study revealed strong population-by-environment interaction in fitness, but, inconsistently with the local adaptation hypothesis, none of the populations had the highest fitness in their geographically and climatically closest site. A potential factor that could explain this pattern is that climatic conditions during the study period at the sites diverged from those of the 1970-2000 reference period for the studied populations. Specifically, all sites exhibited warmer conditions (0.2-6.1°C warmer in summer) in the study years than the 1970-2000 period for their local populations (Table 2). Warmer and drier environments might have contributed to the higher survival observed for the southernmost Spanish population not only in their local environment, but also in Germany, where high temperatures and low precipitation in 2018 were even harsher than those in Spain for the reference period (1970-2000) (Table 2). Warmer conditions were also observed in the rest of the seasons. A milder winter could have fostered the high overwinter survival observed for all populations in the northernmost garden, where freezing temperatures were expected to elicit differential mortality among populations (Lindgren & Nilsson, 1992; Hurme *et al*., 1997; Savolainen *et al*., 2004). In contrast, the low survival registered in Lithuania, in particular for the local population, was related to the anomalous week of heavy rainfall recorded in July of 2017 in this site. Warming and the higher frequency of extreme climatic events are within the main factors driving the decoupling of population variation in fitness and average climate. For example, in a three-year study with ecotypes of two oak species, Etterson *et al*., (2020) suggested that

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the superior performance of southern populations in northern sites was probably a consequence of the warm conditions experienced by the sites during the study years. These apparent adaptation lags underline the need to carry out more studies to reevaluate provenances and ecotypes for restoration and assisted migration purposes (Ukrainetz *et al*., 2011).

Other authors have suggested that patterns of population variation are highly dependent on the life stage in long-lived organisms, and consequently local adaptation might be only observable using long-term studies that integrate fitness components throughout the life cycle (Vizcaíno-Palomar *et al*., 2014; Deacon & Cavender-Bares, 2015; Tíscar *et al*., 2018). In fact, some studies suggest that local adaptation might require decades to be evident (Germino *et al*., 2019), and need to incorporate fitness at the early life stages (e.g. emergence), which has been traditionally ignored in common garden experiments with trees (Gibson *et al*., 2016).

Conclusions

Our study makes three important contributions. First, we detected that seedling mortality in four common gardens of *P. sylvestris* was associated with high temperatures, drought and extreme precipitation events and that these factors differed among sites. Strong selection was observed for heavier seeds, particularly under warm and dry conditions, and also for emergence time and growth rates, but with more complex patterns. Together, these results reinforce the idea that using plant material from nurseries to establish common garden experiments overlooks selection on early life stages and, consequently, our interpretation on how tree populations adapt to climate (McLane & Aitken, 2012; Tíscar *et al*., 2018; Solé-Medina *et al*., 2020). In addition, the strong and non-linear patterns of selection suggest that climate change influence on selection might be complex and trait- and site-specific.

Second, some populations exhibiting the same trait means in a common environment were under different phenotypic selection pressures, probably because of differences among populations in genetic correlations with non-measured fitness traits (i.e. different genetic backgrounds), which points out the need for studies investigating natural selection on integrated phenotypes (Agrawal & Stinchcombe, 2009; Damián *et al*., 2020).

Third, there were differences among populations in phenotypic plasticity (genotype-by-environment interaction), but phenotypic selection analyses did not provide consistent evidence that

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plasticity was adaptive across measured traits and environments. In summary, our results highlight the need for long-term studies replicated in multiple environments that consider early life stages to understand the role of climate change on demographic and evolutionary dynamics of tree populations.

Author contributions

JJR-A, KH, LO, OS, TP, DD conceived the idea. JJR-A, EN and JAR-V designed the experimental layout. JAR-V, AS-M, KH, JS, LO, SC, TP, OS, RK, STK, TAK, DD, JJR-A, RB contributed to the experiment management and/or data collection. JAR-V analyzed the data and wrote the manuscript with inputs from all other authors.

Data availability

The datasets of this study are included in the Supporting Information as Datasets S1.

The R code for the main analyses is included in the Supporting Information as Notes S1.

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Supporting information

Methods S1. Cox-proportional Hazard mixed-effect models and Phenotypic selection on plasticity.

Datasets S1. Phenotypic traits, fitness and climate measured in the common garden experiments (Dataset) and family means for the analyses of plasticity (Dataset_plasticity)

Notes S1. R codes for the statistical analyses.

Fig. S1. Map with the locations of the studied *Pinus sylvestris* populations and common garden sites.

Fig. S2. Standardized coefficients for precipitation for the four studied populations in Spain and Lithuania.

Table S1. Physical and chemical soil properties for the seed collection locations and common garden sites.

Table S2. Standardized linear and quadratic selection differentials per sites for seed mass, emergence time and developmental time

Table S3. Phenotypic selection analyses for individual populations within sites.

Table S4. Standardized linear and quadratic selection differentials for each pair of sites for plasticity of emergence time and developmental time.

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Table 1. Geographical and climatic variables for the seed collection locations (populations) and common garden sites of *Pinus sylvestris* used in this study.

	Country	Longitude	Latitude	Altitude (m)	MAT (°C)	AP (mm)	TWM (°C)
Population	Spain	3° 48' 37'' N	41° 01' 41'' W	1507	8.4	598	25.3
	Germany	13° 08' 15'' N	53° 15' 47'' E	77	8.1	575	21.9
	Lithuania	24° 25' 17'' N	54° 02' 56'' E	139	6.1	635	22.5
	Finland	26° 47' 05'' N	66° 26' 19'' E	192	-0.3	531	19.3
Site	Spain	3° 31' 13'' N	41° 11' 51'' W	1326	9.3	563	26.1
	Germany	8° 46' 00'' N	50° 48' 08'' E	183	9.0	724	23.0
	Lithuania	23° 54' 49'' N	54° 53' 42'' E	33	6.6	614	22.5
	Finland	25° 28' 56'' N	65° 00' 28'' E	16	1.9	456	20.3

MAT: mean annual temperature, AP: annual precipitation, TWM: maximum temperatures of the warmest month. Climatic values correspond to averages for the reference period 1970-2000 (obtained from Worldclim, Fick & Hijmans 2017).

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Table 2. Monthly precipitation (Pp, in mm) and average maximum temperature (T_{max}, in °C) in the four common gardens of *Pinus sylvestris* for three months (July, August, September) in 2017 and 2018 during which emergence and most mortality occurred.

		July			August			September		
Site		Reference	2017	2018	Reference	2017	2018	Reference	2017	2018
Finland	Pp	73.0	56.3	24.7	67.0	100.3	85.5	54.0	40.2	62.8
	T_{max}	17.4	20.6	26.7	14.5	18.0	20.5	9.3	12.5	14.9
Lithuania	Pp	83.0	76.8	138.4	66.0	55.8	65.6	59.0	56.1	88.4
	T_{max}	19.2	21.7	25.4	18.5	23.3	25.0	13.9	20.3	17.4
Germany	Pp	65.0	110.8	20.4	57.0	134.6	24.6	44.0	44.2	52.8
	T_{max}	19.5	23.6	28.6	19.2	22.6	26.7	15.6	17.4	20.7
Spain	Pp	25.0	NA	2.3	23.0	49.3	7.9	42.0	0.3	0.8
	T_{max}	20.8	25.1	23.9	20.9	24.9	25.9	17.6	19.2	22.5

Reference corresponds to the average values for the reference period 1970-2000 at the original source locations of the four assayed provenances (obtained from Worldclim, Fick & Hijmans 2017).

NA: not available data because supplemental watering was added during the first month

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Table 3. Results of the mixed models for fitness variables and phenotypic traits measured in the common gardens of *Pinus sylvestris*.

	df	χ^2	*P*
Emergence			
Site	3	**66.6**	**< 0.001**
Population	3	**77.2**	**< 0.001**
Site × population	9	**206.7**	**< 0.001**
Family (population)	1	**114.8**	**< 0.001**
Survival 2017			
Site	3	**136.8**	**< 0.001**
Population	3	**23.2**	**< 0.001**
Site × population	9	**29.6**	**< 0.001**
Family (population)	1	**4.7**	**0.030**
Survival 2018			
Site	3	**65.4**	**< 0.001**
Population	3	**105.2**	**< 0.001**
Site × population	9	**132.1**	**< 0.001**
Family (population)	1	0.0	0.999
Cumulative survival			
Site	3	**148.5**	**< 0.001**
Population	3	**52.2**	**< 0.001**
Site × population	9	**155.6**	**< 0.001**
Family (population)	1	0.1	0.708
Cumulative fitness			
Site	3	**66.8**	**< 0.001**
Population	3	**84.2**	**< 0.001**
Site × population	9	**158.8**	**< 0.001**
Family (population)	1	**55.4**	**< 0.001**
Seed mass			
Population	3	**564.2**	**< 0.001**
Family (population)	1	**9886.3**	**< 0.001**
Emergence time			

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Site	3	**33.8**	**< 0.001**
Population	3	**107.2**	**< 0.001**
Site × population	9	**36.3**	**< 0.001**
Family (population)	1	**62.8**	**< 0.001**
Developmental time			
Site	3	**122.8**	**< 0.001**
Population	3	**40.0**	**< 0.001**
Site × population	9	**65.1**	**< 0.001**
Family (population)	1	3.3	0.069

Site and Population were fixed-effect factors. Family nested within population was a random-effect factor. Results for random-effect factors Replication, Column, Row and Experimental-unit are not shown for simplicity. Degrees of freedom (df), chi-square (χ^2) and significance *P* values are shown. Significance effects ($P < 0.05$) are typed in bold. Note that 'Site' effect could not be estimated for seed mass because seeds were collected in the populations of origin.

Figure legends

Fig. 1. Least squares means of early-stage fitness variables (panels a-e) and phenotypic traits (panels g-h) of the four studied *Pinus sylvestris* populations (circles) at the four study sites (*x*-axes). Sites: SP: Spain, GE: Germany, LI: Lithuania, FI: Finland. Vertical bars indicate standard errors and asterisks (*) indicate differences among populations within sites. Panel (f) shows least squares means of seed mass for the studied populations, with populations represented in the *x*-axis and different letters indicating significant ($P < 0.05$) differences among populations using post-hoc tests. In f) Standard error bars are hidden behind the dots.

Fig. 2. Seedling survival probabilities for the four studied populations of *Pinus sylvestris*: Spain (SP), Germany (GE), Lithuania (LI) and Finland (FI) at the four common garden sites in 2017 (a-d) and at the southern and northern gardens in 2018 (e, f). Shaded bands represent 95% confidence intervals. Chi-square (χ^2) and *P*-values from the Cox proportional hazards mixed models are shown for population factor. Survival probabilities in 2018 are refered to alive seedlings after winter 2017/2018. Hazard mixed models were not performed for Finland in 2018 due to the low mortality observed that year (see material and methods for details).

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Fig. 3. Effect of temperature and precipitation during the preceding week on *Pinus sylvestris* seedling mortality. Standardized coefficients for temperature (T) (top panels, red lines) and precipitation (PP) (bottom panels, blue lines) of Cox proportional hazard mixed models fitted at the four common garden sites (Spain, Germany, Lithuania and Finland) in 2017 and at the Spanish site in 2018. Shaded bands represent 95% confidence intervals. Chi-square (χ^2) and *P*-values from the hazard models are shown. Coefficients for temperature and precipitation in the Spanish site in 2018 were estimated in separate models due to high variance inflation factor (VIF) when fitting both variables jointly.

Fig. 4. Relationship between *Pinus sylvestris* seed mass and seedling fitness variables measured at four common garden sites in Spain (SP), Germany (GE), Lithuania (LI) and Finland (FI). Estimated values of linear (*S*) and quadratic (*C*) selection differentials are shown for each trait and site. Shaded bands represent 95% confidence intervals. The colour and subscripts of selection differentials indicate the site, SP: Spain, GE: Germany, LI: Lithuania, FI: Finland. Chi-square (χ^2) and *P*-values of *Site × trait* (S×T) and *Site × trait*^{2} (S×T^2) interactions were obtained using mixed models. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$. Significant selection differentials ($P < 0.05$) are underlined. Data points to fit the functions are not shown for simplicity. The *x*-axes show standardized (std) values.

Fig. 5. Relationship between *Pinus sylvestris* seedling fitness variables and emergence time (left panels) and developmental time (right panels) measured at four common garden sites in Spain (SP), Germany (GE), Lithuania LI) and Finland (FI). Shaded bands represent 95% confidence intervals. Estimated values of linear (*S*) and quadratic (*C*) selection differentials are shown for each trait and site. Colour and subscripts of selection differentials indicate the site, SP: Spain, GE: Germany, LI: Lithuania, FI: Finland. Chi-square (χ^2) and *P*-values of *Site × trait* (S×T) and *Site × trait*^{2} (S×T^2) interactions were obtained using mixed models. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$. Significant selection differentials ($P < 0.05$) are underlined. Data points to fit the functions are not shown for simplicity. The *x*-axes show standardized (std) values.

Fig. 6. Linear selection differentials (*S*) for seed mass (a-d), emergence time (e-h) and developmental time (i-l) estimated for each *Pinus sylvestris* population (*x*-axes) within each of the four experimental sites (Spain, Germany, Lithuania and Finnland) obtained using 'cumulative fitness' (for seed mass) or 'cumulative survival' (for emergence time and developmental time). Assayed populations were: Spain (SP), Germany (GE), Lithuania (LI) and Finland (FI). Vertical bars indicate standard errors and asterisks selection differentials (*S*) significantly different from zero (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

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Differences in *S* among populations within sites were tested using trait-by-population interactions obtained from mixed models, the corresponding Chi-square (χ^2) and *P*-values are shown. Population estimates of *S* with different letters were significantly different from each other.

Fig. 7. Linear selection differentials (*S*) for plasticity of emergence time between German and Lithuanian sites (a) and between Lithuanian and Finnish sites (b), and linear selection differentials for plasticity of developmental time between Spanish and German sites (c) and between Spanish and Lithuanian sites (d). Only survival 2017 is represented as fitness component. Shaded bands represent 95% confidence intervals. Asterisks indicate selection differentials (*S*) significantly different from zero (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Plasticity values were standardized and survival rates were relativized (see text for details). See Table S4 for linear and quadratic selection differentials of all site combinations and fitness components. Note that fitness-plasticity functions only represent the linear components.

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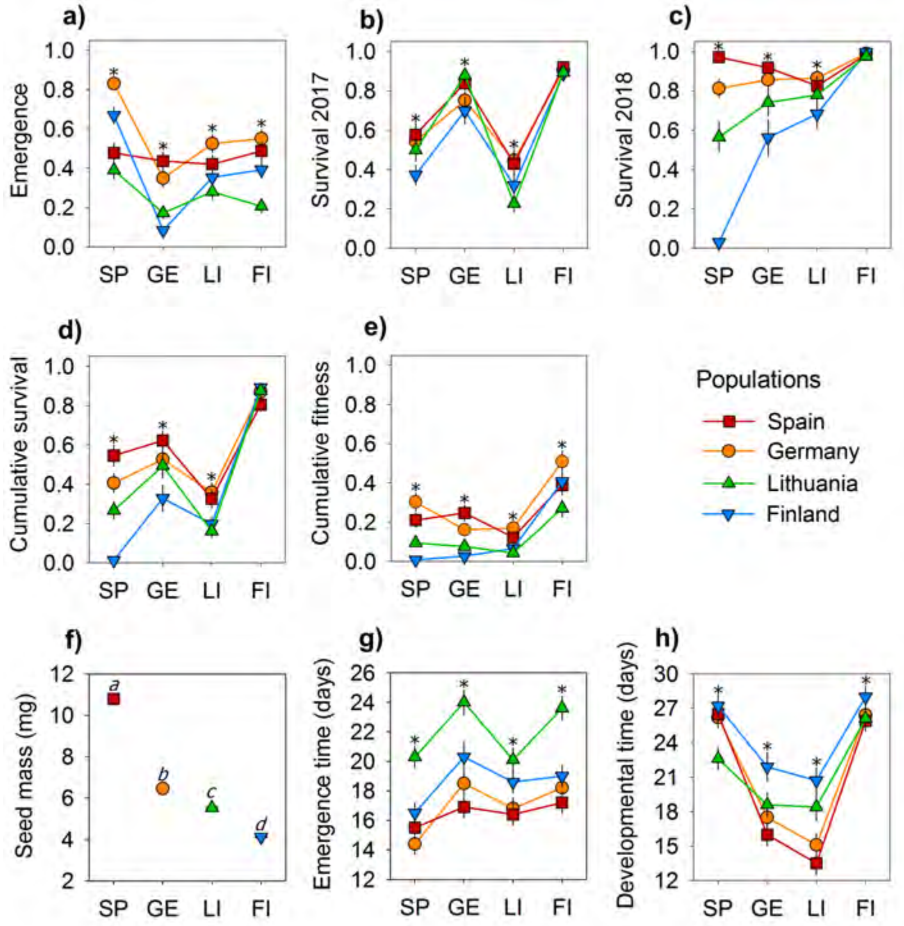

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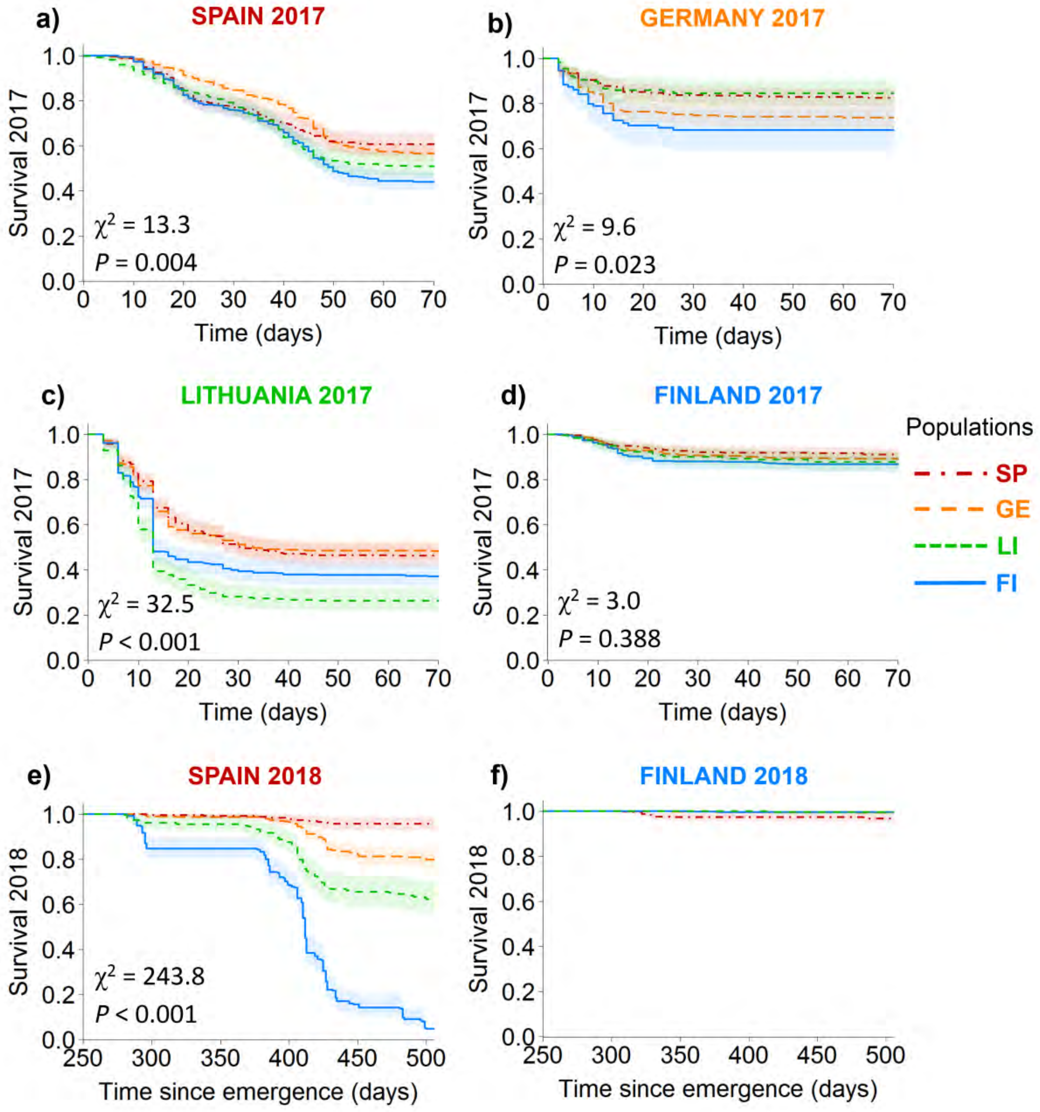

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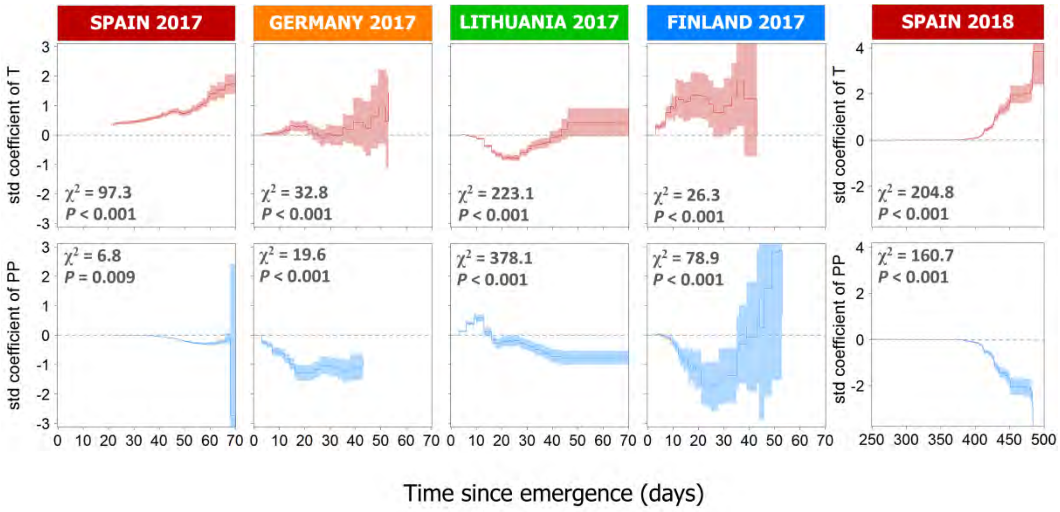

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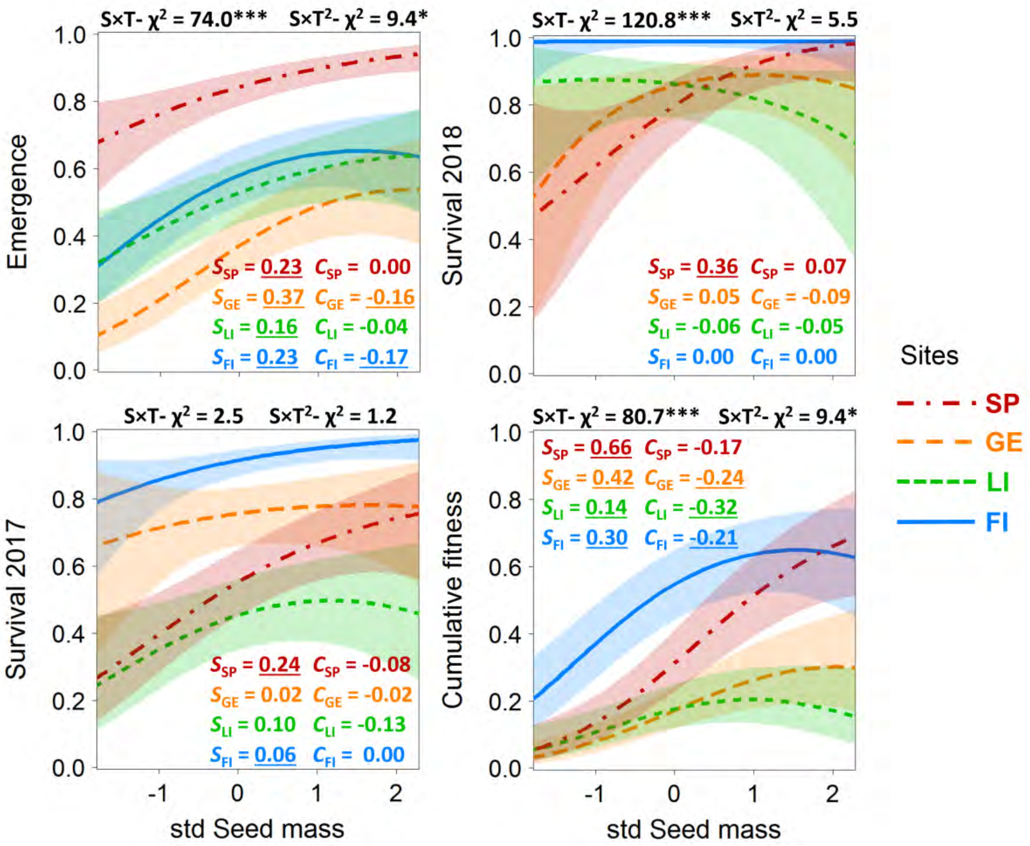

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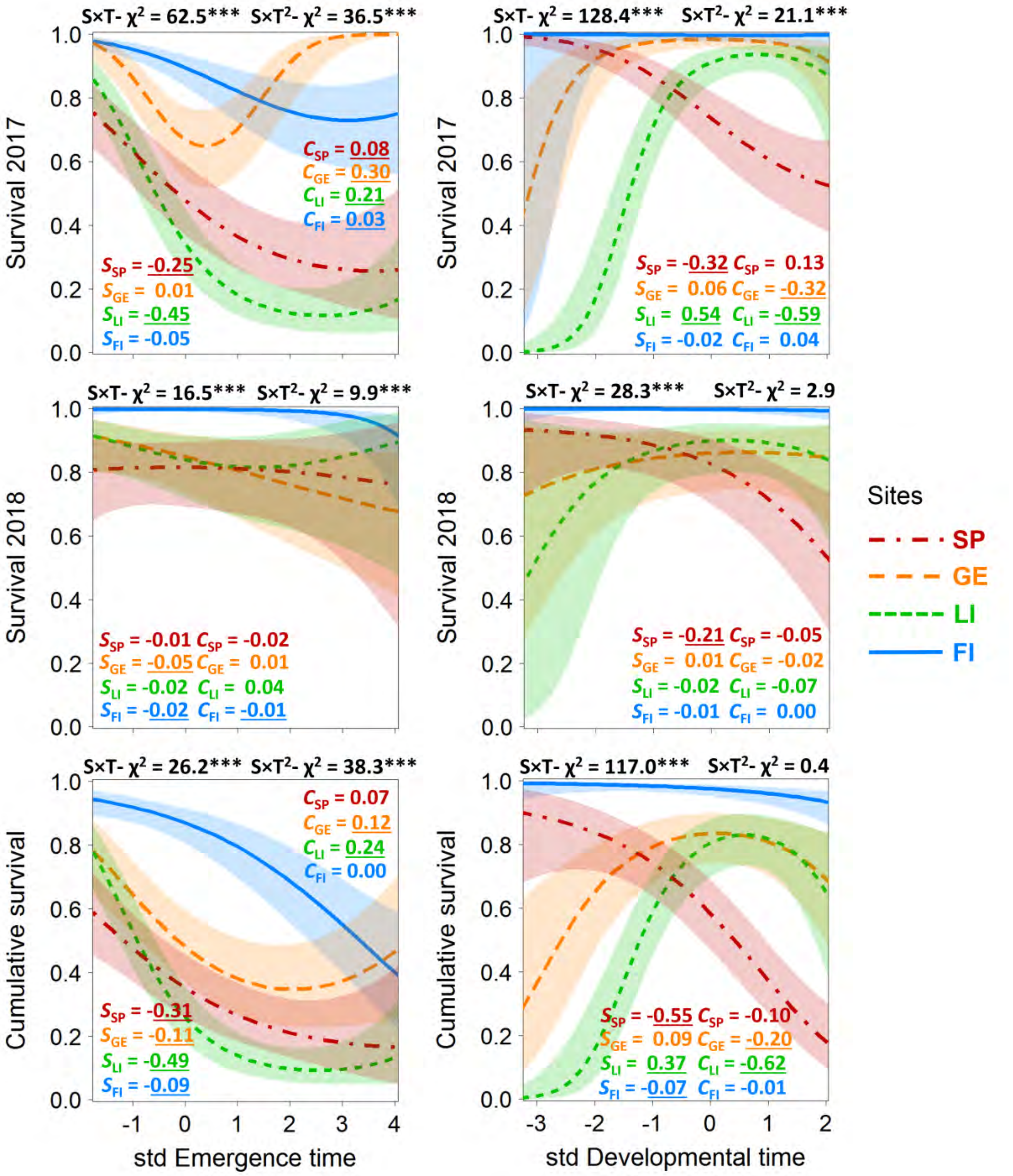

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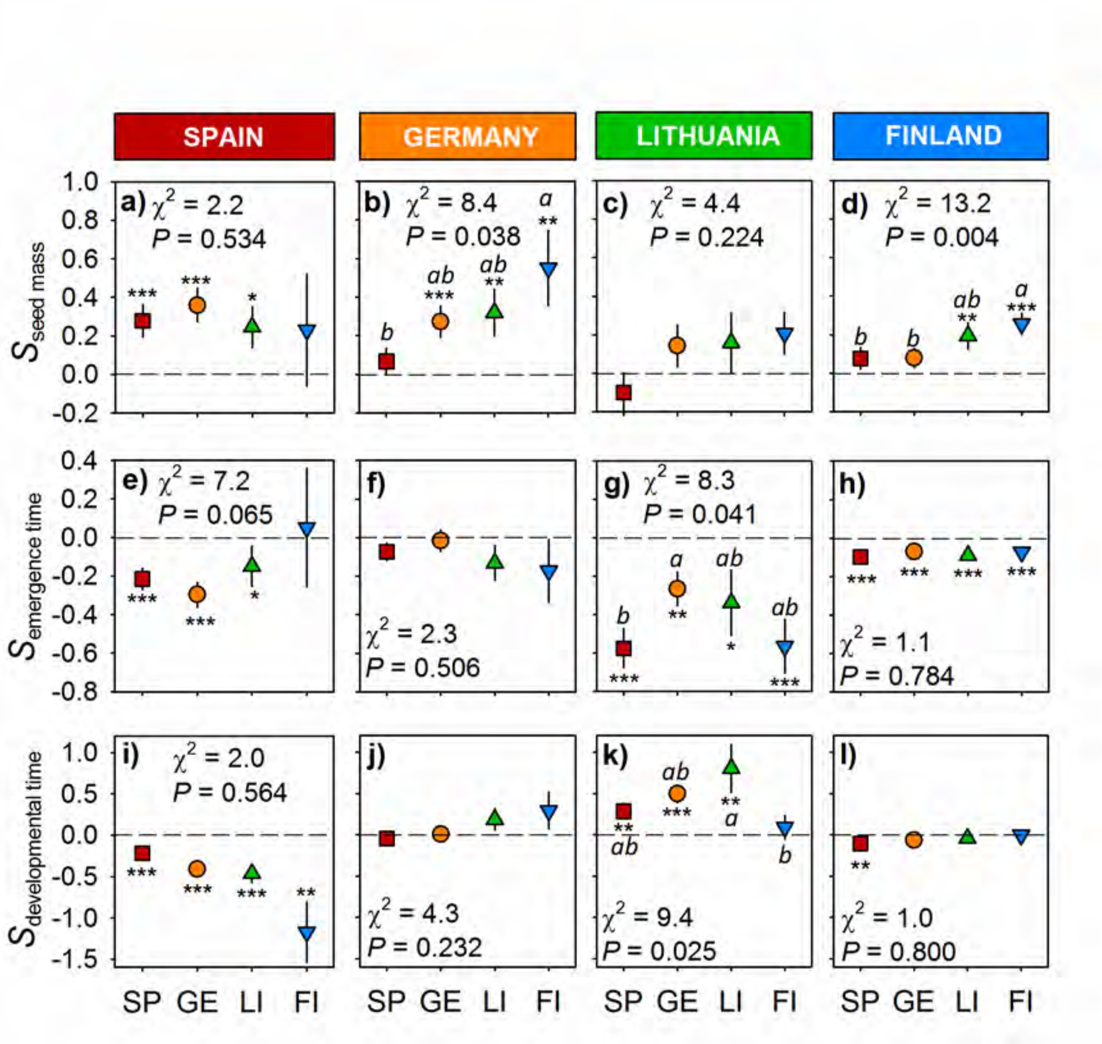

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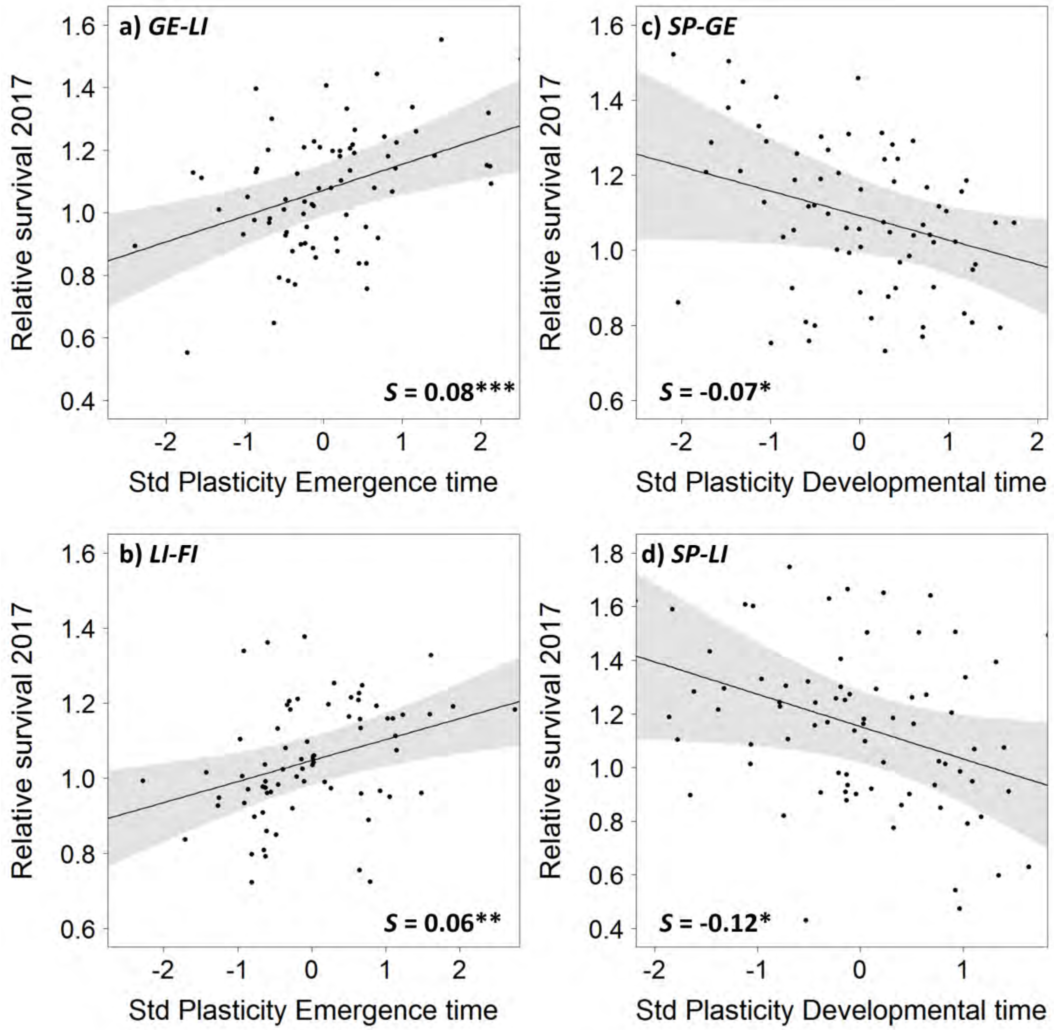

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