

## Variability in seedling emergence traits of Patagonian Cypress marginal steppe populations

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**Abstract** Genetic information on adaptive traits is crucial for prediction of the evolution of natural populations in relation to global climate change. The seedling emergence process together with germination is a key adaptive stage in any seeding species. We aim to analyze variability in seedling emergence traits within and among marginal populations of Patagonian Cypress (*Austrocedrus chilensis* (D. Don) Pic. Ser. et Bizzarri), which have been suggested to be of conservation relevance. We performed an emergence trial in a greenhouse with seeds collected from 177 open-pollinated trees from 10 populations. A sigmoidal curve was fitted to the cumulative emergence data (in percentage of the sown seeds) for each replicate of each family. Variability was estimated using ANOVA for six variables: emergence capacity (*EC*), emergence energy (*EE*), energy period (*EP*), emergence initiation ( $t_{10}$ ), emergence cessation ( $t_{90}$ ) and emergence duration (*Dur*). The overall trial mean for *EC* was 76.2 %, while *EE* was only 27.6 %. Hence, most seedlings emerged after the energy period, which is interpreted as a bet-hedging strategy. Both “population” and “family” factors significantly affected all variables. The proportions of “family” variances were higher than “population” ones for *EC*, *EE*, *Dur* and  $t_{90}$ , but the opposite was found for *EP* and  $t_{10}$ , which is evidence of differentiation among populations. Variability among families may be due to both genetic and environmental causes, including maternal effects. However, the relatively high proportion of family variability in *EC* and *EE* suggests acceptable levels of additive genetic variance, which would not hinder the potential to evolve in these specific traits. Conversely, the chances to adapt in *EP* and  $t_{10}$  are lower, and consequently local extinctions driven by global climate change seem possible.

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## Introduction

The Patagonian Cypress (*Austrocedrus chilensis* (D.Don) Pic. Ser. et Bizzarri) is the most important timber-productive native conifer (Cupressaceae) of the temperate forests of Argentina, where it occupies some 141,000 ha (Bran et al. 2002). Although almost this whole range corresponds to continuous mixed or pure forests, in this study we concentrate in the minimal part of it that surpasses the ecotone with the steppe as scattered stands in a pastureland matrix. The Patagonian Cypress is the tree species of the Sub-Antarctic forest that penetrates furthest into the Argentinean Patagonian steppe, forming extremely isolated patches, totally separated from the western continuous forest of the Andean mountains. With the help of isozyme genetic markers, these scattered stands, each composed of about a hundred small, crooked trees, have already been shown to be the most genetically diverse natural populations of this species (Pastorino et al. 2004; Pastorino and Gallo 2009). Following the concepts of Hampe and Petit (2005) regarding the conservation significance of marginal populations, Arana et al. (2010) and Aparicio et al. (2010) have suggested that these populations constitute a receding edge of the species range. This hypothesis gives central importance to these marginal forest patches, in spite of their silvicultural irrelevance and small size. They could be the reservoir of genetic variants, some of them adapted to drought stress conditions, and thus the key to the species persistence in situ despite global climate change, not only because of the survival of these marginal populations themselves, but also through the introgression of their genes into the western dense forests currently adapted to more suitable conditions.

Several marginal populations from the steppe have already been included in genetic studies with neutral markers (Pastorino and Gallo 2002, 2009; Pastorino et al. 2004; Arana et al. 2010) and quantitative traits such as seed size (Pastorino and Gallo 2000), early height growth (Aparicio et al. 2010), seedling architectural morphology (Pastorino et al. 2010), seedling survival (Aparicio et al. 2012) and water-use efficiency assessed through carbon isotope discrimination (Pastorino et al. 2012). Genetic information on adaptive traits is crucial for the prediction of natural population evolution in relation to global climate change. The aforementioned studies are valuable in this regard, but knowledge about traits of fundamental adaptive significance is still lacking.

Each year, every adult population of trees creates a new generation, combining the allelic variants it possesses and receives through migration. The zygote population is thus the most genetically diverse stage of development of the new generation. From this starting point, different evolutionary factors begin to erode this genetic diversity, concentrating its deepest natural changes in the early stages of development, according to the most drastic reduction of population size of that generation (Finkeldey and Hattermer 2007).

Among the evolutionary processes that model the genetic diversity of the new generation, viability selection is the one with adaptive meaning. Not counting seed abortion, germination is the first stage of viability selection for any seeding plant species, followed by seedling emergence and establishment. In this respect, it has been shown for a Neotropical forest tree that the greatest change in the genetic composition of progeny occurred between mature seeds and established seedlings (Hufford and Hamrick 2003). The germination, emergence and establishment processes thus appear as key adaptive stages, especially in

**Table 1** Location, mean annual precipitation (M. A. P.), number of studied families and seed collection year of *A. chilensis* sampled populations

Population		Latitude (S)	Longitude (W)	Altitude (m asl)	M. A. P. (mm)	No. of famil.	Collection year
Molina	Mo	37°08'	70°36'	1.450	604	14	2003
Rahueco	Ra	37°10'	70°36'	1.500	604	12 (7)	2002 (2003)
Riscos Bayos	Ri	37°59'	70°47'	1.350	246	6	2002
Catán Lil	N	39°21'	70°39'	1.100	308	17	2002
Chacabuco	Ch	40°39'	71°01'	900	450	25 (1)	2003 (2002)
Chacay	Y	40°51'	70°59'	1.250	488	19	2003
Pilca. North	PN	41°13'	70°42'	1.100	330	19	2003
Pilca. South	PS	41°14'	70°42'	1.100	330	16	2003
El Maitén	M	42°02'	71°12'	750	490	26	2003
Leleque	Q	42°20'	71°09'	870	353	23 (9)	2003 (2002)

Number of families between parentheses correspond to those collected in years between parentheses

small-seeded trees (Moles and Westoby 2004), such as *A. chilensis* (Pastorino and Gallo 2000).

The aim of the present study is to analyze variability in seedling emergence traits within and among marginal populations of Patagonian Cypress. The strong selective pressure of their extreme environment, led us to expect them not to differ significantly. Consequently, higher levels of intra- rather than inter-population variation are predicted.

## Materials and methods

Ten marginal populations of *A. chilensis* were sampled along more than five latitudinal degrees in the northwestern Patagonian steppe (Table 1). They all share similar physiology but each one has its own particular environmental features. We presume that a balance effect occurs between some of those features, such as altitude and latitude (higher altitudes at lower latitudes result in similar temperatures to lower altitudes at higher latitudes), or precipitation volumes and soil superficiality or slope (steeper slopes with higher precipitation volumes can lead to similar water availability to gentler slopes with lower precipitation volumes). These trade-offs could bring about a certain similarity among the environmental conditions the trees undergo in all these populations irrespective of their peculiarities related to the environmental parameters we are considering. In any case, they all suffer extreme conditions of drought stress, with strong, desiccating winds and different combinations of rocky soils and steep slopes.

Cones were collected in April from a total of 177 randomly chosen trees, directly from their crowns, maintaining a minimum distance of 30 m between trees in order to reduce the probability of sampling related individuals. Most of the trees (137) were sampled in 2003, but a collection from the previous year was included to enhance the sampling. Cones were dried at room temperature for a week, seeds extracted manually and finally conserved at  $-18\text{ }^{\circ}\text{C}$  till used.

In July 2003 (south hemisphere winter), approximately 130 full, healthy seeds were counted (unfilled and insect-damaged seeds were discarded) per open-pollinated family and subjected to recommended pre-germination treatment (60 days in moist sand in a refrigerator at  $4\text{ }^{\circ}\text{C}$ , Contardi 1995).

Sowing was carried out from 16 to 20 September inclusive, in a greenhouse under controlled conditions in San Carlos de Bariloche city (41°07'S lat., 71°15'W long., 810 m a.s.l.). Seeds were sown at about half centimeter depth directly in 265 cm<sup>3</sup> pots (HIKO™ HV265 tray), filled with 1:1 peat-sand substrate. Temperature did not surpass 10 °C and water was not supplied until sowing was completed. On 20 September the greenhouse windows and door were closed, and temperature was regulated with a heater not to fall below 18 °C during the trial. Thus, the trial was considered to begin on 20 September. Water was provided three times per week assuring adequate moisture availability.

We used a randomized complete block design, with three replicates. Forty-two seeds per replicate per family were sown, occupying 14 pots (three seeds per pot in half HIKO™ HV265 tray). Seedling emergence was recorded every other day from the observation of the first emerged seedling in the whole trial. Visible appearance of the cotyledons was the emergence criterion.

A sigmoidal curve was fitted to the cumulative seedling emergence data (in percentage of the sown seeds, starting when the first seedling of the entire trial emerged) for each replicate of each family, using GraphPad Prism 5.00 (GraphPad Software, San Diego CA, USA). Such a procedure has its first antecedents in germination trials of herbaceous species (e.g. Schimpf et al. 1977; Torres and Frutos 1989). The Gompertz sigmoid function was chosen due to its adequacy for time series with a slow start and finish, but more gradually approaching the right than the left asymptote, and according to previous reported germination studies in forestry species (e.g. Alía et al. 2001). Its mathematical expression is:

$$E_t = a \cdot e^{-e^{-b(t-c)}}$$

where  $E_t$  is the percentage of the sown seeds from which seedlings emerged at each time  $t$  measured in days,  $a$  is the upper asymptote, which in this case represents the fitted emergence capacity ( $EC$ ) at the end of the trial,  $e$  is Euler's number,  $b$  is a parameter of the curve proportional to the maximum emergence rate standardized by the predicted  $EC$ , and  $c$  is the time corresponding to the inflection point of the curve, namely the time in days taken by a replicate of a family to reach its maximum emergence rate, which in germination trials has been called energy period ( $EP$ ; e.g. Ffolliot and Thames 1983; Willan 1985).

Normality of the residuals was revised by means of Shapiro–Wilk  $W$  test for each fitted curve. The accuracy of the fitted parameters was examined via the ratios between the standard errors of estimate and the best-fitted values (Zar 1999).

The fitted curves enabled us to calculate four additional parameters descriptive of the emergence process. We calculated the emergence energy ( $EE$ ), which is the percentage of the sown seeds from which seedlings have emerged within the energy period (i.e. the cumulative emergence at the inflection point of the curve), and also  $t_{10}$  and  $t_{90}$ , which are the times needed to reach 10 % and 90 % of the total emergence predicted for the considered replicate, indicative of emergence initiation and cessation respectively. Finally, the time that elapsed between  $t_{10}$  and  $t_{90}$ , abbreviated as  $Dur$ , was calculated, which is indicative of the duration of the period when emergence was concentrated.

Variability within and among populations was estimated for these six variables [ $EC$  (as the measured % of emerged seedlings),  $EE$ ,  $EP$ ,  $t_{10}$ ,  $t_{90}$  and  $Dur$ ] by means of ANOVA, using the following model with independent and normally distributed residuals:

$$y_{ijklm} = \mu + \alpha_i + \rho_j + \beta_k + \phi_l(\rho_j) + \varepsilon_{ijklm}$$

where  $y_{ijklm}$  is an observation of the variable for the  $m$ th replicate of the  $l$ th family within the  $j$ th population collected in the  $i$ th year and located in the  $k$ th block;  $\mu$  is the overall

mean for the variable;  $\alpha_i$  is the effect (fixed) of the year when seeds were collected,  $\rho_j$  is the effect (random) of the  $j$ th population;  $\beta_k$  is the effect (random) of the  $k$ th block;  $\varphi_l(\rho_j)$  is the effect (random) of the  $l$ th family within the  $j$ th population; and  $\varepsilon_{ijklm}$  is the random residual  $NID(0, \sigma_\varepsilon^2)$ .

The MIXED procedure of SAS software (SAS/STAT<sup>®</sup> Version 9.1 ©2004/2005 SAS Institute Inc., Cary, NC, USA) was utilized to estimate the components of variance through restricted maximum likelihood. All the variables except  $t_{10}$  were transformed in order to achieve normality and homoscedasticity, which were subsequently assessed by a histogram, a normal probability plot of the residuals and by plotting the residuals against the predicted values [ $EC_{tr} = \arcsin(EC/100)^{1/2}$ ;  $EE_{tr} = \arcsin(EE/100)$ ;  $EP_{tr} = \log(EP)$ ;  $t_{90tr} = \log(t_{90}-3.5)$ ;  $Dur_{tr} = \log(Dur-0.83)$ ]. The significance of the factors “population” and “family” was tested by means of likelihood ratio tests (LRT), while that of the factor “year” through a Type III fixed effects test.

Pearson’s product moment correlations were calculated between all variables by means of CORR procedure of SAS software. We additionally performed a multiple regression analysis for the population mean of each seedling emergence trait, in order to explore any possible trend related to the main environmental features we considered, namely latitude, altitude and precipitation. We followed a stepwise “forward selection” procedure as described by Zar (1999). We utilized the statistical package R 2.10.1 (R Development Core Team 2009).

## Results

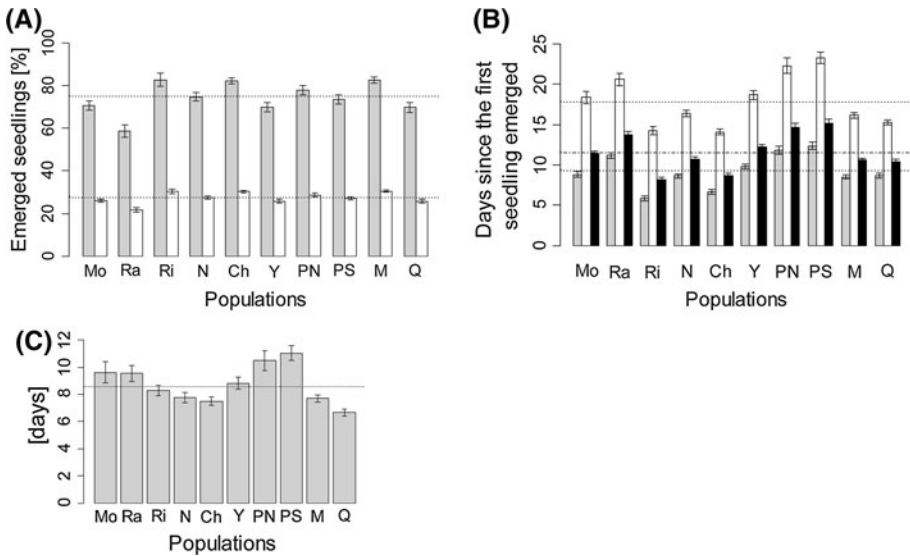
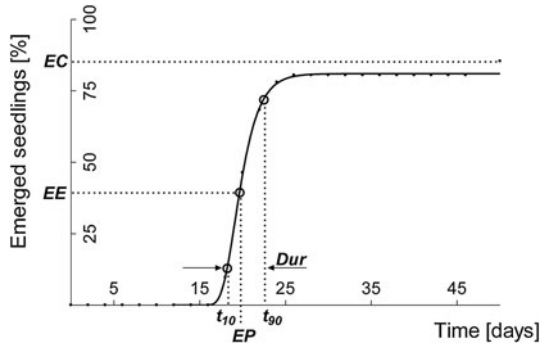
The first seedling emerged on 1 October, 10 days after the beginning of the trial. The trial ended when no new emerged seedlings were observed, which happened on 8 November; hence, the trial lasted 49 days.

The Gompertz function fitted the data very accurately (e.g. Fig. 1), with an average coefficient of determination ( $R^2$ ) of  $0.995 \pm 0.004$  (minimum of 0.971). The average ratios between standard errors of estimate and best-fitted values of the parameters were  $0.013 \pm 0.009$ ,  $0.078 \pm 0.031$  and  $0.015 \pm 0.009$ , for a, b and c, respectively. Residuals were normally distributed for all the fitted curves.

Population means for all variables are shown in Fig. 2. The overall mean emergence capacity ( $EC$ ) of the trial was 76.2 %, the absolute minimum  $EC$  being 11.6 % for one replicate of the Leleque population. On the other hand, the mean emergence energy ( $EE$ ) was only 27.6 % of the sown seeds, and differences of this magnitude recurred in each population. The average maximum emergence rate of the entire trial was reached 11.5 days after the first seedling emerged ( $EP$ ), barely 2 days after 10 % of the seedlings had emerged in the trial ( $t_{10}$  overall mean = 9.2 days), and 6 days before emergence could be considered to have finished ( $t_{90}$  overall mean = 17.8 days). Regarding the time variables, it must be highlighted that these are expressed in days from 1 October; thus, 10 days should be added in order to refer to the beginning of the trial.

Original and transformed variables gave relative results that were not significantly different. Therefore, since the interpretation of the original variables is more direct, we present the results based on these. Significant differences were shown between the collection years only for  $EC$  (means:  $EC_{2002} = 70.13$ ,  $EC_{2003} = 77.98$ ,  $P = 0.010$ ) and  $EE$  (means:  $EE_{2002} = 25.35$ ,  $EE_{2003} = 28.20$ ,  $P = 0.010$ ). Both “population” and “family” factors significantly affected all the variables, while blocking was not significant (Table 2). A minor portion of the variances lay in the residuals of the proposed model for all variables

**Fig. 1** Curve fitted to the cumulative seedling emergence data (as percentage of the sown seeds) of one replicate of an open-pollinated family of *A. chilensis* in a greenhouse emergence trial. Traits analyzed are indicated (*EC* emergence capacity on day 49, *EE* emergence energy, *EP* energy period,  $t_{10}$  emergence initiation time,  $t_{90}$  emergence cessation time, *Dur* emergence duration time)



**Fig. 2** **a** Mean values and standard deviation of emergence capacity (*grey bars and upper dotted line* for the overall mean value) and emergence energy (*white bars and lower dotted line* for the overall mean value) in 10 marginal *A. chilensis* populations assayed in a greenhouse seedling emergence trial (see population names in Table 1). **b** Mean values and standard deviation of emergence initiation (*grey bars and lower dotted line* for the overall mean value), emergence cessation (*white bars and upper dotted line* for the overall mean value) and energy period (*black bars and middle dotted line* for the overall mean value) in 10 marginal *A. chilensis* populations assayed in a greenhouse seedling emergence trial (see population names in Table 1). **c** Mean values and standard deviation of emergence duration time (*dotted line* for the overall mean value) in 10 marginal *A. chilensis* populations assayed in a greenhouse seedling emergence trial (see population names in Table 1)

(with a value of 39.96 %, the duration of the lapse where seedling emergence was concentrated, *Dur*, was the one with the highest variability among replicates within families). The proportions of the variances explained by the family effect were higher than those explained by the population effect for *EC*, *EE*, *Dur* and emergence cessation time ( $t_{90}$ ). However, population was more influential than family for energy period (*EP*, i.e. parameter *c* of the curve) and emergence initiation time ( $t_{10}$ ; Table 2).

All correlations between variables were significant (Table 3). *EC* and *EE* were negatively correlated with all time variables. The highest correlations were those between *EC*

**Table 2** Percentage of variance (%) of the considered factors for all seedling emergence traits studied in 10 marginal *A. chilensis* populations (*EC*: emergence capacity on day 49, *EE*: emergence energy, *EP*: energy period,  $t_{10}$ : emergence initiation time,  $t_{90}$ : emergence cessation time, *Dur*: emergence duration time)

	EC	EE	EP	$t_{10}$	$t_{90}$	Dur
Block	0.00	0.00	0.74	0.87	0.31	0.00
Population	17.62*	17.72*	43.08*	39.12*	36.16*	12.64*
Family (Pop)	64.37*	63.35*	37.73*	32.14*	46.71*	47.40*
Residual	18.01	18.94	18.45	27.88	16.82	39.96

\* Significance effect as revealed by likelihood ratio test ( $P < 0.001$ )

and *EE* ( $r = 0.987$ ), *EP* and  $t_{10}$  ( $r = 0.958$ ), *EP* and  $t_{90}$  ( $r = 0.890$ ) and *Dur* and  $t_{90}$  ( $r = 0.814$ ), while the lowest were those between *Dur* and  $t_{10}$  ( $r = 0.188$ ), *EE* and  $t_{10}$  ( $r = -0.280$ ) and *EC* and  $t_{10}$  ( $r = -0.298$ ).

On the other hand, in the regression analysis, none of the environmental features considered resulted significant to explain the variation of any trait across the populations ( $\alpha = 0.05$ ). Only a marginal association could be considered between *EC* and altitude ( $P = 0.073$ ).

## Discussion

We aimed to draw conclusions about the adaptive success and ability of marginal populations and their open-pollinated families at early stages of development, from an ecological rather than a physiological perspective. This led us to conduct the seedling emergence trial instead of a more classical germination one performed in germination chambers. Germination and seedling emergence are two related concepts, but clearly different developmental stages. Seeds able to germinate are viable seeds, but not all viable seeds have the ability to produce seedlings. Thus, there is an additional viability selection step between these stages, and consequently, the analysis of seedling emergence gives us information on a more advanced stage of the adaptation process. On the other hand, considering the establishment stage (the following developmental stage) would require an experiment carried out under even more natural environmental conditions, i.e. less controlled conditions, which would add variation sources (statistical “noise”) to the variables surveyed.

**Table 3** Pearson’s product moment correlations between six seedling emergence traits of 10 marginal *A. chilensis* populations

	EC	EE	EP	$t_{10}$	$t_{90}$	Dur
EC		<0.001	<0.001	<0.001	<0.001	<0.001
EE	0.9875		<0.001	<0.001	<0.001	<0.001
EP	-0.3748	-0.3449		<0.001	<0.001	<0.001
$t_{10}$	-0.2975	-0.2804	0.9583		<0.001	<0.001
$t_{90}$	-0.4318	-0.3869	0.8902	0.7228		<0.001
<i>Dur</i>	-0.3639	-0.3145	0.4605	0.1876	0.8144	

Correlation coefficient  $r$  and probability value  $P$  below and above diagonal respectively (see text or Table 2 references for the identification of the abbreviated names of traits)

Although we predicted general uniformity among populations, variability was found among them. However, variability among families within populations was higher for most of the variables considered, which was in agreement with our expectations, and is a common result in forest tree populations. Notwithstanding, there were two traits where this relation was inverse: emergence initiation ( $t_{10}$ ) and energy period ( $EP$ ), both related to the rate at which the emergence process occurs.

The germination rate has been shown to be more sensitive to environmental conditions than the proportion of seeds that germinate (Schimpf et al. 1977). Thus, the speed of the germination process of a set of seeds is probably more susceptible to current selective pressures, consequently holding deeper significance in terms of adaptation to present environments. This feature would give rise to directional selection and cause the populations to differentiate.

Higher variability among populations rather than among families within them is evidence of differentiation among populations for  $t_{10}$  and  $EP$ ; in other words, the moments when seedling emergence begins and when it reaches its peak crucially depend on the origin of the seed. However, any clear trend in relation to latitude, altitude and precipitation could not be recognized in the among-population variation of the mean values of these traits (this was the result of the regression analysis). We consequently should discard the idea of any clinal geographic variation.<sup>1</sup> Similarly, evidence supporting ecotypic variation was not found, what becomes obvious just considering some clear examples: under an ecotypic variation scenario, Catán Lil population should not differ in  $EP$  and  $t_{10}$  from Pilcañeu North and South regarding altitude, and Molina and Rahueco should have the same values of  $EP$  and  $t_{10}$  regarding altitude, latitude and precipitation. We consequently assume random geographic variation. According to Wright (1976), such a pattern could be due to (1) adaptation to an unknown, currently existing environmental feature, (2) adaptation to ancient ecological conditions, or (3) genetic drift. Given that we are dealing with small isolated populations, and that genetic drift was already reported in them (Pastorino and Gallo 2009; Pastorino et al. 2010, 2012), the third option seems the most likely.

Marginal populations exposed to extreme environmental conditions are expected to go through processes of directional selection (Stern and Roche 1974). However, regarding the parameters related to the quantity of emerged seedlings ( $EC$  and  $EE$ ) in *A. chilensis* populations, their variation patterns seem to have been modeled with a prevalence of balancing selection, since less than 18 % of their variability lies among populations.

The total relative number of germinated seeds and the proportion of seeds that germinates in the first flush, characterized by increasing germination speed, are the main basic parameters that describe the germination process of a set of seeds of any species. The importance of germination energy was early understood (Haack introduced the term “germinative energy” in 1909, cited in Czabator 1962). It has been mentioned (Folliot and Thames 1983) that seedlings originating from seeds germinating during the energy period make the best quality planting stock, and that only these seeds are capable of producing vigorous seedlings in field conditions (Aldhous 1972).

Various approaches and methods have been developed to evaluate and measure the concept behind the term “germinative energy” (Czabator 1962), from which we analogously derived “emergence energy”. The main difficulty lies in the fact that the energy period varies for each seed lot, even within the same population of a given species. The

<sup>1</sup> We find interesting the marginal significance of  $EC$  with altitude, which drive us to re-consider this possible association in future studies.



first attempts assumed a fixed period for all lots, basically determined from experience with the respective species. However, this procedure involved a bias, not effectively solved till the advance of information technology during recent decades. The use of software made it possible to easily fit a curve to each replication of ordinary germination trials. This procedure permitted better description of the whole germination process (emergence process in our case), and the deduction of some biologically meaningful parameters, the germinative energy among them. In this regard, the Gompertz function was shown to describe the emergence of *A. chilensis* seedlings very accurately.

One of the main results of this study is the great difference between the *EC* and the *EE* (almost three times larger). We found that most of the seedlings emerged after the energy period (*EP* is very much closer to  $t_{10}$  than to  $t_{90}$ ), that is, emergence continues significantly even after the “quick seeds” have already germinated and their derived seedlings emerged. May be in *A. chilensis*, early seedlings are not better than the late ones. Instead, this long emergence period could be a biological strategy to give a “second chance” to the incipient new generation by offering a more ample variety of germination dates for the harsh conditions of the steppe (a sort of bet-hedging strategy), thus allowing some individuals to profit from the winter humidity (precipitation concentrates in winter in northwestern Patagonia) in mild springs and other individuals to avoid spring late frosts. This seems a good way of facing varying environmental factors like those of the Patagonian steppe, and seems to be a strategy common to all the marginal populations, since variation of *EC* and *EE* among populations was relatively low.

In spite of the large difference between *EC* and *EE*, these two traits are highly correlated. This is due to the fact that *EE* is a linear combination of predicted *EC* ( $EE = EC \cdot e$ ), which is obviously the reason for an almost duplicated behavior of both parameters in each statistical test. Both emergence percentage traits are inversely correlated to all time variables; hence, seedlings lots that emerge most also do it quickly. From a biological point of view, one feature could be a consequence of the other, i.e. seeds most likely to germinate would be those with the best “germination machinery” (i.e. enzymatic abilities, reserve storages), which enables their derived seedlings to emerge before the others.

On the other hand, another important result (particularly relevant for nursery management) does not support this speculation. Differences were shown in *EC* and *EE* between collection years. Just 1 year was enough to reduce both parameters significantly. However, this was not the case for the time variables of the emergence process, and thus this linkage between both variable types seems not to last from one to the following year. A specific sampling to test the effect of time over the emergence parameters would be necessary to draw conclusions in this respect.

The residual variation of the ANOVA model reflects low variation among replicates within each family in all parameters in general, except for the duration of the emergence process (*Dur*), whose portion of the entire variability almost reached 40 %. Therefore, the data variation is well enough explained by the effect of the factors “population” and “family”.

The variability among families may be due to both genetic and environmental causes. Maternal effects are expected to be strong at this ontogenetic stage (Roach and Wulff 1987), which contribute to an increase in the family variation. Unfortunately, with our material we cannot distinguish between the effect of nuclear genes and maternal effects [either reciprocal crosses (not possible with *A. chilensis* due to its dioecy) or at least seeds collected from a common garden trial (e.g. El-Kassaby et al. 1992) would be necessary], which meant we could not estimate additive variances and their derived genetic parameters, such as heritability or quantitative differentiation ( $Q_{ST}$ ).

Although we cannot estimate the weight of maternal effects, the relatively high proportion of family variability in *EC* and *EE* leads us to presume sufficiently high levels of additive genetic variance so as not to hinder evolvability [in the sense of Houle (1992)] specifically in these traits. With respect to *EP* and  $t_{10}$ , the opportunities for adaptation seem fewer, and consequently, local extinctions driven by the global climate change would seem to be a possible scenario. This is in agreement with expeditious observations in the studied populations, since recruitment seems normal in some of them, while in others we found almost no regeneration. Perhaps we are already going through such a process. The corroboration of these suppositions would make active intervention through assisted regeneration necessary if we decide to preserve the entire receding edge of the Patagonian Cypress, a task for which scientific research must indefectibly combine with the stakeholder's commitment.

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