

Heritable variation in the survival of seedlings from Patagonian cypress marginal xeric populations coping with drought and extreme cold

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Abstract The rear edges of tree species have begun to be perceived as highly valuable for genetic resources conservation and management. In view of expected climatic changes, the responses of trees at their xeric limits may largely be determined by their capacity to cope with augmented environmental variance. We assess the heritability of early survival of Patagonian cypress in two common-garden field tests with contrasting summer water deficits, comprising 140 and 163 open-pollinated families from 10 marginal xeric populations. The first experiment underwent less rigorous conditions than the average mesic, Mediterranean climatic conditions, which were sufficient to reveal additive genetic effects of summer drought on seedling survival. The second trial suffered strong summer water-deficit stress and a winter extreme cold event. In this harsher environment, the heritabilities of survival under summer water-deficit stress were high in all the populations ($h^2=0.84$ on average), while the heritabilities of seasonal, extreme cold survival were moderate or even nil ($h^2=0.28$ on average). We did not find evidence of genetic differentiation among populations in their capabilities to survive droughts and cold extremes. Our results indicate that even when climatic changes were strong enough to cause the extinction of the most threatened populations, heritable variation for traits underlying drought and cold tolerances may allow the marginal xeric edge of

cypress to persist under augmented environmental variance, without losing overall genetic diversity.

Keywords Mediterranean climate · Southern conifers · Fitness components

Introduction

Trees have evolved many characteristics, such as large within-population genetic variation, which make them highly qualified to withstand gradual, directional climatic changes (reviewed in Hamrick 2004). However, global climate change implies augmented environmental variance, i.e., augmented amplitude and/or frequency of climate extreme events (Mitchell et al. 2006), whose impacts are expected to be stronger at the receding, xeric edges of the distributional ranges of many species. The receding edges of trees could be largely important for future adaptation, since they represent long-term reserves of genetic diversity (Hampe and Petit 2005; Mátyás et al. 2009). Whether these rear-edge populations can persist under augmented environmental variance depends strongly on the extent at which genes control the expression of tolerance-related fitness traits.

Seedling survival is a very important fitness component, which represents the integrative outcome of multiple traits exposed to the environment during this highly sensitive-to-selection stage. From an organismic approach, an extreme event is an episode in which the acclimation capacity is exceeded, thus leading to persistent effects on performance and eventually to death. Extreme events can therefore lead to marked changes in fitness and thus to strong selection (Gutschick and Bassiri Rad 2003).

For many temperate regions, trends of warming through the moderation of daily and annual minimum temperatures

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imply that heat waves and droughts are likely to increment their frequency and severity, while the contrary is expected for cold extremes (Alexander et al. 2006; Nicholls 2008). In spite of the expected reductions in the incidence of cold extremes, the maximum frost tolerance of plants may result diminished as a consequence of mean temperature increases (Bannister 2007). Such trends apply for the Mediterranean zone of northwest Argentine Patagonia (Rusticucci and Barrucand 2004; Castañeda and González 2008). Therefore, tree populations inhabiting the xeric limits of the sub-Antarctic forest could be threatened.

Patagonian cypress (*Austrocedrus chilensis* (D. Don) Pic. Ser. et Bizzarri) (Cupressaceae) is a good model tree species to study the possible outcomes of receding populations. At the east side of the Andes, it grows across an ample, steep precipitation gradient in a Mediterranean climatic zone. The physiognomy of cypress forests spans from marginal-humid mixed stands inside the Andes, with about 3,000 mm in mean annual precipitation (MAP), to small, isolated pure patches immersed in the Patagonian steppe with even less than 400 mm in MAP (Gallo et al. 2004). The marginal xeric populations as a whole were denominated the extreme-marginal forest type (Pastorino and Gallo 2002; Pastorino et al. 2004). The distribution of the selectively neutral genetic variation of the species suggests a relic character of most xeric marginal populations, in relation to the recent glacial history of the region. These populations most probably represent the rear edge of the last post-glacial recolonization process or may even be stable remnants of an ancient distribution range (Pastorino and Gallo 2009; Arana et al. 2010), which have determined the maintenance of the species' genetic diversity during the Quaternary climatic oscillations. As a result of surviving under extremely adverse ecological conditions, the extreme-marginal cypress populations might harbor valuable adaptations for the long-term persistence of the species; therefore, their conservation is of major evolutionary importance.

In Mediterranean regions, summer drought is the most important selective force that trees must face (Larcher 2000). At the xeric limits of cypress, seedling establishment is related to decadal-scale climatic fluctuations, characterized by humid, cool summers; instead, mortality is driven by annual-scale summer droughts (Villalba and Veblen 1997). During droughts, desiccation avoidance mediated by stomata regulation in response to atmospheric water deficit (Gyenge et al. 2007) can lead to seedling death, presumably due to carbon starvation, as in most isohydric plants (McDowell et al. 2008). While summer water deficit can be regarded as the most frequent, strongest selective pressure, there is no evidence of massive mortality due to extreme cold events (Villalba and Veblen 1997), which might represent a low-frequency selection force.

The degree of genetic differentiation of fitness-related traits depends on the ecological range of sampling. Most

research assesses genetic differentiation among tree populations distributed across wide ecological ranges, thus being significant degrees of differentiation commonly reported (reviewed in Savolainen et al. 2007). Our work is instead restricted to the arid margins of cypress, along its latitudinal range in Argentina. Although cypress is largely understudied, previous research suggests that its marginal populations might not genetically differentiate for traits underlying drought tolerance. For the cessation and duration of seedling annual growth (traits that can be linked to drought tolerance via the timing of stomata closure), genetic differentiation was found not to be significant. Instead, for growth initiation, which is associated to cold adaptation in altitudinal gradients (Howe et al. 2003), differentiation proved to be much higher than expected by drift, thus providing evidence of local adaptation (Aparicio et al. 2010).

As a fitness trait, seedling survival can be expected to have low heritability. This may be due to strong selection and/or large functional architecture of tolerance traits, reducing the additive genetic relative to the residual variance (Mousseau and Roff 1987; Merilä and Sheldon 1999). In trees, there is clear evidence of strong natural selection on cold tolerance; nonetheless, high genetic variation generally persists within populations (reviewed in Howe et al. 2003). Instead, evidence of selection acting on heritable variation in traits increasing drought tolerance is lacking (Arcaux and Wolters 2006). Given the complexity of tolerance-related fitness traits and the scarce knowledge of the genetics of marginal forest systems, it is not straightforward to hypothesize on local adaptation and populations' outcomes under stressful conditions.

Our objectives are to determine the degree of among-population genetic differentiation and the heritabilities of seedling tolerance to summer water deficit and winter extreme cold, across the xeric marginal populations of Patagonian cypress. We assess the variation of tolerances (underlying, continuous traits) through seedling survival (observed, binary traits). Our hypotheses are that (1) mechanisms preventing local adaptation for drought tolerance prevail during seedling recruitment; we expect that the variation of seedling survival under summer water deficit is not explained by the effect of population. Instead, (2) marked differences in altitude among populations drive divergent selection for winter frost tolerance; hence, we predict significant among-population variance of seedling survival after a winter extreme cold event. At the population scale, our hypotheses are that (3) strong, frequent directional selection on drought tolerance depletes the genetic relative to the residual variance; thus, we expect low heritabilities of seedling survival under summer water deficit. Instead, (4) infrequent selective events driven by seasonal cold extremes allow the capture and persistence of genetic variants involved in cold tolerance; hence, we expect heritable seedling survival after an extreme cold event.

Materials and methods

Plant material, study sites, and experimental design

We planted two common-garden provenance and progeny (open-pollinated families) field trials. In order to obtain moderate to severe summer water-deficit stresses, we selected two mesic locations (MAP < 900 mm) with xeric soil moisture regimes. As the establishment of cypress depends on plant facilitation (Kitzberger et al. 2000; Letourneau et al. 2004), we installed the trials in the shade of *Pinus ponderosa* Douglas ex. Laws. plantations, beneath which weed competition was negligible.

We used seedlings from ten extreme-marginal populations of cypress, comprising the latitudinal distribution of the species in Argentina (Table 1). We cultivated the seedlings under greenhouse conditions, using a ferti-irrigation regime and inert substrate in 265 cm³ (HIKO™ model HV265) seedling pots. In experiment 1, 2-year-old seedlings were transplanted directly from the greenhouse to the field; in experiment 2, we hardened the seedlings during the third year, by cultivating them outside the greenhouse with a reduced ferti-irrigation regime and moderate shade. At planting, the aerial portions of the seedlings were 22.0 ± 6.9 and 26.0 ± 6.2 cm high, respectively.

We planted experiment 1 in September 2005 near San Martín de los Andes city (40°08'31" S; 71°09'51" W; 1,000 m above sea level, ASL). We used a 16-year-old pine stand located on a deep, well-drained mid-hill of E-NE aspect and 19% slope. The 0–25-cm soil layer is sandy-loam textured, with a moderate capacity of water retention and strong summer water deficit. The pine stand was 1,250 trees per hectare in density (4 × 2 m) and 23 m² ha⁻¹ in basal area. We used a randomized complete block design with three blocks and plots of six seedlings per family (140 families; 2,520 seedlings) (Table 1), planted at 2 × 2 m between the pine rows. Experiment 2 was installed in August 2006, near San Carlos de Bariloche city (41°10'03" S, 71°15'11" W, 900 m ASL), beneath a 25-year-old pine stand, located at the foot of a SW-exposed low hill, of 18% average slope. The topsoil is sandy-loam textured, somewhat excessively drained, with moderate to strong summer water deficit. The pine stand was 500 trees per hectare in density and 26.5 m² ha⁻¹ in basal area. We used a completely randomized single-tree-plot design, comprising 15 seedlings per family (161 families; 2,415 seedlings) (Table 1).

Climate and weather at the study sites

In experiment 1, we assessed the local climate with 1912–1982 monthly precipitation series from Estancia Collún-Co station (40°04' S, 71°10' W, 875 m ASL), located 8 km northwards from the trial. Mean maximum and minimum

temperatures were assessed with the 1981–2006 series from Chapelco airport (40°05' S, 71°09' W, 790 m ASL), located 7 km northwards. For describing the weather during the experiment, we used 2005–2006 daily temperature and rainfall data of the AIC (Interjurisdictional Basin Authority of the Limay, Neuquén, and Negro Rivers); we do not account with relative air humidity (HR percent) data. In experiment 2, we used the 1973–2007 temperature series from Bariloche airport (41°09' S, 71°09' W, 841 m ASL), located 7 km eastwards, and the 1981–2007 precipitation and HR percent series from INTA station (41°07' S, 71°15' W, 785 m ASL), located 4 km northwards, at the same longitude (same isohyet) of the trial.

Following Villalba and Veblen (1997), who found a strong association between peaks of cypress mortality and above the average summer (October–March) aridity at the species' xeric margin; we computed the periodical and historical monthly aridity indexes (De Martonne 1926) with the formula $AI_M = 1.2P / (T + 10)$, where P (millimeters) and T (degrees Celsius) are monthly precipitation and mean temperature¹. In experiment 2, hourly vapor pressure deficit (VPD) was computed with bulk air T and RH percent (Ewers and Oren 2000) and corrected by assuming VPDs 10% lower under the canopy than in the open (climatic stations) (Porté et al. 2004).

At the environs of experiment 1, MAP is 887 ± 190 mm, 80.4% of which cumulates in the cold season (April–September). Mean annual temperature (MAT) is 9.95 ± 1.5°C, while monthly mean maximum and minimum are 24.6 ± 1.5°C (February) and 1.10 ± 0.5°C (July). During the warm season of our experiment, 95% over the average rainfall coupled with low mean temperatures (mainly due to lower than the average minimum) determined below the average ($AI_M = 1.45 ± 0.50$ mm °C⁻¹) seasonal aridity ($AI_M = 2.95 ± 2.06$ mm °C⁻¹). Winter was also colder than the historical; nevertheless, the absolute minimum temperature was -10.5°C.

At the environs of experiment 2, MAP is 831 ± 196 mm (78.7% cumulated in the cold season). MAT is 8.6 ± 0.5°C, and monthly mean maximum and minimum are 15.0 ± 1°C (January) and 2.3 ± 0.5°C (July). During our experiment, the warm season was slightly more arid ($AI_M = 1.41$ mm °C⁻¹) than the average ($AI_M = 1.65$ mm °C⁻¹). Nevertheless, mid-summer–early autumn was much more arid (Fig. 1), with a marked aridity since January (and prolonged till May), due mainly to rainfall (47.4% the normal) deficit. The period January–April was characterized by the occurrence of only one rainfall event potentially capable of recharging the topsoil (see Licata et al. 2010) in mid-February; high

¹ As we do not account with temperature series for all the sampled populations, we do not report their aridity indexes in “Materials and methods,” but see the “Discussion” section.

Table 1 Material used in two *A. chilensis* genetic field trials

Population	Latitude		Longitude		Altitude (m ASL)	MAP (mm)	Experiment 1		Experiment 2			
	S	W					<i>N</i>	<i>n</i>	<i>N</i>	<i>n</i>	<i>N</i> _{PS}	<i>n</i> _{PS}
Cañada Molina	37°08'	70°36'			1,450	604	15	270	11	165	9	113
Cañada Rahueco	37°10'	70°36'			1,500	604	10	180	12	180	9	106
Catán lil	39°21'	70°39'			1,100	246	14	252	14	210	13	166
Chacabuco	40°39'	71°01'			900	308	18	324	20	300	18	206
Chacay	40°51'	70°59'			1,250	488	13	234	16	240	14	173
La Fragua	41°05'	70°57'			1,000	490	10	180	12	180	12	149
Pilcañeu Norte	41°13'	70°42'			1,100	330	16	288	20	300	19	217
Pilcañeu Sur	41°14'	70°42'			1,100	330	12	216	18	270	18	200
El Maitén	42°02'	71°12'			750	490	15	270	19	285	19	217
Leleque	42°20'	71°09'			850	353	17	306	19	285	17	198

MAP mean annual precipitation; *N* and *n* number of open-pollinated families (OPF) and seedlings per population at planting; *N*_{PS} and *n*_{PS} number of families and seedlings after summer, used for *S*_{PW} analysis; 75% of the OPFs are common to both experiments

atmospheric demands ($VPD=2.47\pm 0.65$) were sustained till the beginning of April (Fig. 2), when a maximum temperature extreme was recorded. Winter was colder than normal, and a 4-day cold extreme (absolute minimum temperature = $-18.8^{\circ}C$) was registered in July, which was immediately preceded by a period of mild temperatures (Fig. 3).

Variables measured

We measured seedling survival 1 year after planting (*S*₁). In experiment 2, we also measured post-summer (*S*_{PS}) and post-winter (*S*_{PW}) survival, on 16 May and 16 October 2007, respectively. We defined a binary scale (1, alive; 0, dead) according to visual symptoms that indicate seedling mortality in cypress, i.e., yellowish to brown-reddish foliage that falls easily when touched. In experiment 2, we consider all the seedlings planted in August 2006 for *S*₁ and *S*_{PS} analyses. For *S*_{PW} analysis, we used only those families with more than seven post-summer living seedlings, thus accounting with 1,745 seedlings from 148 families (12 individuals per family on average) (Table 1).

We analyzed *S*_{PW} under the hypothesis that it was not dependent on seedling performance during the growing season. To test this hypothesis, we used the post-planting relative height growth (RG) as a measure of summer individual performance. We measured height increment on 16 May 2007, in a random sample of 258 seedlings. Although primary growth in cypress is potentially continuous, inter-annual limits can be recognized by morphological marks (Grosfeld and Barthélémy 2004). We analyzed the association between *S*_{PW} and the square root transformation (Zar 1999) of RG with logistic regression.

In order to determine whether tolerance to summer dehydration is associated to winter freezing tolerance, we performed Spearman's rank correlations for the by-family ranks of *S*_{PS} and *S*_{PW}. We performed all the analyses in R 2.8.1 (R Development Core Team 2008).

Analyses of variance and heritability estimates

The estimation of the heritability of survival entails the problems of survival being a non-normal (binary) variable

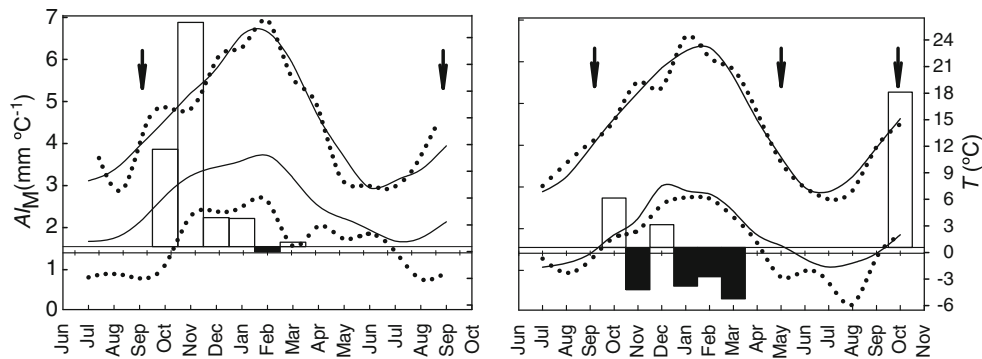


Fig. 1 Climatic diagrams of experiments 1 (left) and 2 (right). The bars are the De Martonne's aridity indexes (*AI*_M) of the warm, dry season, scaled as deviations from the regional average as in Villalba and Veblen (1997); black indicates moisture deficit. The lines are the

historical (solid) and the periodical (dashed) mean maximum and minimum temperatures. The arrows show the dates of planting and of survival measurements

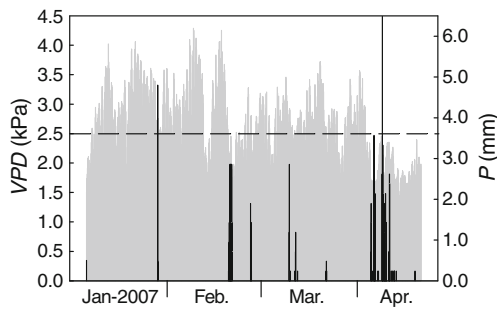


Fig. 2 Summer atmospheric demand during experiment 2. The dashed line shows a threshold in diurnal, hourly VPD (gray bars), at which *A. chilensis* seedlings close their stomata (Gyenge et al. 2007). Precipitation (*P*) pulses are shown in black

and of random effects being involved. Common shortcuts are data transformations (e.g., the arcsine–square root of proportions) and/or the use of ordinary linear models. The inadequacy and failures of such shortcuts (Jaeger 2008) can be overcome with the generalized linear mixed models (GLMMs) (reviewed in Bolker et al. 2008). GLMMs apply to members of the exponential family distributions and are based on finding a relationship (link function) between the expected value of a random variable and a linear combination (linear predictor) of fixed and random explanatory variables. Here, we used the mixed logistic model to estimate the variance components for liability to survival. This implies underlying continuous distributions of phenotypes with polygenic base. For example, the ability of plants to cold acclimate (liability variable) and survive frosts may involve up to 25% of the transcriptome (Krebs et al. 2002). Working on the liability scale allows the direct comparison of estimates across populations and experiments with different incidences of the binary trait (Dempster and Lerner 1950; Chambers et al. 1996). Also, the logistic model captures the fact that changes in the probability of the response around $\mu=0.5$ matter less than equal changes close

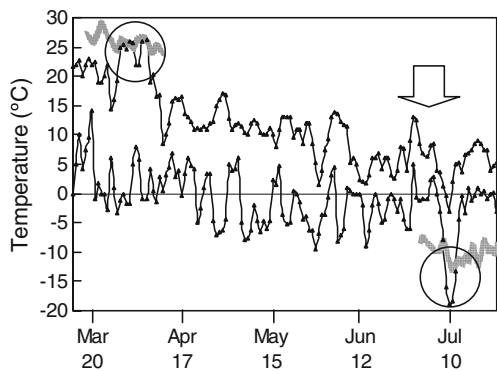


Fig. 3 Temperature extremes in experiment 2 (circled); the thin lines are the daily absolute maximum and minimum temperatures; the thick lines are the 95th and 5th percentiles; the arrow highlights a period of daily minimum absolute temperatures above the 50th percentile (mean=0.78°C) just before the cold extreme

to $\mu=0$ or $\mu=1$ (Jaeger 2008). This overcomes the possible biases in heritability estimates for incidences outside the 0.25–0.75 range, attributable to other statistical models (see Lopes et al. 2000). The mixed logistic model can be specified as:

$$\eta_i = \text{logit}(\mu_i) = \mathbf{x}'\boldsymbol{\beta} + \mathbf{z}'\mathbf{b}; \mathbf{b} \sim N(0, \sigma^2 \boldsymbol{\Sigma}),$$

where the linear predictor η_i is linked to the expected value μ_i of the binary outcome Y_i (Bernoulli distributed) via the logit function. The design matrices \mathbf{x}' and \mathbf{z}' contain the values of the explanatory variables for the fixed (the predictors) and random effects (e.g., family and population); $\boldsymbol{\beta}$ is a vector of unknown parameters; the vector of random coefficients \mathbf{b} is characterized by a multivariate normal distribution centered on zero, with the variance–covariance matrix $\boldsymbol{\Sigma}$ to be estimated (Jaeger 2008). The conditional expectation, i.e., the probability μ_i that the binary response $Y_i=1$ given the random effects, is:

$$E(Y_i|\mathbf{x}', \mathbf{z}') = \frac{\exp(\eta_i)}{1 + \exp(\eta_i)}.$$

A model for the unobserved underlying variable (e.g., drought tolerance) can be specified as $Z_{ij} = \eta_i + e_{ij}$, where Z_{ij} is the liability to survival of individual j , and e_{ij} is the random residual component associated with the observation from seedling j , with logistic cumulative density function and thus mean=0 and variance= $\pi^2/3$ (Rabe-Hesketh and Skrondal 2008).

In experiment 1, the linear predictor was:

$$\eta_{ijk} = \alpha_0 + p_i + f_{j(i)} + b_k + d_{ijk}, \tag{1}$$

where η_{ijk} is the logit of the expected S_1 variable, α_0 is a fixed intercept, p_i is the random effect of population ($i=1, \dots, 10$), $f_{j(i)}$ is the random effect of family (j varying between 1, ..., 10 and 1, ..., 18) nested within population, b_k is the random effect of block ($k=1, 2, 3$), and d_{ijk} is the plot error.

In experiment 2, the linear predictor was:

$$\eta_{ij} = \alpha_0 + p_i + f_{j(i)}, \tag{2}$$

where η_{ij} is the logit of the expected S_1 , S_{PS} , or S_{PW} , α_0 is an intercept, p_i is the random effect of population ($i=1, \dots, 10$), and $f_{j(i)}$ is the random effect of family (j varying between 1, ..., 9 and 1, ..., 20) nested within population.

We performed the analyses in R 2.8.1 with the *lmer* function in *lme4* package (Bates et al. 2008), using full maximum likelihood estimation of the parameters of the models with the Laplace approximation to the marginal likelihood. For each population, we estimated the additive genetic variance and the narrow sense heritability (h^2) of

liability to survival (hereafter heritability) removing the effect of population from the linear predictors 1 and 2, thus being heritability:

$$h^2 = V_A/V_P = 3 \cdot V_{fam}/(V_{fam} + V_b + V_d + V_e) \text{ and } h^2 = V_A/V_P = 3 \cdot V_{fam}/(V_{fam} + V_e)$$

in experiments 1 and 2, respectively, where V_A and V_P are the additive genetic and the phenotypic variances. We estimated V_A as three times the family variance component (V_{fam}), as suggested by Squillace (1974) for open-pollinated families that are likely a mix of mid- and full-siblings (Pastorino and Gallo 2006), V_b and V_d are block and plot variances, and $V_e = \pi^2/3$ is the implicit residual variance. The standard errors of h^2 are:

$$SE_{h^2} = 3 \cdot \sqrt{\frac{2 \cdot (1 - \frac{1}{3} \cdot h^2)^2 \cdot [1 + (n - 1) \cdot \frac{1}{3} \cdot h^2]^2}{n \cdot (n - 1) \cdot (N - 1)}}$$

where N and n are the number of families and seedlings per family (Falconer and Mackay 1996).

We tested the significance of the additive genetic variances (V_{AS}) via sampled randomization tests (Sokal and Rohlf 1995). Within each population, the 0–1 responses were reassigned 1,000 times without replacement (i.e., keeping the incidence of survival constant), in order to obtain random distributions of V_A . Observed V_{AS} in the upper 5% tails of the random distributions were considered significant. We carried out the resamplings with a routine written ad hoc in R language. The same method was used to test the significance of the random sources of variation in 1 and 2.

In this work, we do not use the coefficient of additive genetic variation (CV_A), useful for analyzing the potential of populations to respond to natural selection, since that coefficient does not apply to transformed scales (Houle 1992).

Results

In both trials, the among-population variance components were zero (Table 2); therefore, the calculation of genetic differentiation was meaningless.

In experiment 1, S_1 ranged between 85.7% and 92.2% across populations (overall $S_1=89.9\%$). The family variance component in model 1 was low (4.70% of the total variance) but significant (Table 2). Within populations, V_{AS} was significant only for one northern (Cañada Molina) and two southern (Pilcañeu Norte and P. Sur) populations. Given the absence of specific adverse events and the low seedling mortality, we do not go further with details on the environmental conditions.

Table 2 Partitioning of the variance of liability to seedling survival (in percentages) in two *A. chilensis* field trials; S_1 , S_{PS} , and S_{PW} are 1-year, post-summer, and post-winter survival

Sources of variation	Experiment 1		Experiment 2	
	S_1	S_1	S_{PS}	S_{PW}
Populations	0	0	0	0
Families within populations	4.7	24.5	25.17	12.58
Blocks	1.39	–	–	–
Within-plot	18.27	–	–	–
Residual	75.64	75.5	74.28	87.42

In experiment 2, S_1 ranged between 44.1% and 63.8% across populations (overall $S_1=54.4\%$). Overall seasonal survival rates were $S_{PS}=76.5\%$ and $S_{PW}=72.1\%$. The variance components analyses for model [2] showed relatively high, significant family variance components for the three survival traits (Table 2). Heritability of S_1 was high for most populations, with the exception of the southern Leleque, for which it was moderate ($h^2=0.23 \pm 0.13$). A similar pattern was found for S_{PS} , while for S_{PW} , the heritabilities were lower in general. Chacay, Pilcañeu Sur, and Leleque populations had no significant heritabilities of S_{PW} (Table 3). The heritabilities of S_{PS} were higher in the northern ($h^2=0.84$ on average) than in the southern ($h^2=0.28$ on average) populations, while for the heritabilities of S_{PW} , the pattern was population specific (Fig. 4). S_{PW} was not associated ($\chi^2_{0.05, 256}=303.52, p=0.78$) to relative growth ($RG=43.8 \pm 25.9\%$), proving that it was not dependent on seedling performance during the growing season.

The overall by-family ranks of S_{PS} and S_{PW} were correlated ($r_{s0.05, 161}=0.58, p<0.001$). Nevertheless, the correlation was variable among populations, ranging those significant between $r_{s0.05, 15}=0.45$ (Chacabuco) and $r_{s0.05, 11}=0.86$ (Catán lil); it was not significant for the two northernmost populations, Cañada Molina and Cañada Rahuco, and for the southern Pilcañeu Norte and Leleque.

Discussion

Environmental causes of seedling mortality/survival

Our experiments showed unexpected survival rates due to opposite-sign summer rainfall anomalies. In experiment 1, plants had not been hardened prior to planting; thus, we expected moderate to low survival. Although we could not assess the seasonal evolution of soil moisture and VPD, which are key to seedling responses under drought stress (Gyenge et al. 2007), it is likely that abnormally high summer rainfall coupled with low mean temperatures enhanced the chances of seedling acclimation. Hence, the

Table 3 One-year (S_1), post-summer (S_{PS}), and post-winter (S_{PW}) seedling survival (percent), additive genetic variances (V_A), and heritabilities of liability to survival (h^2) of ten extreme-marginal populations of *A. chilensis* in two field experiments

Population	Experiment 1				Experiment 2				S_{PW}	$h^2 \pm SE$	V_A	$h^2 \pm SE$
	S_1	V_A	$h^2 \pm SE$	S_1	V_A	$h^2 \pm SE$	S_{PS}	V_A				
Cañada Molina	88.9 (72.2–100)	1.04	0.28±0.15	56.2 (7.1–84.6)	2.85	0.67±0.30	81.7 (42.9–100)	3.41	0.77±0.32	71.4 (38.5–100)	1.42	0.38±0.26
Cañada Rahueco	91.4 (77.8–100)	0	0	44.1 (0–86.7)	6.78	1.22±0.34	68.8 (9.1–100)	5.71	1.10±0.33	64.0 (20.0–92.8)	2.12	0.53±0.32
Catán Ili	92.1 (75.0–100)	0	0	61.2 (7.7–100)	7.42	1.29±0.32	81.4 (23.1–100)	6.02	1.13±0.32	76.5 (22.2–100)	3.76	0.83±0.31
Chacabuco	92.2 (77.8–100)	0	0	57.9 (13.3–93.3)	2.99	0.70±0.22	74.4 (33.3–100)	5.11	1.02±0.26	78.7 (45.5–93.3)	0.96	0.26±0.17
Chacay	89.4 (72.2–100)	0	0	52.1 (0–94.1)	3.30	0.75±0.26	76.1 (26.7–100)	4.32	0.91±0.28	69.9 (45.4–94.1)	0.49 ns	0
La Fragua	88.1 (66.7–100)	0	0	63.8 (20.0–93.3)	2.85	0.67±0.28	84.2 (57.1–100)	1.75	0.45±0.23	75.8 (27.3–100)	1.85	0.47±0.26
Pilcañeu Norte	91.1 (55.7–100)	3.55	0.71±0.24	54.0 (13.3–100)	2.73	0.65±0.21	75.4 (40.0–100)	2.40	0.59±0.20	72.6 (25.0–100)	2.40	0.58±0.23
Pilcañeu Sur	85.7 (41.7–100)	3.06	0.68±0.27	54.7 (13.3–93.3)	1.50	0.40±0.17	74.4 (20.0–100)	2.63	0.63±0.21	72.5 (25.0–93.3)	0.08 ns	0
El Maitén	90.6 (58.3–100)	0	0	54.1 (15.4–100)	2.73	0.65±0.21	76.5 (20.0–100)	2.55	0.62±0.21	70.0 (28.6–100)	1.51	0.40±0.19
Leleque	88.4 (66.7–100)	0	0	46.2 (0–73.3)	0.84	0.23±0.13	75.6 (26.7–100)	1.16	0.32±0.15	62.9 (25.0–91.6)	<0.01 ns	0

In brackets are the minimum and maximum per-family survival rates

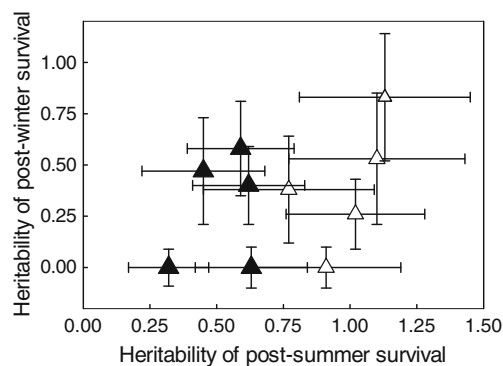


Fig. 4 Ten extreme-marginal populations of *A. chilensis* grouped according to the heritabilities of post-summer and post-winter seedling survival in experiment 2; open and solid triangles are the northern and the southern populations, respectively. The bars are the standard errors of h^2

scarce seedling mortality could be attributed to suboptimal hardening rather than to any specific adverse climatic condition. The significant heritabilities found for the Cañada Molina, Pilcañeu Norte, and P. Sur populations suggest that even under less rigorous than the average mesic summer conditions, our plant material may reveal non-random variation for tolerance to water deficit.

In experiment 2, we expected a high degree of acclimation to summer water deficit, since the seedlings were hardened for 1 year prior to planting. Indeed, our seedlings quickly resumed growth, as evidenced by large height increments (also observed in the dead plants, although not measured). However, strong early–mid-summer rainfall deficit coupled with high atmospheric demands led to moderate, non-random survival rates in all the populations. Although seasonal aridity at the experimental locations does not indicate by itself that plants suffered drought stress, the aridity indexes for the period November–February ($AI_M = 1.18 \pm 0.4 \text{ mm } ^\circ\text{C}^{-1}$) agree with those shown in Villalba and Veblen (1997) for peaks of massive mortality in marginal cypress populations. The period January–February was particularly stressful, since VPD exceeded 2.5 kPa over 60% of the diurnal time..

A second mortality wave occurred after a winter cold extreme, when plants were probably not fully hardened. Most temperate trees, including several Cupressaceae species that display potentially continuous growth (Hawkins et al. 2001), have the ability to increase their degree of cold tolerance in response to low temperatures (Krebs et al. 2002; Howe et al. 2003; Bannister 2007). Cold acclimation can be rapidly lost when plants return to non-acclimating temperatures (Bigras et al. 2001). Although the thresholds for acclimation and deacclimation of cypress have not yet been determined, rapid dehardening may have contributed to make seedlings prone to freezing. Even assuming that the threshold for acclimation was relatively high, the maximum cold tolerance of cypress

would be between -6.7 and -12.1°C (Bannister and Neuner 2001, according to the USDA hardiness zone system), i.e., quite higher than the minimum temperature registered during our experiment (-18.8°C).

Patterns of genetic variation and heritability

According to our hypothesis (1), we expected similar inter-population survival rates under summer water deficit and thus not significant among-population variances. This prediction was confirmed in both experiments, supporting the hypothesis that processes preventing local adaptation for drought tolerance traits prevail during seedling recruitment. Our prediction (2) of significant among-population genetic variance of survival after winter extreme cold was not fulfilled, suggesting that local adaptation for winter frost tolerance is prevented, despite the altitudinal differences between the populations.

Among the processes preventing local adaptation, homogenizing selection due to regional-scale ecological homogeneity could be the most relevant. Unlike most published research (reviewed in Savolainen et al. 2007), our work was restricted to a narrow, xeric strip, along which homogeneous, strong summer aridity can be regarded by far as the key ecological selection driver. Although the mean annual rainfall in the northern Cañada Molina and Rahueco populations is almost twofold that of several other populations, their temperature regime is warmer, thus their summer aridity being ($IA_M=0.52\pm 0.33\text{ mm }^{\circ}\text{C}^{-1}$) similar to that of drier populations such as Pilcañeu Norte and P. Sur ($IA_M=0.70\pm 0.40\text{ mm }^{\circ}\text{C}^{-1}$). Likewise, the predicted altitude-related cold adaptation might turn to be counteracted by warmer winter temperatures at the higher, northern populations, thus homogenizing selection being the regional outcome.

Another process that may lower genetic differentiation is genetic drift. Even if high regional-scale environmental variation was the scenario, drift may confound local adaptation by causing non-adaptive genetic variance (Kawecki and Ebert 2004). In cypress, previous research shows that demostochastic processes may have impacted differentially on within-population neutral genetic diversity, having a greater impact at the eastern limits of the species (Pastorino and Gallo 2009; Arana et al. 2010). Evidence of demostochasticity has been also found at the adaptive level, given contrasting patterns of additive genetic variance of seedling growth traits between neighbor populations (Aparicio et al. 2010). The role of gene flow preventing differentiation may be downplayed, due to physical isolation from the species distributional center, as well as between marginal populations. Indeed, even very close populations such as Pilcañeu Norte and P. Sur were found to maintain genetic isolation due probably to restricted latitudinal pollen flow (Pastorino

and Gallo 2009; Arana et al. 2010). Also, molecular evidence (ibid.) suggests very limited effects of pollen arrival from windward, humid and mesic locations.

The second finding of our work (refuting our hypothesis 3) is that the variation of seedling survival under summer water deficit was moderately to highly heritable. This suggests that the long-term persistence of populations at the rear edge of cypress relies on maintaining high genetic variation for seedling drought tolerance, even under strong, frequent selective drought events. This may reflect fine-scale spatial heterogeneity of natural selection (Linhart and Grant 1996), coupled with grouped mating (Pastorino and Gallo 2006) and probably with short-distance seed dispersal, modeling the genetic structures within the populations. Variation in drainage, moisture regime, aspect, or wind exposure may determine that the extent and/or the timing at which droughts affect seedlings can substantially vary over a fine spatial scale, in relation to the heterogeneous micro-topography of the rocky islands cypress inhabits within the steppe.

Our prediction (4) was partially confirmed. Although lower than those of post-summer survival, the heritabilities of post-winter survival were substantial in most populations. A population-specific pattern (i.e., not structured by latitude or altitude), with several cases of low or zero heritability, suggests ancient, strong selective events for cold-tolerance and/or demostochastic events followed by genetic drift depleting the genetic variation. For the Pilcañeu Sur population, the latter hypothesis can be supported by the contrastingly high heritability observed in its neighbor P. Norte population, and has been previously suggested given similar patterns of neutral variation (Pastorino and Gallo 2009). An alternative hypothesis explaining the low to nil heritabilities of winter survival in some populations could be the superimposing of several mortality factors other than extreme cold, e.g., fire and long-lasting grazing, reducing the genetic and/or augmenting the residual variance, as expected for complex fitness traits (Mousseau and Roff 1987; Houle 1992; Merilä and Sheldon 1999). Nevertheless, this hypothesis seems quite unlikely for our sampled populations (Anchorena and Cingolani 2002).

Our estimates of heritability of seedling survival under extreme cold are consistent with cold-tolerance heritabilities of many conifers assessed during acclimation and/or dehardening (Hawkins et al. 2001; Aitken and Hannerz 2001). The overall correlation between the summer and winter by-family survival ranks and the high heritabilities of 1-year survival suggest links between the physiological mechanisms of cold and drought tolerances. However, the association was variable across populations, suggesting population-specific genetic correlations.

We found a clear north–south structuring of the populations according to the extent of genetic control of survival under water-deficit stress. This structure is consistent with previous results regarding the patterns of genetic variation

of early growth rhythm traits (Aparicio et al. 2010) and agrees also with those of selectively neutral variation of the species (Pastorino et al. 2004; Pastorino and Gallo 2009; Arana et al. 2010). This suggests a disproportional relevance of the northern range of cypress in Argentina for a genetic resources conservation program, targeting both adaptive and neutral variation.

Possible performance under climate changes

Only for Pilcañeu Norte and P. Sur populations we account with precise climatic data (Pilcaniyeu INTA station, 41°07' S, 70°44' W, 920 m ASL). At this extreme location, the monthly aridity of the warm season is $IA_M = 0.70 \pm 0.40 \text{ mm } ^\circ\text{C}^{-1}$, while temperatures below -12°C are frequent in winter. These data show that the xeric margins of cypress are still far harsher than our experimental conditions. Under a scenario of directional increases in aridity accompanied by augmented environmental variance, the ranges for phenotypic plasticity could be rapidly exceeded. Also, rear-edge stands of cypress are restricted to specific habitat islands within a matrix of unsuitable steppe landscape; thus, migration is being impeded. Therefore, in situ adaptive responses may still play a crucial role. Although more studies are needed, including comparisons with mesic and humid populations, we suggest that some population features and modeling processes at the rear edge of Patagonian cypress may differ from the rear-edges Northern Hemisphere model (Hampe and Petit 2005), in which among-population differentiation (due to local adaptation) coupled with reduced within-population variation (due to small size and long-lasting isolation) are dominant processes.

The extreme-marginal cypress populations would be not genetically limited in their capacity for responding to augmented severity of droughts. Although more severe droughts can produce steeper demographic declines, adaptation might be restored effectively due to higher intensities of selection acting on heritable variation. Probably, the effect of droughts on long-term mean fitness will depend on its frequency rather than its severity. Selective extreme events at shorter intervals and/or shorter duration of favorable-for-recruitment periods might reduce the total per-generation supply of beneficial mutations, thus leading to mean fitness decline (Bell and Collins 2008). Regarding extreme cold tolerance, some populations may be unable to respond adaptively if their maximum frost tolerance diminishes as a consequence of augmented mean winter temperature (Bannister 2007) or altered seasonality promoting dehardening in winter. Nevertheless, most populations seem to harbor substantial heritable variation for cold tolerance. In summary, the high contribution of genes to the variation of seedling survival in most populations suggests that marginal cypress can survive climatic changes affecting the environmental variance at the

recruitment stage, without losing overall genetic diversity, even when changes were strong enough to extinguish the most threatened populations.

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