Genetic variation in seedling water-use efficiency of Patagonian Cypress populations from contrasting precipitation regimes assessed through carbon isotope discrimination

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Abstract

Water-use efficiency (WUE) is a physiological parameter that plays a significant role in the evolutionary dynamics of many forest tree species. It can be estimated indirectly through carbon isotope discrimination (Δ). In general, plants of more arid origins have lower values of Δ. In order to study the degree of genetic control of this parameter and the genetic variation in Δ of Patagonian Cypress seedlings, three Argentinean natural populations chosen to represent two contrasting precipitation regimes were sampled in a common garden trial. The dry situation was represented by two neighboring marginal forest patches from the steppe, while the humid condition was represented by a population with 1,200 mm higher mean annual precipitation. Height (H) and Δ were measured in 246 five-year-old seedlings from 41 open-pollinated families. The factor 'family' had a significant effect on both variables; however heritability for Δ was found not to be significant in two out of the three populations. This could be explained by low sample size in one of them and by a real evolutionary effect in the other. An inverse association between H and Δ was verified, which is interpreted as evidence of an adaptation process at the intra-population level. The studied populations were not shown to discriminate carbon isotopes differently; hence evidence of adaptation to current environmental conditions could not be obtained. On the other hand, the arid populations proved to be quite different in terms of genetic variation, which seems to be the consequence of genetic drift and isolation.

Key words: *Austrocedrus chilensis*; differentiation; adaptation; genetic drift.

Resumen

Variación genética de poblaciones naturales de Ciprés de la Cordillera con regímenes de precipitación contrastados, en la eficiencia del uso del agua de plántulas, a través de la discriminación isotópica del carbono

La eficiencia en el uso del agua es un parámetro fisiológico que desempeña un rol significativo en la dinámica evolutiva de muchas especies forestales. Puede estimarse indirectamente a través de la discriminación isotópica del carbono (Δ). En general, las plantas de orígenes más áridos tienen valores de Δ más bajos. Con el propósito de estudiar el grado de control genético de Δ y la variación genética en este parámetro en plántulas de Ciprés de la Cordillera, tres poblaciones naturales elegidas para representar dos regímenes de precipitación contrastados fueron muestreadas en un ensayo de ambiente común. La condición árida estuvo representada por dos fragmentos de bosque esteparios marginales, vecinos entre sí, mientras que la condición húmeda fue representada por una población con una precipitación media anual 1.200 mm superior a la de las áridas. Se midió altura total (H) y Δ en 246 plántulas de 5 años de edad correspondientes a 41 familias de polinización abierta. El factor 'familia' tuvo un efecto significativo en ambas variables; sin embargo, la heredabilidad para Δ no resultó significativa en dos de las tres poblaciones. En una de ellas esto podría explicarse por el restringido tamaño muestreal, mientras que en la otra por un verdadero efecto evolutivo. Asimismo se verificó una asociación inversa entre H y Δ, la cual es interpretada como evidencia de un proceso de adaptación a nivel intra-poblacional. No se observó que las poblaciones estudiadas discriminaran los isótopos del carbono de un modo diferencial, y por lo tanto no se obtuvieron evidencias de adaptación a las condiciones ambientales actuales. Por otro lado, las poblaciones áridas probaron ser muy diferentes entre sí en términos de variación genética, lo que parece ser la consecuencia de deriva y aislamiento genéticos.

Palabras clave: *Austrocedrus chilensis*; diferenciación; adaptación; deriva genética.

Introduction

Some knowledge of the genetic variation of natural populations in ecophysiological parameters may be the key for correct interpreting the evolutionary pathways and strategies for adaptation to extreme environmental conditions of species distributed in strongly contrasting environments. Furthermore, ecophysiological studies in seedlings of forest tree species may help us to understand and predict their performance during installation in productive afforestation. Such studies become particularly relevant when dealing with species involved in a domestication process, particularly those which are potentially productive but lacking the technological development necessary for confidence in a productive business.

The Patagonian Cypress (*Austrocedrus chilensis* (D.Don) Pic. Ser. et Bizzarri, Cupressaceae) is the target of a domestication program currently being carried out in NW Argentine Patagonia by the Instituto Nacional de Tecnología Agropecuaria (INTA) in San Carlos de Bariloche. Promising growths have been reported for the species in natural forest (8.3 $m^3 ha^{-1} y^{-1}$ without intervention, Goya *et al*., 1995; 16 $m^3 ha^{-1} y^{-1}$ with thinning, Constantino, 1949, 1958), and also in exploratory afforestation (12.2 $m^3 ha^{-1} y^{-1}$ with no genetic improvement or intermediate silviculture intervention, Loguercio *et al*., 2005). In addition, its wood, still obtained only from natural forests, has high technological and aesthetic qualities, which are recognized in the market since its price is among the highest in Argentina. There is also a profound ecological interest in this species, which is classified as vulnerable by the IUCN (Conifer Specialist Group, 1998). This is evidenced by its use in some restoration experiences (e.g. Gallo, 1996; Oudkerk *et al*., 2003; Rovere, 2008), a purpose that also makes important the optimization of its afforestation technology.

Some important contributions to the understanding of Patagonian Cypress physiology are already available. Gyenge *et al*. (2005, 2007, 2008) have shown that it presents several features typical of water-spending species, such as high vulnerability to xylem cavitation and high cell water content at turgor loss point. However, they also observed that the species compensates for those vulnerabilities by means of rapid stomatal response, since even when the soil water content is not limiting, just a moderate evaporative demand (vapor pressure deficit ≈ 2.5 kPa) is enough for the species to close its stomata, thus preventing water potential to drop to critical limits. This is not a costless strategy, given that early stomatal closure in the morning leads to a low fixation rate in the day. It also pushes the species to dependence on "nurse plants" at the installation stage. Only in the shadow of other plants are Patagonian Cypress seedlings able to open their stomata, allowing growth and the activation of water flow through the xylem, which seems to be crucial to the refrigeration of their delicate stems, avoiding cell damage due to over-heating at the collar level.

Long-term water-use efficiency (WUE) is a physiologic parameter likely important for the domestication of a wild species. This parameter is defined as the ratio of total plant dry matter produced to total water used over the same period, and can be indirectly estimated by measuring the stable carbon isotope composition of the plant, $\delta^{13}C$ (Farquhar and Richards, 1984). Plants with C3 metabolism discriminate against the ^{13}C isotope because it is heavier than ^{12}C, which is then preferred by the carboxylation enzyme (Rubisco) and also has faster diffusion in the air (Farquhar *et al*., 1982). When closing the stomata, the plant impedes the entrance of new air into the leaves and continues fixating carbon from the air of the inter-cellular spaces and sub-stomatal chambers. Consequently, the ^{13}C concentration starts to increase in the inter-cellular air spaces and the discrimination in favor of ^{12}C diminishes. Thus, the enrichment of ^{12}C of the plant tissues decreases, making the proportion of both isotopes in the plant less distant from that of the atmospheric CO_2. Hence, plants showing higher stomatal control show lower carbon isotope discrimination when photosynthesis is not limited by non-stomatal factors. From the relation between the carbon isotope composition of the plant and that of the atmospheric CO_2, the carbon isotope discrimination parameter Δ can be derived, which is inversely correlated to WUE (Farquhar *et al*., 1989).

Plants of more arid origins have repeatedly been found to have lower values of Δ, i.e., higher WUE (Pennington *et al*., 1999), which has been interpreted as adaptation to water-limited environments. However, the lack of this association has also been reported (e.g. Zhang *et al*., 1993, 1995), and even the inverse relationship was shown for some species (e.g. Read and Farquhar, 1991; Aranda *et al*., 2010), casting doubt on this early paradigm.

Genetic variation in Δ has been reported for several species (e.g. Zhang *et al*., 1994; Sun *et al*., 1996; Prasolova *et al*., 2000), and in some of them Δ was shown to be related to productivity (e.g. Sun *et al*., 1996; Zhang *et al*., 2004; but see Bonhomme *et al*., 2008 for

an opposite result). Likewise, in some forest tree species, genotype-by-environment interaction was ruled out (e.g. Zhang and Marshall, 1994; Guy and Holowachuk, 1993). Thus, Δ seems to be a good selection criterion for breeding with productivity purposes, at least for some species.

Genetic differentiation in quantitative traits related to early height growth (Aparicio *et al.*, 2010) and seedling architectural morphology (Pastorino *et al.*, 2010) has been shown in natural populations of Patagonian Cypress. However, to our knowledge, differentiation studies based on traits related to WUE have never been conducted for the species. Thus, the aim of the present work is to test differences between Patagonian Cypress natural populations in seedling water-use efficiency through carbon isotope discrimination, and also to estimate the genetic control of this parameter. We concentrate here on the study of three populations with contrasting precipitation regimes.

Materials and methods

Sampling

Foliage samples of five-year-old seedlings were collected from a Patagonian Cypress progeny trial growing near San Carlos de Bariloche, in Argentine North Patagonia (41°10'03" S, 71°15'11" W, 890 m a.s.l.). The trial included 185 open pollinated families corresponding to 12 natural populations and had been installed two years before (August 2006), under a 25-year-old afforestation of *Pinus ponderosa* Douglas ex. Laws., in which (five years previous to our trial) the trees had been thinned to 500 trees ha^{-1} (26.5 m^2 ha^{-1} basal area at the moment of the trial) and had been completely freed of branches up to a height of 6 m. The pines provided homogeneous cover, with the shade required for Patagonian Cypress seedling installation. The soil was covered by a 2 cm thick layer of dead pine needles, and there was no undergrowth (not even grass) under the pines. We used a completely randomized single-tree plot design in our trial, with 15 replicates (i.e., 15 seedlings per family), and plants were 2 × 2 m spaced. Mortality occurred during the first year after summer and winter stresses, which is the subject of another study (Aparicio *et al.*, in press).

In May 2007, we measured the heights of the plants (*H*) with 0.5 cm precision. The following year, we choose three populations from the 12 included in the trial, in order to measure carbon isotope composition. The three natural populations chosen represented two contrasting precipitation regimes, with more than 1,200 mm difference in mean annual precipitation (Table 1). The humid condition was represented by the Río Azul population, which is a continuous, dense forest, mixed with *Nothofagus dombeyi* (Mirb.) Oerst., with trees taller than 25 m. Two forest patches from the steppe represented the dry situation: Pilcañeu North and Pilcañeu South. They are at the arid extreme of the species range, located some 130 km to the northeast of Río Azul. These two patches, each containing around a hundred trees, are separated by only 1,200 m of grassy steppe. Trees are 4 to 6 m tall, tortuous, centenary, and scattered around the tops of two rocky hills, over an area of about 4 ha each. Previous studies with neutral markers (isozymes: Pastorino and Gallo, 2009; and microsatellites: Arana *et al.*, 2010) have shown that in spite of their physical closeness, they are genetically very distant, which has been interpreted as evidence of genetic drift and isolation.

From the three populations described we sampled a total number of 41 open-pollinated families (six seedlings per family) towards the end of summer (7 March 2008), when seedlings had completed the fifth growing season, making a total sample size of 246 seedlings (the number of families per population is shown in Table 2). Sampling started after dawn and was completed during the morning, so as to minimize variation due to recent assimilation (Brendel, 2001). Leaves of the year were collected from the tips of the seedling's upper third, so that the samples were representative of the yearly growth conditions.

Chemical analyses

Samples were oven-dried for 48 hours at 60 °C and then ground into fine powder with a mill (Retsch™ Mixer Mill MM301, with steel balls inside 0.5 ml plas-

Table 1. Location and mean annual precipitation (m.a.p.) of the sampled *Austrocedrus chilensis* populations

Population	Latitude S	Longitude W	Altitude [m asl]	m.a.p. [mm]
Río Azul	42° 06'	71° 40'	300	1,600
Pilcañeu North	41° 13'	70° 42'	1,100	330
Pilcañeu South	41° 14'	70° 41'	1,100	330

tic tubes). Five mg of powder per sample were sent to Centro de Aplicaciones de Tecnología Nuclear en Agricultura Sostenible (CATNAS) of the Facultad de Agronomía de la Universidad de la República (Montevideo, Uruguay) in order to analyze the stable carbon isotope composition ($^{13}C/^{12}C$) using a mass spectrometer with an analytical precision of 0.25 ‰ standard deviation. $\delta^{13}C$ was calculated as:

$$\delta^{13}C = \left[\frac{R_{sample}}{R_{PDB}} - 1\right] \cdot 1{,}000\,‰,$$

where R_{sample} is the sample $^{13}C/^{12}C$ ratio and R_{PDB} the $^{13}C/^{12}C$ ratio of the international PDB standard.

Carbon isotope discrimination (Δ) of each seedling was estimated according to Farquhar and Richards (1984) as:

$$\Delta = \frac{\delta^{13}C_a - \delta^{13}C_p}{1000 + \delta^{13}C_p} \cdot 1{,}000,$$

where $\delta^{13}C_a$ is the $\delta^{13}C$ of the atmospheric CO_2 and $\delta^{13}C_p$ that of the plant measured.

Although for free air experiments performed in recent years $\delta^{13}C_a$ is commonly assumed to be constant with a value of –8 ‰, a gradient of CO_2 isotopic composition have been shown in the air close to the ground (Berry *et al.*, 1997), which is ascribed to soil respiration. In an ambient similar to that of our trial (*Pinus resinosa* Sol. ex Ait. 32-years-old plantation, with the soil surface covered with a thick layer of pine needles), Berry *et al.* (1997) measured the isotopic composition of air samples taken at 15, 12, 9, 6, 3, 0.5, 0.2 and 0.05 m above ground, and they showed significant differences below 3 m. In order to estimate a value of $\delta^{13}C_a$ particular for each plant of the trial according to its height, we fitted a mathematical curve to Berry's *et al.* (1997) data by using non-linear regression with the help of GraphPad Prism 5.00 (GraphPad Software, San Diego, CA, USA). We fitted the data to a three-parameter non-rectangular hyperbola:

$$\delta^{13}Ca_x = \frac{a_1 \cdot x + a_0 - \sqrt{\left(a_1 \cdot x + a_0\right)^2 - 4x \cdot a_0 \cdot a_1 \cdot a_2}}{2 \cdot a_2},$$

where x is the height above the ground, a_0 is the curve asymptote (maximum $\delta^{13}C_a$ in this case), a_1 is the initial slope of the curve and a_2 is the sharpness at the inflection point.

Stressful conditions

In case water availability were not limiting at all, or on the contrary, totally lacking, all populations and progenies would have the same behavior. Moderate drought stress conditions are a requisite for the plants to express WUE differences between genetic entities. The climate of the region is Mediterranean, with 831 ± 196 mm mean annual precipitation, 78.7 % occurring in the cold season. This feature is a guarantee of drought stress each summer. However, to have a better understanding of the stressful conditions during the trial, rain pulses were registered and vapor pressure deficit (VPD) was estimated with local climate data, considering that, at the species level, 2.5 kPa is the threshold beyond which Patagonian Cypress closes its stomata even when water soil content is not limiting (Gyenge *et al.*, 2007).

Rain pulses, air temperature and relative air humidity were registered every 15 minutes from 20 December 2007 to 7 March 2008 with an automatic weather station (Hobo ® Weather Station Logger, Onset Computer Corp., USA) located 8.5 km southeast of the trial site.

Statistical analysis

The statistical significance of differences between populations for Δ and H, and the variability between families were tested by means of ANOVA using a nested mixed model with independent and normally distributed residuals:

$$y_{ijk} = \mu + \rho_i + \varphi_j(\rho_i) + \varepsilon_{ijk},$$

where y_{ijk} is an observation of the variable for the kth seedling, from the jth family nested within the ith population; μ is the overall mean for the variable; ρ_i is the effect (fixed) of the ith population; $\varphi_j(\rho_i)$ is the effect (random) of the jth family within the ith population, and ε_{ijk} is the overall error of the model NID(0, $\sigma^2\varepsilon$).

A Tukey test was utilized to identify homogeneous groups for the fixed effects factor. The analyses were carried out with the MIXED procedure of SAS 9.1 (SAS/STAT® software), with the use of the REML algorithm. The significance of the factor 'family' on both variables was tested through a likelihood ratio test (LRT) between the complete and the without-family models. Normality assumption was assessed by examining a histogram and a normal probability plot of the

residuals. Homocedasticity was checked by plotting the residuals against the predicted values.

In order to examine the association between Δ and H, we performed a Pearson's product moment correlation analysis, utilizing the statistical package R 2.10.1 (R Development Core Team 2009). The genetic correlation between those variables was also estimated using the following formula:

$$r_A = \frac{Cov_{f_{xy}}}{\sqrt{\sigma^2_{f_x} \cdot \sigma^2_{f_y}}},$$

where $\sigma^2_{f_x}$ and $\sigma^2_{f_y}$ are the family variances of both variables and $Cov_{f_{xy}}$ is the family covariance of the variables, calculated by

$$Cov_{f_{xy}} = \frac{\sigma^2_{f_{x+y}} - \sigma^2_{f_x} - \sigma^2_{f_y}}{2},$$

where $\sigma^2_{f_{x+y}}$ is the family variance of a synthetic variable built by the sum of both variables (O'Neill *et al.*, 2001). The standard error of the genetic correlation was estimated by

$$SE_{r_A} = \sqrt{\frac{(1 - r_A^2)^2}{f - 1}},$$

where f is the number of families.

Genetic variation parameters

The genetic variation of a population in a quantitative trait (within population variation) is measured by the additive genetic variance of the trait (σ^2_A) in that population, estimated by:

$$\sigma^2_A = \frac{1}{2\theta} \cdot \sigma^2_f,$$

where σ^2_f is the family variance in the analyzed population obtained through the ANOVA (the described model is obviously reduced to a family model without a "population" factor) and θ is the coefficient of coancestry, which is half the coefficient of relatedness (r) for diploid individuals (Wright, 1976; Lynch and Walsh, 1998). For half-sibs θ is 0.125, while it is double this value for full-sibs. Our trial was installed with open pollinated families of a dioecious species, namely a species where auto-pollination is impeded, that is, self full-sibs are not

possible. On the other hand, we know from a previous study (Pastorino and Gallo, 2006) that *A. chilensis* mates not randomly but in groups, what makes some level of biparental inbreeding likely (i.e. mating between relatives, Ritland, 2002). Recently, θ was estimated in Pilcañeu North (PN) population and in a humid population with a physiognomy similar to Río Azul (Pastorino and Marchelli, data not published) by means of isozyme genotypes corresponding to embryo samples, with the help of MLTR 3.2 software (Ritland, 2002): $\theta_{PN} = 0.148$; $\theta_{humid\ pop.} = 0.140$. The first value of coancestry was considered for the estimation of σ^2_A of both Pilcañeu populations, and the second one for that of the Río Azul population.

The values of σ^2_A are a function of the magnitude and units utilized, so that a sort of standardization is recommendable, such as that achieved by the parameter additive genetic variation coefficient (CV$_A$, Houle, 1992):

$$CV_A = 100 \frac{\sqrt{\sigma^2_A}}{\mu},$$

where μ is the variable mean.

Narrow sense heritability (h^2) is another kind of standardization for σ^2_A, and was similarly estimated for each population:

$$h^2 = \frac{\sigma^2_A}{\sigma^2_P},$$

where σ^2_P is the phenotypic variance, which in this case is the sum of σ^2_f and σ^2_ε (the error variance).

Standard error of heritability was estimated by:

$$SE_{h^2} = \frac{1}{2\theta} \cdot \sqrt{\frac{2 \cdot \left[1 + (n-1) \cdot h^2 / \frac{1}{2\theta}\right]^2 \cdot \left(1 - h^2 / \frac{1}{2\theta}\right)^2}{n \cdot (n-1) \cdot (f-1)}},$$

where f is the number of families and n the number of individuals within each family (Falconer and Mackay, 1996).

Genetic variation between populations can be estimated through the partition of the phenotypic variance of the complete model (with "population" and "family" factors) into its intra- and inter-population components (Spitze, 1993). Thus:

$$Q_{st} = \frac{\sigma^2_{pop}}{\sigma^2_{pop} + 2(\sigma^2_A)},$$

where Q_{st} is the inter-population variation, σ^2_{pop} is the component of the population variance estimated when considering populations as a random effects factor in the statistical model, and σ^2_A is the additive genetic variance estimated across populations jointly.

Results

Only eight rain pulses occurred during the 83-day period previous to leaf sampling, and just two of these accumulated more than 5 mm. Irrespectively of the soil water content, the vapor pressure deficit (VPD) proved stressful for *A. chilensis* (> 2.5 kPa) mainly in February, but also in December and January, as shown in Figure 1.

The non-rectangular hyperbola fitted the data of Berry *et al.* (1997) very accurately ($R^2 = 0.994$), being its best-fitted parameters: $a_0 = -7.787$, $a_1 = 26.97$ and $a_2 = 0.8633$. The ratios between the standard errors of estimate and the best-fitted values of the parameters were 0.002, 0.156 and 0.006 for a_0, a_1 and a_2 respectively; the residuals were normally distributed. Thus, a particular value of carbon isotope composition of the atmospheric CO_2 ($\delta^{13}C_a$) was estimated with this equation for each plant as a function of its height, and subsequently, together with the measured value of carbon isotope composition of the plant, its isotope discrimination (Δ) could be estimated.

Mean values of height (H) and carbon isotope composition ($\delta^{13}C_p$) and discrimination (Δ) per population,
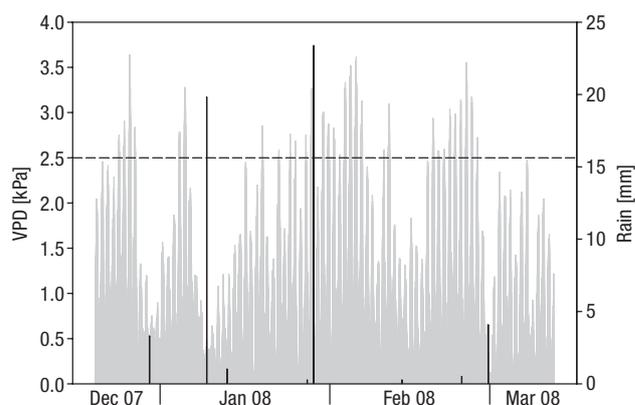

Figure 1. Rain pulses (black lines) and vapor pressure deficit (VPD, grey lines) registered every 15 minutes before the trial, in the days previous to leaf sampling. The dashed line shows the VPD drought stress threshold for *Austrocedrus chilensis,* irrespective of soil water content (built with the demo version of Sigma Plot 11 software).

together with inter- and intra-population variation parameters, are presented in Table 2. Differences between populations for Δ were not significant or, at most, were "marginally" significant ($P = 0.0886$). A low value of differentiation supported this result ($Q_{st}\,\Delta = 3.47$ %). Given the closeness of the probability value to the rejection threshold, pair-wise Q_{st} were calculated (Table 3). This additional procedure revealed considerable differences only between Río Azul and Pilcañeu North ($Q_{st}\,\Delta_{\text{Az-PN}} = 13.5$ %). Conversely, for H, significant differences between populations could be seen ($P = 0.0386$), which through the Tukey test were shown to be deter-

Table 2. Number of families, mean carbon isotope composition *($\delta^{13}C_p$)*, mean carbon isotope discrimination (Δ), mean height (H), additive genetic variance (σ^2_A), coefficient of additive variance (CV_A) and heritability (h^2) of the analyzed *Austrocedrus chilensis* natural populations (standard errors between brackets). Genetic differentiation between populations (Q_{st}) is indicated in the last column

		Río Azul	Pilcañeu North	Pilcañeu South	Q_{st}
	Nº famil.	10	16	15	
	$\delta^{13}C_p$ [‰]	−29.08 (0.142)	−29.60 (0.098)	−29.35 (0.116)	
Carbon isotope discrimination	Δ [‰]	21.92 (0.150)	22.46 (0.103)	22.20 (0.122)	
	σ^2_A	0.784[#]	0.146[#]	0.881	0.0347[#]
	CV_A [%]	4.0[#]	1.7[#]	4.2	
	h^2	0.58[#] (0.468)	0.14[#] (0.262)	0.65 (0.370)	
Height	H [cm]	34.7 (1.240)	28.2 (0.720)	29.6 (0.982)	
	σ^2_A	126.960	69.981	150.723	0.0354
	CV_A [%]	32.5	29.6	41.5	
	h^2	1.31 (0.552)	1.47 (0.405)	1.81 (0.399)	

not significant.

Table 3. Pair-wise differentiation (Q_{st}) between three *Austrocedrus chilensis* natural populations by means of carbon isotope discrimination.

Q_{st}	Río Azul	Pilcañeu North
Pilcañeu North	13.55 %	
Pilcañeu South	0.11 %	1.20 %

mined exclusively by the differences between Río Azul and Pilcañeu North ($P_{Az-PN} = 0.0367$; $P_{Az-PS} = 0.1020$; $P_{PS-PN} = 0.8748$).

The factor 'family' had a significant effect on both variables (LRT: $P_\Delta = 0.0022$ and $P_H < 0.0001$), and explained 13.6 % and 45.28 % of the total variance of Δ and H respectively.

Intra-population variation in both traits is presented in Table 2, in terms of σ^2_A and CV_A as well. For Δ, the family model was significant only in Pilcañeu South population ($P_{\Delta Az} = 0.1030$; $P_{\Delta PN} = 0.5509$; $P_{\Delta PS} = 0.0179$), while for H it was significant in the three studied populations ($P_{HAz} = 0.0012$; $P_{HPN} < 0.0001$; $P_{HPS} < 0.0001$). The significance of the family model in each population can be perceived in the standard errors of the derived heritability values. Thus, Río Azul and Pilcañeu North h^2_Δ could not be shown to be different from nil. On the other hand, heritability was found to be high in Pilcañeu South for Δ, and very high in the three populations for H.

Unfortunately, we lost the identification numbers of some samples (but not the family identification), thus for the phenotypic correlation analysis we counted only with 172 samples (46 from Azul, 73 from Pilcañeu North and 53 from Pilcañeu South). This analysis showed an inverse association between Δ and H ($r_P = -0.467$; $P < 0.0001$; SE $= 0.068$); the linear regression between both variables ($R^2 = 0.213$; $P < 0.0001$) is presented in Figure 2. The genetic correlation was found to be even higher ($r_A = -0.684$; SE $= 0.093$).

Discussion

The family effect was found to be significant for carbon isotope discrimination (Δ) in the complete model with the three surveyed populations included. On the other hand, the heritability of Δ was not significant in two out of the three populations, while the third one showed high heritability. Heritability estimations of physiological plant traits usually have low precision due to the impossibility of handling large
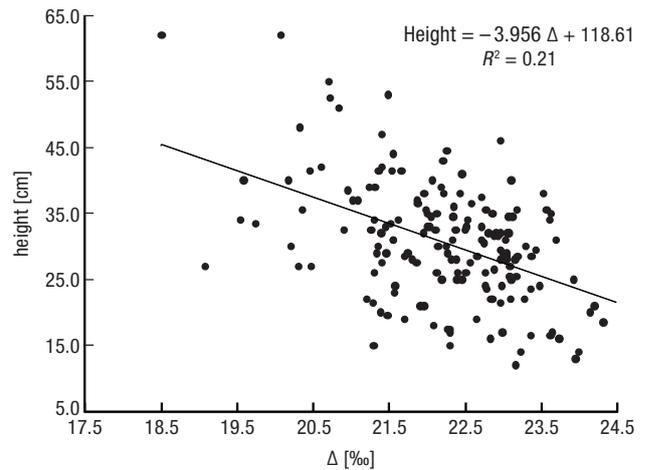

Figure 2. Linear regression between carbon isotope discrimination (Δ) and height of *Austrocedrus chilensis* seedlings included in a common garden trial.

enough samples to reduce standard errors. Río Azul population showed a high h^2_Δ, but this estimation was not significant, which could be due exclusively to its low sample size (the lowest of the three populations). Pilcañeu North h^2_Δ was also not significant, although its sample size is the highest of the three populations. In addition, its estimated h^2_Δ is low (the lowest of the three populations), and consequently, the lack of significance of $h^2_{\Delta PN}$ seems to be the effect of a real evolutionary process more than a sampling artifact. We will come back to this point in a later paragraph. In summary, we proved a family effect for Δ, but we could not demonstrate a general level of genetic control over this trait. Even with similar sample sizes and in quite physiognomically similar populations, like Pilcañeu North and Pilcañeu South, h^2_Δ estimates were different, and more precisely, contrasting.

A family effect was also reported for Δ in other conifers, for instance *Larix occidentalis* Nutt. (Zhang *et al.*, 1994), *Pinus ponderosa* Dougl. ex Laws. (Olivas-García *et al.*, 2000) and *Araucaria cunninghamii* Ait. ex D Don (Prasolova *et al.*, 2000). Likewise, moderate to high heritability values were estimated in conifers such as *Pinus pinaster* Ait. ($h^2 = 0.17$; Brendel *et al.*, 2002), *Araucaria cunninghamii* Ait. ex D Don ($h^2 = 0.20$ in a dry site, and $h^2 = 0.33$ in a wet site; Xu *et al.*, 2003); and *Picea mariana* (Mill.) B. S. P. ($h^2 = 0.54$; Johnsen *et al.*, 1999).

On the other hand, we verified an important inverse association between Δ and H, both with regard to their phenotypic expression and also their genetic bases. Similar results were reported in previous studies: i) an

inverse association of Δ with dry matter production of *Picea glauca* (Moench) Voss seedlings (Sun *et al.*, 1996), ii) an inverse association of Δ with height and diameter at breast-height of six-year-old saplings of the inter-specific hybrid between *Pinus elliottii*. Engelm var. *elliottii* and *Pinus caribaea* var. *hondurensis* Barr. et Golf. (Prasolova *et al.*, 2003), and iii) an inverse association of Δ with dry matter and height of one-year-old *Castanea sativa* Mill. seedlings (Lauteri *et al.*, 2004).

The Δ and H inverse association indicates that plants with larger height increments were those with higher WUE. This could be interpreted as an adaptation strategy at the intra-population level. Taller plants (those with more above-ground biomass exposed to the desiccating environment) need to use water more efficiently. That is, they close their stomata sooner as water availability decreases, thus resigning carbon gain. Their higher productivity should be associated with other mechanisms, for example a more efficient photosynthetic apparatus, by means of which they would fixate more C than shorter plants even with a longer stomatal closure period. On the other hand, shorter plants can bear being not so efficient in their water consumption because they are not so exposed to desiccation. Hence, drought stress would drive selection to favor tall but efficient or inefficient but short plants within the same population.

In the case of seedling height, heritability surpassed the theoretical maximum value (1). This is a little-reported but not uncommon result. It must be taken into account these are estimations of the true heritability, in which additive epistatic interactions are potentially included in the estimates of the genetic variances (Lynch and Walsh, 1998). But more than this, low estimation precision seems to be the main reason. The large standard errors estimated for the three populations as a consequence of the small sample size (determined by the sampling restrictions of the carbon isotope composition measurement) indicate that the true heritability values lie within a confidence interval that includes the value of 1. In any case, it is evident that the true values of these heritabilities are rather high.

Besides the inverse association between Δ and H, our other main result is the absence of significant differences between populations in their ability to discriminate carbon isotopes, in spite of their deeply contrasting precipitation regimes. In fact, if a slight differentiation could be accepted, contrary to the regular expectation, the most discriminating population was an arid one. However, due to the low sampling size of the humid population in particular (only 10 families), this result should be considered cautiously.

A divergence between arid populations must also be highlighted. Although significant differences could not be shown between them, they did not behave in a similar way regarding the humid population. Pilcañeu North was significantly different from Río Azul in terms of H and "marginally" significantly different in terms of Δ, but Pilcañeu South was not different at all. Furthermore, both Pilcañeu populations proved to be quite different in terms of genetic variation, since in spite of their similar sample sizes, h^2_Δ was high in PS while the family factor was not even significant in PN. This result is surprising given the potentially adaptive condition of the analyzed traits and the environments of these arid populations, which are basically the same. They are also supposed to have a common origin due to the scarce distance that separates them. This supports the idea of an isolation ("isolation by wind", Gallo and Pastorino, 2010) and genetic drift effect on the considered traits in these small populations. In fact, our findings confirm previous reports in other quantitative traits (Aparicio *et al.*, 2010; Pastorino *et al.*, 2010) and also in neutral markers (Pastorino and Gallo, 2009; Arana *et al.*, 2010).

In conclusion, the differentiation between the genetic pools of Pilcañeu North and Río Azul seem not to have been the result of an adaptation process but the effect of genetic drift causing the simple stochastic reduction of the intra-population variation level of Pilcañeu North. This variability decrement would have increased its chance of differentiation. On the other hand, the high variability of Pilcañeu South would have preserved it from being different.

This inference has relevant consequences for any management decision in relation to the genetic resources of the species, both with respect to conservation and utilization strategies. If genetic patterns governing characters with phenotypic expression could be modeled by genetic drift processes in a similar or even deeper way than by adaptive processes, then populations growing in similar environments will not necessarily have a similar adaptive genome. At least, this seems to be true for populations of small size, that is, more likely to be affected by drift. A joint analysis of the different kinds of available data could shed light on this disquieting hypothesis.

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