Life-history correlations with seasonal cold hardiness in maritime pine[1]

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PREMISE OF THE STUDY: Plants have developed mechanisms to withstand stressful environmental conditions, but the high energetic cost of these mechanisms may involve exchanges with other key functions. While trade-offs between cold hardiness and growth rates are a general assumption, we lack information regarding genetically based trade-offs between cold hardiness and other life-history traits. Such information has strong implications for tree conservation and breeding, especially in the context of ongoing climate change.

METHODS: We used a common garden progeny test to examine the relationships between seasonal cold hardiness and life-history traits of growth, reproduction, juvenile ontogeny, and phenology in 75 families of six maritime pine (*Pinus pinaster* Ait.) populations, three of continental and three of coastal origins.

KEY RESULTS: We found a clear differentiation among populations with regard to cold hardiness and life-history traits. Two continental Iberian populations showed high cold tolerance and slower growth, but faster ontogenetic development in relation to both vegetative heteroblastic change in juveniles and the onset of female reproduction. The coastal populations displayed the opposite behavior, while the continental Moroccan population presented a unique combination of traits. We confirmed trade-offs between cold-hardiness and growth at the population level, but not within populations. There were no trade-offs with other life-history traits at either level.

CONCLUSIONS: Relevant local adaptation syndromes were identified in the relationship between cold hardiness and life-history traits. These should be considered in developing tree management guidelines aimed at increasing productivity or adaptability under the expected conditions of climate change.

KEY WORDS   cold hardiness; families; global warming; life-history traits; local adaptation; phenology; *Pinus pinaster*; populations; trade-offs

Plants have developed mechanisms for maintaining growth and productivity even under stressful circumstances (Wang et al., 2003). This is seen especially in temperate regions in response to low temperatures as a predictable, seasonal periodic stressor (Hofmann and Bruelheide, 2015). To minimize high energetic costs (Huner et al., 1998), cold hardening mechanisms such as phenological synchronization are regulated according to environmental conditions (Weiser, 1970, Charrier et al., 2011). It has long been hypothesized that a trade-off or cost exists between cold hardiness and growth rates (Levitt, 1980; Aitken and Hannerz, 2001; Howe et al., 2003) and that growth rates, and thereby competitive ability, might increase in the absence of cold hardiness (Grime, 1977). However, we lack information about trade-offs with other life-history traits related to plant fitness, particularly in long-lived forest trees. Reproduction is the basic plant function most directly related to fitness (Stearns, 1992) and may be linked to abiotic stress tolerance (Obeso, 2002), although it may also deplete resources necessary for growth and maintenance (Bazzaz et al., 1987). Tree populations adapted to stressful conditions tend to invest more in reproduction, at the expense of vegetative growth (see, for example Santos-del-Blanco et al., 2012, 2013). While many works address the negative, direct effects of frost on reproduction (see, for example Morin et al., 2007), analysis of genetic correlations—those with an evolutionary basis—between cold hardiness and reproduction is generally lacking.

Apart from reproduction, other life-history traits that could trade-off with cold hardiness include those linked to heteroblastic changes in plants. The mechanism by which ontogenetic development

influences cold hardiness has been the subject of substantial consideration but relatively little research (Kalberer et al., 2006). Maintenance of juvenile structures and a habit of continuous shoot growth may favor foliage production, thereby maximizing growth and enhancing competitiveness (Zotz et al., 2011), but may trade-off with lower cold hardiness in juveniles (Sakai and Larcher, 1987; Climent et al., 2009, 2011; Pardos et al., 2014). This is not completely clear, because examples exist of higher cold tolerance in juveniles (Bannister et al., 1995) or similar cold hardiness between saplings and adults (Greer et al., 1989).

Common-garden progeny tests provide an excellent experimental setting for observing trade-offs between plant functions, because we can disentangle environmental and genetic effects. Similarly, *Pinus pinaster* Aiton (maritime pine) is a good tree species for addressing seasonal cold hardiness trade-offs, because low temperatures are a particular concern in this and other Mediterranean species (Climent et al., 2009; Corcuera et al., 2011; Larcher, 2000; Díaz et al., 2009). The fragmented distribution of maritime pine extends from the Mediterranean basin to the European Atlantic coast, covering a wide range of elevations and contrasting climates. In a previous work, we found genetic variation in cold hardiness for autumn, but not spring, among and within six *P. pinaster* populations (Prada et al., 2014). Population differentiation has also been reported for some life-history traits (see, for example Santos del Blanco et al., 2012).

In this paper, we focus on the trade-offs between spring and autumn cold hardiness and life-history traits of growth, reproduction, and juvenile ontogeny by analyzing half-sib families of six *P. pinaster* populations in a common garden. Based on the life-history theory, we expected the more cold-tolerant genotypes to grow less, reproduce more, and transition earlier to vegetative adults (Stearns, 1992; Aitken and Hannerz, 2001; Zotz et al., 2011); while those less cold tolerant would show opposite trends. The specific objectives of this study were (1) to estimate genetic variation for cold hardiness and life-history traits among and within populations; (2) to determine the existence of trade-offs between these traits at family and population levels, taking shoot phenology variation into account; and (3) to look for indications of local adaptation by analyzing how cold hardiness and life history traits relate to geoclimatic data from the place of origin.

MATERIALS AND METHODS

Plant material—We selected saplings from several half-sib families in six *P. pinaster* populations with contrasting climatic conditions of origin, in the distribution range covering 34–44° N, 1–9° W, and 0–1600 m a.s.l. (Fig.1; see Table 1 for details). Three of the populations came from the Atlantic coast (Sergude (SERG), Leiría (LEIR), and Mimizán (MIMI)), while the other three came from the Mediterranean continental climate area (Bayubas (BAYU), Cuellar (CUEL), and Tamrabta (TAMR)). Half-sib families in natural populations were sampled from trees spaced at least 100 m apart. The saplings were situated in four blocks of a provenance-progeny common garden test site established in 2005 in Ourense (42°14′N, 7°56′W, and 460 m a.s.l., Galicia-Spain), with 10–16 half-sib families per population (10 families from MIMI, 11 from LEIR, 12 from BAYU, 15 from TAMR, 15 from CUEL, and 16 from SERG) and four contiguous plants from the same family per block.

Freezing damage traits—We assessed cold hardiness for twigs excised from the last lateral growth of well-exposed branches of four trees per family from each of the six populations (Table 2). We collected the twigs on 5 November 2010 (5 yr after planting) and 1 April 2011, corresponding to the acclimation phase in autumn and the deacclimation phase in spring, when the risk of freezing damage is greatest. Four twigs from every tree in each sampling period were collected: one twig for each of three freeze temperatures (−14.5, −17.0, and −19.5°C in autumn and −8.0, −13.0, and −15.5°C in spring) and one for the control. The freeze temperatures were chosen based on previous tests to attain medium percentages of damage, which would vary depending on the season and type of tissue (see Prada et al., 2014). We collected all twigs for each sampling period on a single day, transported them on ice to the laboratory, and cold-stored them at 4°C overnight. The next day, we wrapped them in damp cheesecloth and aluminum foil and placed them into a computer-controlled freezer that was programmed to cool down to the desired test temperature—a rate of 2°C h⁻¹. After 3 h at this temperature, samples were thawed at a rate of 5°C h⁻¹ until they reached 4°C. Freezing damage was assessed using fluorometry (FN) for the needles, and by visual scoring of buds (FB) and stems (FS).

We estimated minimum (F_0) and maximum (F_m) chlorophyll fluorescence in dark-adapted needle samples by means of a pulse amplitude modulated fluorometer (MINI PAM, Walz, Effeltrich, Germany) equipped with a 2030-B leaf clip holder. We also calculated variable chlorophyll fluorescence (Fv) as $F_v = F_m − F_0$ (see Genty et al., 1989) and dark-acclimated, maximum potential photosystem II (PSII) efficiency as F_v/F_m (Peguero-Pina et al., 2008). High F_v/F_m values reveal undamaged tissue, while low values indicate freezing damage (see Appendix 1). We expressed this variable as a percentage of maximum damage, as follows:

$$F = 100 - \left(100 * \frac{Fv/Fm}{Fvc/Fmc} \right)$$

where F is the percent of damage estimated by measurement of fluorescence, F_v/F_m is the maximum potential PSII efficiency of the sample, and F_{vc}/F_{mc} is the maximum potential PSII efficiency of the control.

To visually estimate freezing damage in buds and stems, we placed the samples in trays with substrate and maintained them in greenhouse conditions (20–25°C and 90% relative humidity; using a cooling/heating system and a fog device) for 10 days to allow visible signs of freezing damage to develop. To determine the extent of damage, we sliced buds and stems longitudinally and scored them according to tissue discoloration on a scale of 0–3 (0 = 0%, no damage; 1 = 1–33% damaged; 2 = 34–66% damaged; 3 = 67–100% damaged). Additional information on cold hardiness assessment methods can be found in Prada et al. (2014).

Life-history traits—Six years after planting, we measured diameter at breast height, total height, juvenile ontogeny, shoot phenology, and male and female reproduction on all the live plants in the progeny test for the six populations studied. The number of trees per family varied from 5–16, totaling 878 plants in 2005, down to 790 plants by 2010. See Table 2 for acronyms and summary information.

We estimated the relative height growth rate (RHGR6) and the absolute height growth rate (AHGR6) from the total height
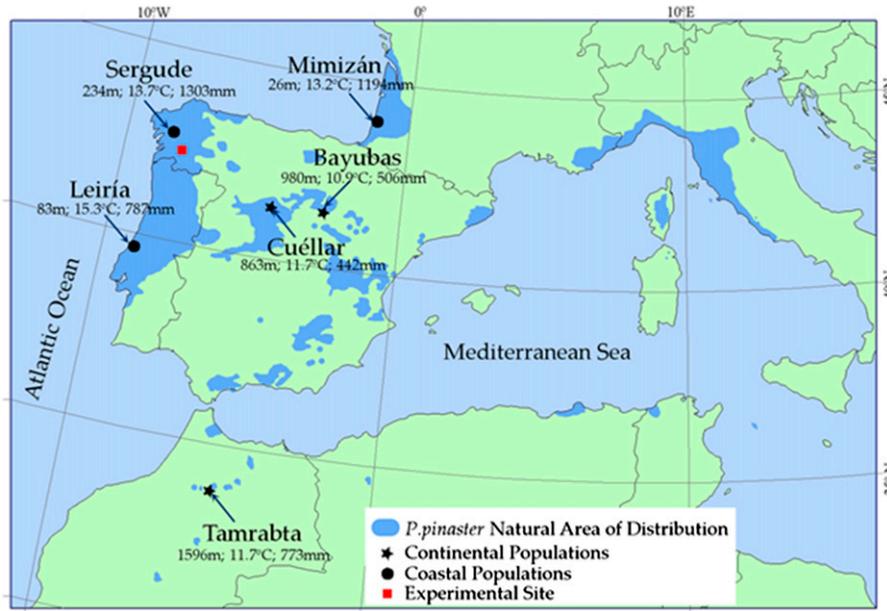
FIGURE 1 Location of the six *P. pinaster* populations sampled in the study, and the experimental site (EUFORGEN, 2009).

measured at the end of the growing season for each tree in 2005 and 2010. The RHGR is the maximum intrinsic growth rate in height, and AHGR is the total height that a tree accumulates in a year. Plants with a shorter growing season may grow faster in early spring, and thus have higher RHGR, but may have accumulated less AHGR by the end of the year (Koehler et al., 2012). We calculated RHGR (cm cm^{-1} yr^{-1}) as RHGR= $(lnHi - lnHj)/(ti-tj)$, where H is the height in time i and j (ti and tj respectively, with $i > j$) (Sánchez-Gómez et al., 2010). We calculated AHGR (cm yr^{-1}) as AHGR= $(Hi - Hj)/(ti-tj)$ (Koehler et al., 2012).

Two years after planting, we assessed juvenile ontogeny (ONT2) linked to shoot heteroblastic change in early stages of development (Climent et al., 2006). Using a three-level scale, we assigned a category value of 1 for the juvenile stage (only primary needles), 2 for the transitional stage (dwarf shoots in the axils of primary needles), and 3 for the adult stage (dwarf shoots in the axils of scales, cataphylls). Because ontogenetic stage is strongly related to seedling size (Climent et al., 2006), we also calculated a derived variable unbiased by growth differences: HAd corresponds to the height at which the probability for a tree within a population to have reached the adult stage was 99%. To estimate HAd, we fitted a logistic regression for ontogeny (pooled in two categories: 0 for juveniles and transitional stage, 1 for adults) with height as the independent variable and population as the categorical factor, obtaining one HAd value for each population.

Each plant in the field trial was assigned a phenological stage (shoot phenology, SP6) on 21 April 2010, based on a categorical, six-level scale with the following values: 0 for dormant buds, 1 for elongating buds, 2 for elongating internodes, 3 for emerging secondary needles, 4 for secondary needles not yet completely developed, and 5 for completely developed secondary needles.

We estimated female reproductive biomass as the sum of all immature cones at age 5 (FRB5). Because the *P. pinaster* female cone development cycle is biennial, this variable includes two consecutive flowering years (Climent et al., 2008). We estimated the male reproductive biomass at age 6 as the number of twigs with male strobili that could be counted in 15 s (MRB6). Again, to remove the size effect on reproduction, we used the median threshold size for first reproduction (FTSFR for female reproduction, MTSFR for male reproduction), or the size at which the probability was 50% for a tree within a population to have reached sexual maturity (Santos del Blanco et al., 2012).

Climate data—Climatic variables were generated with the ClimateEU v4.63 software package (Hamann et al., 2013), which is freely available in beta at https://sites.ualberta.ca/~ahamann/data/climateeu.html. The following variables were selected for their potential biological relevance to population adaptation to climate: mean annual temperature (MAT), continentality (TD, equivalent to the mean of the warmest month temperature minus the mean of the coldest month temperature), growing degree-days above 5°C (DD5), mean annual precipitation (MAP), mean summer (May to September) precipitation (MSP), annual heat-moisture index (AHM= (MAT+10)/(MAP/1000)) and Hargreaves reference evaporation (Eref). Other directly obtained and derived climate variables were also analyzed and are included in Appendix 2.

TABLE 1. Geographic and climatic parameters of the experimental site and population origins. Climatic data were generated from the ClimateEU v4.63 software package.

Parameter	Experimental site	Population					
		BAYU	**CUEL**	**LEIR**	**MIMI**	**SERG**	**TAMR**
Longitude West (Long, dec degrees)	7.93	2.87	4.32	9.02	1.22	8.67	5.02
Latitude North (Lat, dec degrees)	42.23	41.52	41.40	39.68	44.20	42.73	33.60
Altitude (m)	460	955	858	50	20	150	1760
Mean Annual Temperature (MAT, °C)	13.5	11.2	11.9	15.5	13.4	14.3	12.5
Temperature difference (TD, °C)	14.5	18.7	18.7	9.6	14.5	11.2	26.0
Degree-days above 5°C (DD5, growing degree-days)	3188	2514	2716	3917	3154	3471	3116
Mean Annual Precipitation (MAP, mm)	1092	487	440	785	1203	1154	555
Mean summer precipitation (MSP, mm)	266	195	158	133	443	275	63
Annual heat-moisture index (AHM)	21.5	44.8	51.2	34.0	19.9	22.3	43.3
Hargreaves reference evaporation (Eref)	892.0	943.5	955.3	809.3	832.1	731.1	1344.5

Note: BAYU: Bayubas; CUEL: Cuéllar; LEIR: Leiría: MIMI: Mimizán; SERG: Sergude; TAMR: Tamrabta.

TABLE 2. Summary of assessed variables: description, unit, measurement year (Year), plant age (Age (yrs.)), number of populations (P), number of families per population (F(P)), total number of families (F), and number of plants (Pl).

Variable	Description	unit	Year	Age	P	F(P)	F	Pl
*Freezing damage traits**								
FN_A, FS_A, FB_A	Freezing damage traits in autumn	%	2010	6	6	9–16	75	300
FN_S, FS_S, FB_S	Freezing damage traits in spring	%	2011	6	6	9–16	75	300
Life-history traits								
DBH6	Diameter at Breast Height	mm	2010	6	6	9–16	75	790
RHGR6	Relative Height Growth Rate	cm cm^{-1} yr^{-1}	2005 - 2010	6	6	9–16	79	790
AHGR6	Absolute Height Growth Rate	cm yr^{-1}	2005 –2010	6	6	9–16	79	790
ONT2	Juvenile Ontogeny	scale: 1-3	2007	2	6	9–16	79	878
HAd	Adult Height	cm	2007	2	6	—	—	878
SP6	Shoot Phenology	scale: 0–5	2010	6	6	9–16	79	790
FRB5	Female Reproductive Biomass	no	2008; 2009	5	6	9–16	79	816
MRB6	Male Reproductive Biomass	no	2010	6	6	9–16	79	790
FTSFR	Female Median Threshold Size for First Reproduction	cm	2008-2010	5–6	6	—	—	790
MTSFR	Male Median Threshold Size for First Reproduction	cm	2008-2010	5–6	6	—	—	790

Note: * Chlorophyll fluorescence assessment method in needles (FN_A and FN_S), visual damage assessment method in stems (FS_A and FS_S), and buds (FB_A and FB_S).

Data analysis—The variables analyzed were related to growth (DBH6, RHGR6, AHGR6, HAd, ONT2, SP6), reproduction (FRB5, MRB6, FTSFR, MTSFR), and freezing damage (FN_A, FS_A, FB_A, FN_S, FS_S, FB_S). Three of these variables (HAd, FTSFR, MTSFR) were calculated on a population basis only, and therefore were not analyzed at the family level.

At the family level, we estimated the mean population (μ) and coefficient of variation (CV) for 300 individuals for the freezing variables, and for 790–878 individuals for the other traits (Table 2). Prior to analysis, we transformed the categorical traits per field block using a normal score transformation to restore normality and homogeneity of variances, because of an underlying increase in injury severity or advance in development from one class to the next (Johnsen et al., 2005). Then, to examine the spatial structure of field traits, we used the SAS VARIOGRAM procedure to construct an empirical semivariogram for the residuals, which was adjusted for family effects. We corrected spatially dependent variables (DBH6, RHGR6, AHGR6, ONT2, SP6, and MRB6) with the iterative spatial analysis (ISA) method (Zas, 2006). Spatial adjustment was applied to the location of every tree in the common garden, and the corrected values were used in subsequent analyses.

We used the restricted maximum likelihood (REML) algorithm to develop the following mixed model for analyzing these variables:

$$X_{ijkl} = \mu + P_i + F(P)_{j(i)} + B_k + \varepsilon_{ijkl}$$

where X_{ijkl} is the value of the variable for the lth tree from the jth family within ith population and kth block, μ is the overall mean, P_i is the effect of the ith population (i = 1 to 6), $F(P)_{j(i)}$ is the effect of the jth family within the ith population (j = 1–9 to 1–16 depending on the population, B_k is the effect of the field block (k = 1–4) and ε_{ijk} is the experimental error. All factors except 'family within population' were considered fixed. After testing several covariance structures to model the residuals, we selected a first-order autoregressive (AR(1)) structure.

The LSMEANS (Least square means) and BLUPs (Best Linear Unbiased Predictors) were used to obtain point estimates of fixed and random effects, respectively, in the mixed-effect model, to predict an individual tree's expected phenotype based on phenotypic information collected from its relatives (Gilmour et al., 2009).

We estimated the difference among populations (Dap) for each trait studied by subtracting the minimum from the maximum population LSMEANS. Similarly, we estimated the mean value of the differences among families within populations (Daf) and calculated the differences within each population by subtracting the minimum from the maximum family BLUP estimates.

Spearman correlations among populations (populations LSMEANS) and within populations (family BLUP after removing the population mean effect) were obtained to explore the relationships between freezing damage traits, life-history traits, and origin parameters. All statistical analyses were done with the SAS System (SAS 9.1, SAS Institute Inc., 2004).

RESULTS

Genetic variation at different levels—*Freezing damage*—Among the 18 freezing damage traits assessed (three organs × three temperatures per season × two seasons), we selected those in an intermediate level (40–70%) and with a coefficient of variation between 49 and 70% for subsequent analyses and inferences (Table 3).

Variation among families was significant (df = 69, P < 0.05) for all freezing traits in autumn and for freezing damage in needles in spring. Variation among populations was significant (df = 5, P < 0.001) for all freezing traits in autumn and spring, except for buds in spring (Table 3). The differences among families (Daf) and among populations (Dap) were high for all freezing traits (Table 3).

Life-history traits—We found significant variation (P < 0.05) among (df = 5) and within populations (df = 73) for all traits except juvenile ontogeny (ONT2), which only showed significant differences between populations (Table 3). The difference among families was lower than the difference among populations for all life-history traits (Table 3).

Correlation between cold hardiness and life-history traits—We considered in subsequent analyses freezing damage in needles in autumn and spring (FN_A and FN_S) as representative traits for seasonal freezing damage, because damage in needles was well correlated with damage in the other organs (data not shown). We found a significant positive correlation between freezing damage in

TABLE 3. Treatment freezing temperature (T, °C), Population mean (μ), range, coefficient of variation (CV, %), fixed effect estimation for population (P) and block (B), variance components of the random effects ($\sigma_{F(p)}^2$ and σ_e^2 are the family variance components nested to population and error, respectively) and differences among (Dap) and within (Daf) populations for freezing damage and life-history traits.

Variable	T (°C)	μ	Range	CV	Pa	Bb	$\sigma_{F(p)}^2$	σ_e^2	Dap	Daf
Freezing damage traits in autumn										
FNA	−17.0	61.4	0-100	49.4	8.11***	1.53	140.88*	623.99	35.7	34.1
FSA	−19.5	15.4	0-100	144.7	15.95***	2.28	55.66*	313.59	35.9	24.7
FBA	−19.5	62.8	0-100	52.1	12.17***	1.30	158.01*	615.48	44.8	30.5
Freezing damage traits in spring										
FNS	−15.5	40.3	0-100	62.1	7.53***	2.66*	99.27*	430.53	35.3	24.3
FSS	−15.5	60.2	0-100	70.0	3.36**	7.96***	271.61	1316.68	29.8	44.5
FBS	−15.5	65.6	0-100	66.6	2.00	6.76***	222.27	1573.02	29.7	31.2
Life-history traits										
DBH6		38.8	4-112	52.6	28.00***	1.88	0.02*	0.39	0.8	0.4
RHGR6		0.5	0.2-1.2	14.9	15.44***	1.85	0.04**	0.59	1.0	0.5
AHGR6		54.5	8-112	32.4	27.87***	2.69*	0.05**	0.42	1.0	0.4
ONT2		2.7	1-3	16.6	8.96***	1.06	0.00	0.38	0.3	0.0
SP6		1.8	1-3	29.8	9.23***	0.96	0.07**	0.39	0.9	0.8
FRB5		1.0	0-22	223.0	7.92***	0.50	0.03*	0.61	0.6	0.4
MRB6		16.6	0-61	98.4	32.08***	0.38	0.03*	0.54	1.3	0.3

Notes: Significance levels: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$.
a $F_{5,69}$ (freezing traits).$F_{5,73}$ (life-history traits).
b $F_{3,221}$ (freezing traits).$F_{3,707-796}$ (life-history traits).

needles in autumn, and absolute height growth rate at the population level (df = 4, $r = 0.83$, $P < 0.05$, Table 4), indicating that the genotypes with more freezing damage in autumn tended to have more absolute growth. However, this relationship was not significant at the family level (df = 73, $r = −0.11$, $P > 0.1$). We found a barely significant negative correlation between freezing damage in needles in spring and female reproductive biomass at the population level (df = 4, $r = −0.77$, $P < 0.1$), suggesting that the genotypes with less freezing damage in spring tended to have a greater female reproductive biomass. This correlation was not significant at the family level (df = 73, $r = −0.01$, $P > 0.1$). No relationship was found at the population or family levels between freezing damage traits and the other life-history traits studied: relative growth, juvenile ontogeny, shoot phenology, or male reproductive biomass.

Shoot phenology showed a significant negative correlation with absolute height growth rate (df = 4, $r = −0.94$, $P < 0.01$, Table 4), and with male reproductive biomass (df = 4, $r = −0.89$, $P < 0.05$), indicating that the genotypes with delayed budburst tended to have more absolute growth and more male reproductive biomass. However, these relationships were not significant at the family level (df = 77, $r = −0.04$, $P > 0.1$). We found a positive correlation between male reproductive biomass and absolute height growth rate, which was barely significant at the population level (df = 4, $r = 0.77$, $P < 0.1$) and significant at the family level (df = 77, $r = 0.35$, $P < 0.01$), suggesting that the genotypes with higher male reproductive

biomass tended to have more absolute growth. The correlation between male reproductive biomass and juvenile ontogeny was barely significant and negative at the population level (df = 4, $r = −0.77$, $P < 0.1$) but significant and positive at the family level (df = 77, $r = 0.23$, $P < 0.05$). This suggests that as seedlings, the genotypes with higher male reproductive biomass tended to delay heteroblastic change.

Correlation with geoclimatic data—We found significant positive relationships between freezing traits and the mean annual temperature (df = 4, $r = 0.89$, $P < 0.05$) and between freezing traits and degree-days with temperature above 5°C (df = 4, $r = 0.89$, $P < 0.05$), indicating that populations with greater freezing damage came from areas with higher mean annual temperature and more degree-days above 5°C (Table 5). We did not find significant correlations between freezing damage and mean annual precipitation, annual heat-moisture index, or Hargreaves reference evaporation. For geographic data (longitude and latitude), we found only a barely significant positive correlation between seasonal freezing traits and the longitude of origin (df = 4, $r = −0.77$, $P < 0.1$).

The correlations observed between life-history traits and climatic data of origin showed that populations with higher absolute growth rate (AHGR6), delayed shoot phenology (SP6), and higher threshold for female flowering (FTSFR) tended to come from areas with higher mean annual temperature (MAT), less continentality (TD), more degree-days above 5°C (DD5), and minor Hargreaves reference evaporation (Eref) (Table 5). Shoot phenology (SP6) was also strongly correlated with almost all temperature variables (see Appendix 2) and presented a barely positive correlation with altitude (Alt) (Table 5). Juvenile ontogeny (ONT2) showed significant positive correlations with altitude and annual heat moisture index (AHM), but negative correlations with mean annual precipitation (MAP) (Table 5).

TABLE 4. Spearman correlations for freezing damage and life-history traits: within populations (among families) under diagonal and among populations above diagonal; abbreviations are as defined in Table 2.

Variable	FNA	FNS	RHGR6	AHGR6	ONT2	SP6	FRB5	MRB6
FNA			0.31	**0.83***	−0.37	−0.66	−0.66	0.49
FNS			0.31	0.66	−0.54	−0.54	−0.77a	0.43
RHGR6	−0.03	0.12		0.03	0.20	0.26	−0.77a	−0.54
AHGR6	−0.11	0.02	**0.27***		−0.54	**-0.94****	−0.43	0.77a
ONT2	−0.03	−0.14	−0.03	0.05		0.60	0.43	−0.77a
SP6	0.05	−0.05	−0.17	−0.04	−0.02		0.20	**-0.89***
FRB5	0.06	−0.01	−0.01	0.16	0.04	0.02		−0.03
MRB6	0.11	0.13	−0.12	**0.35***	**0.23***	−0.04	**0.27***	

Note: Significance levels: ** = $P < 0.01$, * = $P < 0.05$, a = $P < 0.1$.

TABLE 5. Spearman correlations for freezing damage traits, life-history traits and population geoclimatic characteristics; abbreviations are as defined in Tables 1 and 2.

Variable	Long	Lat	Alt	MAT	TD	DD5	MAP	MSP	AHM	Eref
FN$_A$	0.77[a]	−0.09	−0.31	**0.89***	−0.58	**0.89***	0.60	−0.09	−0.60	−0.60
FN$_S$	0.77[a]	−0.20	−0.31	**0.89***	−0.64	**0.89***	0.60	−0.20	−0.60	−0.60
RHGR6	0.43	−0.77[a]	0.14	0.31	0.17	0.31	−0.09	−0.77[a]	0.09	0.43
AHGR6	0.54	0.31	−0.71	**0.89***	**-0.84***	**0.89***	0.66	0.31	−0.66	**-0.83***
ONT2	0.03	−0.54	**0.83***	−0.66	0.72	−0.65	**-0.88***	−0.54	**0.88***	0.71
HAd	0.37	0.54	−0.71	0.71	**-0.93****	0.71	0.66	0.54	−0.66	**-1.00*****
SP6	−0.43	−0.49	0.77[a]	−0.77[a]	**0.93****	−0.77[a]	−0.60	−0.49	0.60	**0.94****
FRB5	−0.54	0.43	0.31	−0.77[a]	0.35	−0.77	−0.49	0.43	0.49	0.14
FTSFR	0.60	0.09	−0.60	**0.94***	**-0.81***	**0.94****	0.77[a]	0.09	−0.77[a]	−0.77[a]
MRB6	0.09	0.77[a]	−0.77[a]	0.60	**-0.81***	0.60	0.77[a]	0.77[a]	−0.77[a]	**-0.94****
MTSFR	0.14	−0.66	0.09	0.20	0.23	0.20	0.03	−0.66	−0.03	0.49

Note: Significance levels: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, [a] = $P < 0.1$.

Adult height (Had) had very strong negative correlations with TD and Eref (Table 5). Female reproductive biomass (FRB) presented barely significant negative correlations with MAT and DD5 only, whereas the unbiased-by-growth-differences variable (FTSFR) had strong significant relationships with MAT, TD, DD5, MAP, AHM, and Eref (Table 5). Male reproductive biomass (MRB) displayed strong negative correlations with TD and Eref, but there was no evidence of a relation between the unbiased-by-growth-differences variable (MTSFR) and geoclimatic variables (Table 5 and Appendix 2).

Population differentiation—The continental Iberian populations (BAYU and CUEL) had the lowest freezing damage values in both seasons, whereas the coastal Iberian populations (LEIR and SERG) had the highest freezing damage values (Fig. 2A, B, Appendix S1 (see Supplemental Data with the online version of this article)).

The Atlantic coast populations showed distinctly higher absolute growth (AHGR6), delayed juvenile ontogeny (ONT2) and shoot phenology (SP6), and more abundant male reproductive biomass (MRB6) compared to the continental southern populations (Fig. 2D-F, H). Female reproductive biomass (FRB5) was highest in the two continental Iberian populations, and intermediate in the Moroccan population (Fig. 2G). Both height growth rates showed different population-level response patterns (Fig. 2C, D). Specifically, we observed that the Moroccan population had the maximum relative height growth rate, but one of the lowest absolute rates.

DISCUSSION

We found clear differentiation between the Atlantic coast and the continental southern populations with regard to both seasonal freezing damage and life-history traits, suggesting local adaptation. We also uncovered genetic correlations indicative of underlying evolutionary trade-offs, along with significant environmental correlations.

Genetic variation of cold hardiness and life-history traits—We detected significant differences among maritime pine families and populations for most of the freezing and life-history traits analyzed, broadly confirming previous research (Prada et al., 2014; Santos del Blanco et al., 2012). However, differences among populations were higher than those among families for almost all traits, in contrast with other works reporting differences mainly at the family level for winter stress under highly stressful conditions (Corcuera et al., 2011). Within-population adaptive variation may result from gene flow among sites with differing selective pressures (Howe et al.,

2003) or from temporally variable local selection (Salmela et al., 2011) in response to the variations and multiple interactions of temperature, light, and nutrients (Skrøppa, 1991). This intrapopulation variation is highly relevant and general for many adaptive traits, as it ensures the evolvability of populations in response to new environmental conditions imposed by a changing climate (Howe et al., 2003).

Trade-offs between cold hardiness and life-history traits—The more cold-tolerant populations generally confirmed our expectations based on life-history theory: they grew more slowly and showed faster ontogenetic development in relation to both seedling vegetative heteroblastic change and the onset of female reproduction. By contrast, male reproduction tended to be more intense and precocious in the less cold-tolerant populations. However, those results were not confirmed at the intrapopulation level, indicating that different factors influence population divergence and within-population selection.

The results also confirmed our hypotheses that the onset of female reproduction would occur later in less cold-tolerant populations, especially in spring. This is highly consistent with the high resource allocation to growth in the maritime pine of coastal origin. In these favorable environments, early investment in costly female reproduction is disadvantageous because it decreases competitiveness (Santos-del-Blanco et al., 2012, 2013).

As expected, we found faster juvenile ontogenetic development in the more cold-tolerant populations. Conversely, pine seedlings from coastal regions tended to maintain continuous vegetative growth longer by delaying the heteroblastic change that culminates in the formation of a terminal bud and subsequent preformed growth. This delay can favor competitiveness, but can also increase the risk of frost damage in growing tissues, where higher water content makes the plant more susceptible to mechanical damage from extracellular ice and promotes lethal intracellular freezing (Levitt, 1980). This genetic correlation corroborates the well-established evolutionary conflict between stress tolerance and competitiveness (Grime, 1977).

We did not find a significant correlation between spring cold hardiness and shoot phenology. Although these two traits are strongly correlated for most species in the relevant literature (Howe et al., 2003), exceptions also exist (Cannell and Sheppard, 1982). The continental populations showed earlier onset of growth (shoot phenology) and were more cold hardy than the coastal populations. To break out of dormancy, populations from continental environments are likely to have lower chilling requirements, lower heat sum requirements, or a combination of both (Howe et al., 2003).

Notably, we found no correlation between the end of growth and cold hardiness. The Moroccan population stopped growing before the three coastal populations, probably due to summer drought. Contrary to what might be expected, however, the Moroccan population presented autumn freezing damage values similar to those of the coastal populations. Natural selection may have favored cold hardiness at higher latitudes, even in coastal areas like at the French population, which would explain its intermediate cold tolerance behavior. However, at lower latitudes, the shorter growing season
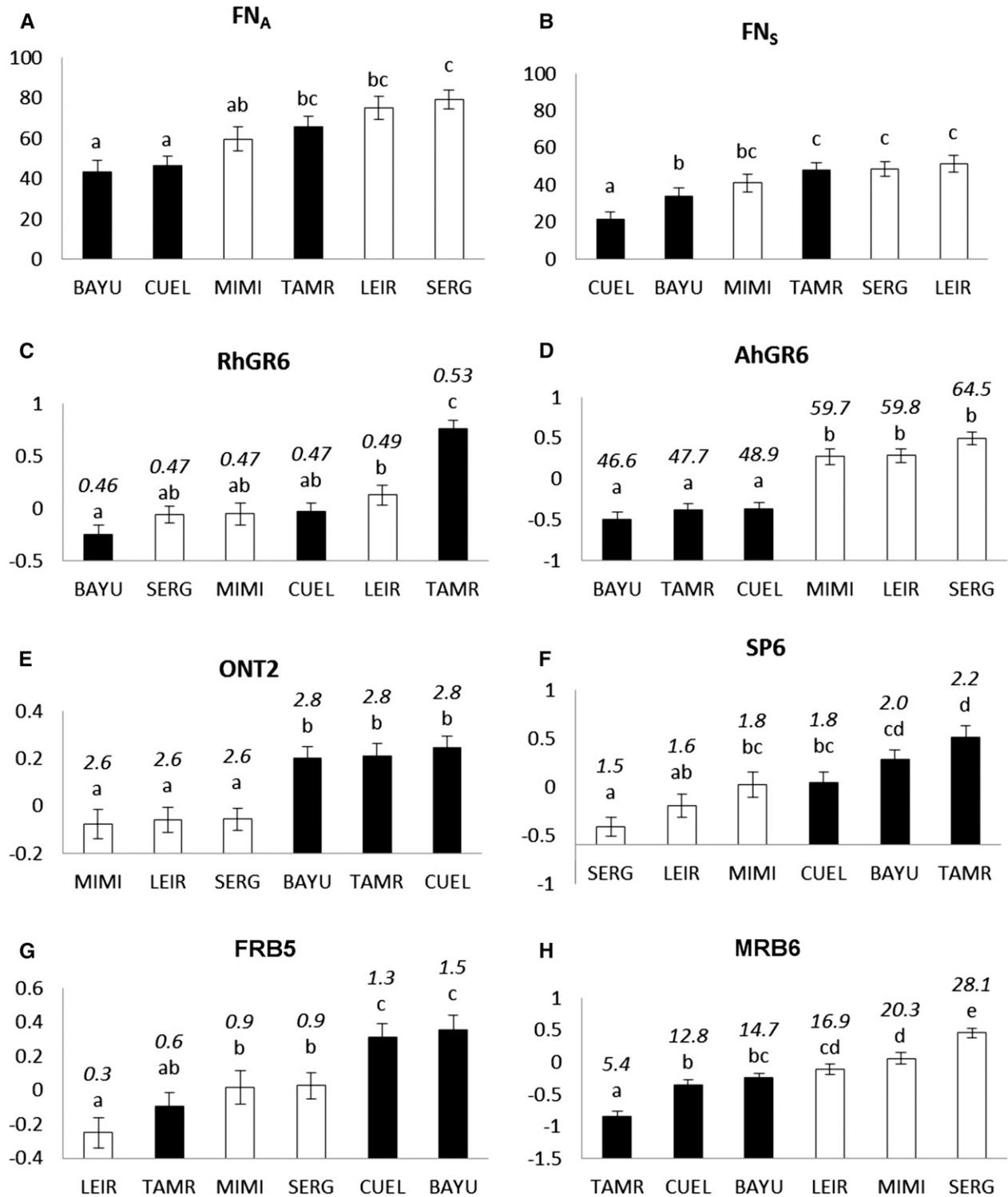
FIGURE 2 Differences among populations for field and freezing damage traits: (A) Freezing damage in Needles in autumn (FN_A), (B) Freezing damage in Needles in spring (FN_S), (C) Relative Height Growth Rate (RHGR6), (D) Absolute Height Growth Rate (AHGR6), (E) Juvenile Ontogeny (ONT2), (F) Shoot Phenology (SP6), (G) Female Reproductive Biomass (FRB5), and (H) Male Reproductive Biomass (MRB6)—obtained from the mixed model. Black bars represent LSMeans for the transformed variables in continental populations, white bars represent LSMeans for the transformed variables in for coastal populations, and vertical lines represent standard errors (see Appendix S1 Supplemental Data with the online version of this article). Different letters indicate significant differences ($P < 0.05$). Numbers in italics above letters indicate mean untransformed values.

seems to restrict fitness more than the risk of seasonal freezing damage (Vitasse et al., 2014). In contrast with findings for Douglas fir (O'Neill et al., 2001) or Norway spruce (Skrøppa, 1991), our results suggest that shoot phenology is not as relevant a criterion for cold hardiness estimation among maritime pine populations.

Correlation with geoclimatic data—The differences we observed in relation to freezing damage and life-history traits among *P. pinaster* populations reveal a pattern of genetic variation consistent with local adaptation to different environments (e.g., Alía et al., 1997; Sánchez-Gómez et al., 2010). We found consistent relationships between cold hardiness traits and climatic variables. Gradients in degree-days above 5°C and mean annual temperature revealed cold hardiness clines in *P. pinaster* populations. Cold hardiness was not associated with latitude and presented only a marginally significant correlation with longitude.

Cold and drought are considered the most important factors in shaping the geographical aspects associated with the genetic structure of the species (Corcuera et al., 2011). Indeed, both environmental stressors often display similar geographic variation patterns (Howe et al., 2003). Contrary to expectations, however, we found no coherent relationship between cold hardiness and drought-related variables (annual heat-moisture index and Hargreaves reference evaporation).

The Iberian continental populations showed significantly higher cold hardiness than the coastal populations in both autumn and spring. This supports the hypothesis that trees from harsher continental regions are less susceptible to autumn frosts than trees from milder coastal regions, but contradicts the assumption that trees from continental regions are more susceptible to spring frosts (Morgenstern, 1996; Aitken and Hannerz, 2001; Howe et al., 2003). Our finding suggests much more intense selection for cold hardiness in continental areas, compared to the more moderate coastal environments (Darychuk et al., 2012).

The Atlantic coast populations showed distinctly higher absolute growth, delayed juvenile ontogeny, and more abundant male reproduction than the continental southern populations, while female reproduction was more precocious and abundant in the two Iberian continental populations (BAYU and CUEL). The Moroccan population (TAMR) also presented precocious onset of female reproduction (low threshold size). This shift of resource allocation from vegetative growth toward female reproduction in more stressful environments has also been reported for *Pinus halepensis* in relation to ecotypic variation and phenotypic plasticity (Climent et al., 2008; Santos-del-Blanco et al., 2013). It is solidly supported by the life-history theory and can be seen in many plants and other organisms (Stearns, 1992).

In regions where drought is not limiting, higher temperatures and increased levels of CO_2 are expected to increase productivity, and therefore competition; although there is heated debate regarding the persistence of these changes (see for example Schelhaas et al., 2015). Such conditions would favor coastal maritime pine populations because of their high-growth strategy, but carry the risk of damage from out-of-season frost events. Conversely, while the risk of suffering frost damage is low in continental populations, their conservative growth strategy makes them less competitive in frost-free conditions. Our results showed that maritime pine under controlled conditions tolerated temperatures that were lower than usual for its natural habitat. However, we still suggest caution when considering seed movement from mild to harsh climate areas, to minimize the risk of damage from early autumn or late spring frost events.

This work highlights the importance of relationships between cold hardiness and key life-history traits. Such correlations point to relevant local adaptation syndromes that should be included in breeding, conservation, and assisted migration programs or transfer guidelines aimed at optimizing productivity or adaptability.

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APPENDICES

APPENDIX 1 Variation of the maximum potential PSII efficiency (Fv/Fm) in needles by population mean (BAYU–Bayubas-, CUEL–Cuéllar-, LEIR–Leiría-, MIMI–Mimizán-, SERG–Sergurde-, and TAMR–Tamrabta-) with decreasing controlled temperature; (A) autumn 2010, (B) spring 2011.
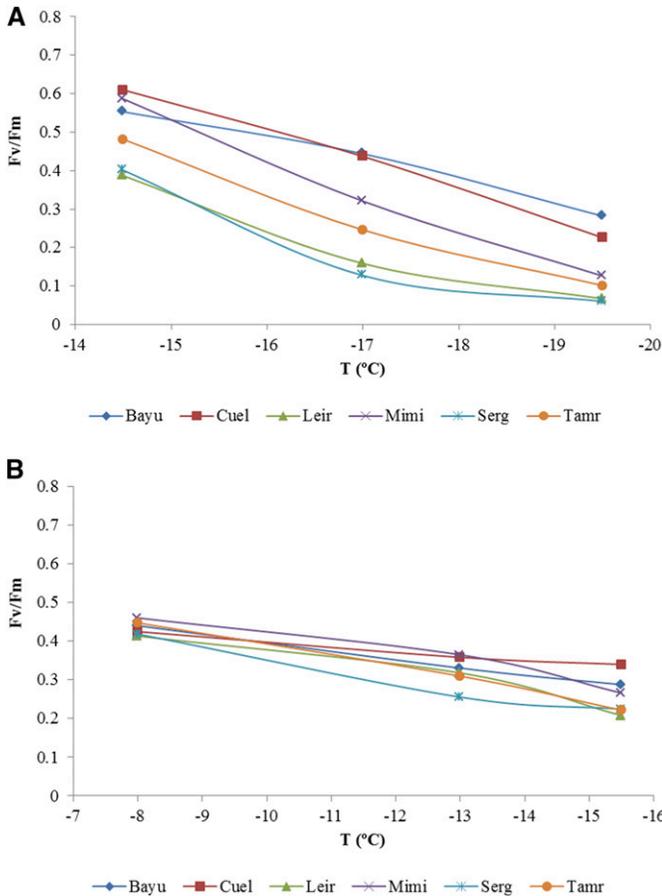
APPENDIX 2 Spearman correlations for freezing damage traits, life-history traits, and population geoclimatic origin traits; abbreviations are as defined in Table 2.

Variable	Tmin_wt	Tmin_sp	Tmin_sm	Tmin_at	MWMT	MCMT	EMT	DD0	NFFD	PPT_wt	PPT_sp	PPT_sm	PPT_at	CMD
FN$_A$	0.60	0.60	**0.89***	0.60	−0.60	0.60	0.60	−0.60	0.60	0.77	0.77	−0.09	0.43	−0.31
FN$_S$	0.60	0.60	**0.89***	0.60	−0.60	0.60	0.60	−0.60	0.60	0.71	0.71	−0.20	0.49	−0.31
RHGR6	−0.09	−0.09	0.31	−0.09	0.43	−0.09	−0.09	0.09	−0.09	−0.14	−0.14	−0.77	−0.37	0.49
AHGR6	**0.89***	**0.89***	**0.89***	**0.89***	**-0.83***	**0.89***	**0.89***	**-0.89***	**0.89***	0.77	0.77	0.31	0.60	−0.66
ONT2	−0.66	−0.66	−0.66	−0.66	0.71	−0.66	−0.66	0.66	−0.66	−0.77	−0.77	−0.54	**-0.94****	**0.89***
HAd	**0.89***	**0.89***	0.71	**0.89***	−1.00	**0.89***	**0.89***	**-0.89***	**0.89***	0.77	0.77	0.54	0.77	**-0.83***
SP6	**-0.94****	**-0.94****	−0.77	**-0.94****	**0.94****	**-0.94****	**-0.94****	**0.94****	**-0.94****	−0.71	−0.71	−0.49	−0.66	0.77
FRB5	−0.37	−0.37	−0.77	−0.37	0.14	−0.37	−0.37	0.37	−0.37	−0.43	−0.43	0.43	−0.26	0.09
FTSFR	0.77	0.77	**0.94****	0.77	−0.77	0.77	0.77	−0.77	0.77	**0.83***	**0.83***	0.09	0.71	−0.60
MRB6	0.77	0.77	0.60	0.77	**-0.94****	0.77	0.77	−0.77	0.77	**0.83***	**0.83***	0.77	**0.89***	**-0.94****
MTSFR	−0.20	−0.20	0.20	−0.20	0.49	−0.20	−0.20	0.20	−0.20	−0.14	−0.14	−0.66	−0.20	0.37

Note: TMIN_wt (winter mean minimum temperature (°C)), TMIN_sp (spring mean minimum temperature) (°C), TMIN_sm (summer mean minimum temperature (°C)), TMIN_at (autumn mean minimum temperature (°C)), MWMT (mean warmest month temperature (°C),), MCMT (mean coldest month temperature (°C), EMT (extreme minimum temperature over 30 yr), DD0 (degree-days below 0°C, chilling degree-days), NFFD (number of frost-free days), PPT_wt (winter precipitation (mm)), PPT_sp (spring precipitation (mm)), PPT_sm (summer precipitation (mm)), PPT_at (autumn precipitation (mm)), CMD (Hargreaves climatic moisture deficit).