

# Genetic versus environmental contributions to variation in seedling resprouting in *Nothofagus obliqua*

Alejandro Gabriel Aparicio · Sebastián Maximiliano Zuki ·  
María Marta Azpilicueta · Fernando Álvaro Barbero ·  
Mario Juan Pastorino

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**Abstract** Resprouting is an almost universal functional trait in temperate angiosperms and confers persistence at an individual level after the loss of above-ground biomass. The importance of genetic versus environmental sources of intraspecific variation in resprouting traits is largely unknown. We conducted two genetic field trials, 400 m apart in altitude, in order to sequentially assess seedling resprouting in four environmentally contrasting Argentinean populations of *Nothofagus obliqua* Mirb. (Oerst). We also performed one nursery test to determine whether populations differed in early root/shoot biomass partition, a key trade-off affecting resprouting. Initial resprouting vigour and final survival were higher in our warmer test site, located 300 m below the core range of the species. The main contrasts between populations were found for pre-clipping seedling size and resprouting profusion, the latter trait showing a clear trade-off with resprouting vigour. Site × population interactions were due mainly to the behaviour of the highest altitude population, suggesting its divergent adaptive trajectory and higher plastic-

ity for resprouting traits. Within populations, trait heritability was low, in general. Episodic frost, which may limit resprouting vigour and final success, had a lower incidence in the altitudinal and xeric limit populations. Overall, our work revealed genetic variation between populations of *N. obliqua* in traits that determine the success of seedling resprouting, probably associated with divergent selection. Low trait heritability suggests limited in situ micro-evolutionary capacities for resprouting traits under ongoing climate warming; phenotypic plasticity may play an important role in population persistence at the low positions of the elevation gradients *N. obliqua* currently inhabits.

**Keywords** Mediterranean climate · Southern angiosperms · Fitness traits · Population differentiation · Heritability

## Introduction

Resprouting is an almost universal syndrome in temperate angiosperms (Del Tredici 2001). This mechanism confers persistence at an individual level in response to recurrent events that cause the loss of above-ground biomass, especially in ecosystems prone to disturbances mediated by fire and drought (Bond and Midgley 2001; Paula and Pausas 2006). Variation in plant resprouting ability has been studied from bio-geographical (e.g. Vesk and Westoby 2004) and phylogenetic (e.g. Pausas and Verdú 2005; Poorter et al. 2012) approaches, but little is known about intraspecific genetic variation, particularly in trees (Clarke et al. 2013).

Resprouting ability is determined by the development, protection and resourcing of a viable bud bank (Clarke et al. 2013). This implies trade-offs involving energy and time, such as nutrient and carbohydrate allocation to storage organs to the

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A. G. Aparicio (✉) · M. M. Azpilicueta · M. J. Pastorino  
Grupo de Genética Ecológica y Mejoramiento Forestal, INTA EEA  
Bariloche, Modesta Victoria 4450 - INTA, 8400 San Carlos de  
Bariloche, Río Negro, Argentina  
e-mail: aparicio.alejandro@inta.gob.ar

A. G. Aparicio  
e-mail: aleaparcioar@yahoo.com

S. M. Zuki · F. Á. Barbero · M. J. Pastorino  
Consejo Nacional de Investigaciones Científicas y Técnicas  
(CONICET), Buenos Aires, Argentina

detriment of resource investment in aerial growth (Bellingham and Sparrow 2000; Bond and Midgley 2001). Consequently, genotypes with the highest fitness for growing and outcompeting may not have the highest fitness for successful resprouting after disturbance. Resprouting ability may be under selection pressure not only from disturbance regimes but also from site productivity and climate (e.g. Clarke et al. 2005). Therefore, the selective pressure on resprouting ability would change between and within populations across a species' distributional range. Whereas resprouting of adult trees allows the extension of lifespan and contributes to maintaining population genetic diversity, seedling resprouting may have more profound effects on population fitness<sup>1</sup>, since it implies the survival of novel, recombined genotypes. This may enhance opportunities for adaptive evolution under climate warming and associated changes in the frequency and severity of disturbances.

In this work, we use *Nothofagus obliqua* Mirb. (Oerst) (Nothofagaceae) as the experimental model species to assess intraspecific genetic variation in seedling resprouting. *N. obliqua*, locally known as 'roble pellín', is a Southern Hemisphere deciduous beech, whose collar resprouting capacity is a recognized form of persistence (Donoso Zegers 1993). In the context of a native tree domestication program (INTA project; <http://inta.gob.ar/proyectos/pnfor-1104063>), we have installed a network of provenance and progeny tests with *N. obliqua*, in which herbivore foraging allowed us to observe variable resprouting responses among seed sources and across test sites, suggesting genetic variation.

In the Argentinean distribution of *N. obliqua*, there is a latitudinal division in drought and fire regimes, which are key determinants of resprouting life histories in Mediterranean ecosystems (e.g. Paula and Pausas 2011). The 38–43° S range, which encompasses most of the species distribution, is characterized by frequent severe droughts and high fire activity (Whitlock et al. 2006). North of 38° S, seasonal rainfall is enhanced during El Niño (fire-promoting dry cycles), thus humidity limiting the role of fire (Markgraf et al. 2009). These coarse-scale environmental and disturbance patterns, with superimposed climatic and local terrain features, determine the presence of *N. obliqua* across disjoint lake basins, from humid dense mixed forests to pure open stands in xeric locations (Donoso Zegers 1993). As evidenced by cpDNA and nSSR phylogeography, there is also a latitudinal pattern of neutral genetic variation, with two main clusters delimited approximately at 39° 30' S (Azpilicueta et al. 2013). Our empirical background, contextualized in a regionally variable environment and considering possible evolutionary imprints, led us

to our main research questions: (1) has the evolutionary history of *N. obliqua* driven divergent resprouting syndromes among populations? and (2) is genetic variation in resprouting ability constrained within populations, or is there still a possibility for micro-evolution under environmental changes?

As a syndrome, resprouting can be assessed as a complex set of plant qualities and sequential events which determine the persistence of a genotype after a disturbance causing the loss of aerial biomass. Here, we use a step mechanistic model that proved useful in the sequential analysis of environmental factors driving intraspecific responses in Mediterranean subshrubs (Moreira et al. 2012). We enrich the model by including genetic factors that may explain the variation in the resprouting capacity of *N. obliqua*. Firstly, we assess the between-population differences and within-population variance of seedling biomass allocation; it is widely known that by allocating to root mass fraction, plants fund future resprouting capacity in a trade-off with current aerial growth and thus competitive ability (Clarke et al. 2013). Next, we focus on spatial variation in seedling resprouting ability and final success. Our objectives were: (1) to quantify the resprouting success of *N. obliqua* during the seedling stage and (2) to evaluate the relative contribution of genetic and environmental controls to the variability of resprouting-related traits. The implications of our results for the conservation and management of *N. obliqua* in the climate change scenario are discussed.

## Materials and methods

### Plant material and early biomass partition experiment

We planted a nursery common garden trial with the objective of determining between- and within-population patterns of biomass allocation (shoot/root) in seedlings of *N. obliqua*. To obtain the plants, we used open-pollinated seed samples from 12 randomly selected trees from each of four wild populations. Our population sampling was intended to capture several contrasting features of *N. obliqua* along its Argentinean distributional range. We sampled: (1) Epulauquen (E) population, located at the northern extreme and the highest altitude of the species' distribution range; (2) Pilo-lil (PL), the most xeric population of the species and (3) Yuco (Y) and Catritre (CA), i.e. the north and south banks of Lake Lácar, around which *N. obliqua* forms its largest forest patches. Lake Lácar is the southern limit of the species in Argentina (Sabatier et al. 2011). While Epulauquen and Pilo-lil are mono-specific isolated populations, in Lake Lácar basin, *N. obliqua* inhabits altitudinal gradients along which it mixes with *Nothofagus dombeyi* (Mirb.) Oerst. 'coihue' and *Nothofagus alpina* (Poepp. & Endl.) Oerst. 'raulí'. Our sample comprises the two main genetic zones

<sup>1</sup> Seedling resprouting may be especially important to population fitness in species that resprout but do not propagate free-living ramets such as *N. obliqua*. Clonality can play a major role in micro-evolution (e.g. Dodd et al. 2012; Pan and Price 2002)

defined for the species at the eastern side of the Andes (Azpilicueta et al. 2013; Table 1).

The seeds were stratified according to standard protocols developed for the species (Azpilicueta et al. 2010). Two weeks after germination (20 October 2008), we planted the seedlings in plastic 25-l pots filled with organic soil material (four seedlings per pot). The pots were arranged in a randomized complete (single-tree plot) block design, with eight replicates of 12 open-pollinated families (OPF) per population. We bred the seedlings during one growing season in our experimental nursery (INTA Bariloche), under moderate sunshine protection (50 % transmittance shade cloth) and lateral wind defence; the pots were well watered throughout the experiment. In June 2009 (winter dormancy, plants without leaves), we measured seedling shoot height (sh) and basal diameter (sbd) and we obtained the root (Rdw) and shoot (Sdw) dry biomass by drying these plant fractions for 48 h at 72 °C.

#### Field resprouting experiments

We planted two common garden trials in September 2009: (i) Bariloche (41° 07' 24" S, 1° 14' 58" W) and (ii) Golondrinas (41° 59' 57" S, 71° 31' 36" W). Bariloche is located at 790 m.a.s.l., i.e. the altitude at which most of *N. obliqua* distributional range lies in Argentina; mean annual temperature is 8.6 °C (mean minimum=3.5 °C; mean maximum=13.8 °C), and mean annual precipitation is 840 mm. Golondrinas is located at 415 m.a.s.l.; mean annual temperature is 9.8 °C (mean minimum=3.6 °C; mean maximum=16.2 °C), and mean annual precipitation is 950 mm. During our experiment, the Bariloche trial experienced an episodic summer frost (−3 °C on 23 December 2011), which caused moderate to severe apical and leaf damage; seasonal autumn frosts also caused apical damages. Such injuries were not observed in Golondrinas.

In each trial, we used 240 1-year-old seedlings (24 plants × 10 OPFs) from each *N. obliqua* population described in Table 1, corresponding to the same seed collection and sowing of the seedlings used in the early biomass partitioning

experiment. These plants were cultivated under greenhouse conditions, in 265 cm<sup>3</sup> seedling containers (HIKO™ HV265) filled with inert substrate (volcanic sand+sphagnum peat in equal proportions), using a ferti-irrigation regime. At planting in the ground, seedlings were, on average, 44.9±12.8 cm high. We installed both trials in row–column single-tree-plot designs, with a 0.25×0.25 m configuration; the crossed blocking ( $h=4\times q=6$ ) factors corresponded to lateral shadow gradients provided by conifer windbreaks. The seedlings grew under field conditions for 2 years; just before the third growing season (15 September 2011), we clipped their aerial biomass to the ground level. We did not apply any agrochemical product to the cuts. Weeds were hand-removed to keep the plants free of competition during the first post-clipping growing season.

In both trials, we measured the first-resprouting traits once the resprouts entered their first dormant season (June 2012). We surveyed resprouting following the step-model proposed by Moreira and colleagues (2012), which considers pre-disturbance condition, resprouting ability and post-resprouting survival. Specifically, we measured pre-clipping seedling height (Sh) as an indicator of pre-disturbance condition. Resprouting ability was estimated by means of initial resprouting capacity (IRC, binary), resprouting vigour (RV, the percentage of Sh reached by the dominant resprout) and resprouting profusion (RP, the number of dominant and co-dominant shoots per plant). Finally, post-resprouting survival (RS, binary) was measured at the beginning of the second spring (i.e. one growing and one dormant season after clipping). In Bariloche (the colder site), we also recorded the damage caused by an episodic summer frost (SFD, the percentage of green tissue damaged) and seasonal autumn cold (AFD, dominant and/or co-dominant apices damaged or not) as a binary trait. Our measure of RV was intended to explain how seedlings recovered aerial size rather than biomass. Given that species of low maximum height can temporarily outpace taller ones (Falster and Westoby 2005), we argue that for tree seedlings, a height-based measure can better reflect vigour and correlate with the final success of the resprouts.

**Table 1** Geographic location, mean annual precipitation (MAP) and genetic zone of four wild populations of *Nothofagus obliqua* used for evaluating seedling resprouting capacity in two common garden genetic trials

Population	Latitude S	Longitude W	Altitude (m.a.s.l.)	MAP (mm year <sup>-1</sup> )	Genetic zone
Epulauquen (E)	36° 49' 07"	71° 04' 06"	1,500	1,500	North
Pilo-lil (PL)	39° 33' 12"	70° 57' 20"	780	680	North
Yuco (Y)	40° 09' 07"	71° 30' 39"	800	1,900	South
Catritre (CA)	40° 10' 32"	71° 24' 07"	700	1,600	South

MAP data were provided by the *Administración Interjurisdiccional de Cuencas* (AIC); for details on the definition of genetic zones of *N. obliqua*, see Azpilicueta et al. (2013)

Other proxies of RV involve biomass estimates, e.g. the product of the number of resprouts and maximum height (Moreira et al. 2012).

In order to evaluate the maintenance of resprouting capacity (MRC), we re-clipped the resprouts in the second growing season after the first disturbance (22 January 2013). We measured MRC as a binary trait in the plants that survived the first disturbance ( $N=866$  in Bariloche;  $N=868$  in Golondrinas); MRC was not surveyed immediately (i.e. during the same growing season as clipping), but once the plants had undergone the rest season and resumed growth the following summer (1 November 2013). We also analysed cumulative resilience (CR) as survival computed over the total initial seedlings.

## Data analysis

### Seedling root/shoot biomass partitioning

To determine whether our populations differed in average root/shoot (R/S) and the relative contribution of the ‘family’ effect to its variance, we fitted a linear mixed-effect model:

$$y_{hij} = \mu + \theta_h + P_i + F_{j(i)} + e_{hij} \quad (1)$$

$$e_{hij} \sim N(0, \sigma^2) \text{ and mutually independent,}$$

where  $y_{hij}$  is the value of R/S measured on a seedling of Family  $j$  (random) nested in Population  $i$  (fixed) and located on block  $h$  (random), with  $i$  (Population  $P$ ) = 1, ..., 4;  $k$  (Family  $F$ , nested in Population  $P$ ) = 1, ..., 12 and  $h$  (block  $\theta$ ) = 1, ..., 8, and  $e_{hij}$  is the residual error. For post hoc population mean comparisons, we applied simultaneous multiple Tukey contrasts with the ‘glht’ function in the ‘multcomp’ package (Hothorn et al. 2008) in R 2.15.1 (R Development Core Team 2012).

We described the direction of the ontogenetic/size drift in biomass partitioning by calculating the slope of the regression between the root and shoot dry weight fractions for each population. We used ordinary least squares (OLS) regression forced through the origin (Zar 1999), with root dry weight as the explanatory variable. The choice of OLS regression is valid since the correlation coefficients for our data were  $r > 0.95$  in all populations; this means that slope parameters equivalent to Model Type II regression (e.g. simple orthogonal regression) would be obtained (Niklas 2004). We did not consider data transformation after checking linearity of model fit. We compared the regression slopes using  $t$  tests for all the pairwise contrasts of model coefficients, using the ‘estimable’ function of the ‘gmodels’ package (Warnes 2013) in R 2.15.1, which obtains the degrees of freedom from the regression model applied simultaneously to all the populations.

### Phenotypic correlations between resprouting traits

In order to determine the level of integration between traits, we calculated the phenotypic correlations at individual level (pooled meta-population); we used Pearson ( $r$ ) correlation for continuous variables, Spearman ( $r_s$ ) for continuous-count traits, point-biserial ( $r_{pb}$ ) for continuous-binary traits and phi ( $r_\phi$ ) correlation for two binary traits.

We also described size–vigour relationships (between sites and populations), fitting OLS regression models:

$$\ln(\text{RV}) = \alpha + \beta \cdot \ln(\text{Sh}),$$

where  $\alpha$  is  $\ln(\text{RV})$  when  $\text{Sh}=1$  cm and  $\beta$  describes the slope of the allometric line; we used Prism4 (GraphPad Software, San Diego, CA, USA).

### Variance components analysis of resprouting traits

We performed variance components analyses using linear (continuous traits: Sh, RV, RP, SFD) and logistic (binary traits: IRC, AFD, RS and MRC) mixed effect models. In the case of RP (count), the use of a linear model (followed by MCMC sampling, see below) is preferred, since zero is not included within the range of resprouted shoots, thus violating one main assumption of Poisson models. We used the ‘lmer’ function of the ‘lme4’ package (Bates et al. 2011) in R 2.15.1. For model fit to the continuous traits, we used restricted maximum likelihood (REML) estimation. For the binary traits, we set the ‘lmer’ function to implement the Laplace approximation to the marginal likelihood, with the logit link function. The general form of the generalized linear mixed model (GLMM) for our row–column single-tree-plot design is (Dean and Voss 1999):

$$y_{hqijk} = \mu + \theta_{h(i)} + \varphi_{q(i)} + S_i \times P_j + F_{k(j)} + e_{hqijk} \quad (2)$$

$$e_{hqijk} \sim N(0, \sigma^2), \text{ and mutually independent;}$$

where  $y_{hqijk}$  is the phenotypic value of the response variable measured on a seedling located in column  $h$  (random) and row  $q$  (random) of Site (trial location)  $i$  (fixed), and belonging to the Family  $k$  (random) nested in Population  $j$  (fixed), with  $h$  (column-block  $\theta$  nested in Site  $i$ ) = 1, ..., 4;  $q$  (row-block  $\varphi$ , nested in Site  $i$ ) = 1, ..., 6;  $i$  (Site  $S$ ) = 1, 2;  $j$  (Population  $P$ ) = 1, ..., 4;  $k$  (Family  $F$ , nested in Population  $P$ ) = 1, ..., 10; and  $e_{hqijk}$  is the residual error. Genotype  $\times$  environment interaction was evaluated at population level ( $S_i \times P_j$  fixed effects term); we did not include random Family  $\times$  Site interactions, since we did not focus on the stability of within-population genetic effects between sites (which could be a breeding objective). In the case of SFD and AFD, the models did not include the

$S \times P$  interaction term. Although in some cases using continuous covariates may seem prescriptive (e.g. SFD for explaining RV), we discarded their use to avoid collinearity with the categorical explanatory variables.

After checking the lack of (or poor) correction of non-normality of some models' residuals, we decided not to use data transformations, e.g. the arcsin transformation of proportions (Zar 1999) such as SFD. In the case of linear REML fits, we implemented Markov chain Monte Carlo samplings from the posterior distribution of the models' parameters, with the 'pvals.fnc' function of the 'languageR' package (Baayen 2011). MCMC sampling is an efficient technique to evaluate the stability of linear model parameters and to distinguish between robust and superfluous ones. We obtained the 95 % highest posterior density (HPD) interval for each of the fixed and random effects and used them to evaluate statistical significance: HPD intervals that do not include zero provide statistical evidence of the significant effect of the specified parameters, with the interval range indicating variability of the response (Baayen et al. 2008).

To evaluate the contribution of random effects to the model fit to the binary traits, we performed likelihood ratio tests (LRTs) for the null hypotheses that simpler models fitted after removing a random effect of interest from the complete models were sufficient (Faraway 2006). The contribution of the fixed effects was assessed via  $z$  statistics, which approximates the relative qualities of one model with and one model without the fixed term in question.

#### Differentiation between populations in resprouting-related traits

We estimated the coefficients of between-population genetic differentiation, i.e. Wright's  $Q_{ST}$  (Spitze 1993), for R/S and the continuous resprouting traits at each study site through the partition of the phenotypic variance into its intra- and inter-population components. For this, we rearranged the mixed models with Population as a random effect:

$$Q_{ST} = \frac{V_{POP}}{(V_{POP} + 2V_A)}$$

where  $V_{POP}$  is the between-population additive variance component and  $V_A$  is the mean additive genetic variance within populations. We performed non-parametric bootstrap (O'Hara and Merilä 2005) to calculate 95 % confidence intervals (CI95) for the  $Q_{ST}$  estimates. This was done by resampling  $n=1,000$  times with replacement from the original data, both at family and population levels, and maintaining the experimental designs. With

the empirical  $Q_{ST}$  values, we calculated the CI95 intervals using the 'quantile' (Type-8) function in R 2.15.1; this quantile type is approximately median unbiased, regardless of the distribution of the variable (Hyndman and Fan 1996).

We quantified the between-population genetic differentiation at presumptively neutral marker loci by the  $F_{ST}$  parameter (Wright 1951). The  $F_{ST}$  CI95 was estimated by bootstrapping using FSTAT v.2.9.3 (Goudet 2001). The  $F_{ST}$  values were obtained with the genotypic information provided by the analysis of 30 to 36 adult trees per population, at seven nuclear micro-satellite loci. For details on plant material, laboratory methods and data analyses, see Azpilicueta et al. (2013).

If a random set of potentially adaptive traits has  $Q_{ST}$  values greater than a mean  $F_{ST}$ , we might infer that the sampled populations experience geographically variable selection, sufficiently strong relative to genetic drift and mutation (Whitlock 2008). We compared both measures of genetic differentiation for our populations to test the hypothesis that the resprouting traits have diversified more than would be expected by random processes alone.  $Q_{ST}$  and  $F_{ST}$  were considered to be statistically different when their 95 % confidence intervals did not overlap (Sahli et al. 2008).

#### Trait heritability

Heritability allows comparison of the relative importance of genes and environment to variation in quantitative traits. For those traits that showed significant 'family' variance components in model Eq. 2, we estimated the narrow-sense heritability:  $h^2 = V_A/V_P$ , where  $V_A$  is additive genetic variance and  $V_P$  total phenotypic variance (i.e. the sum of genetic, design and residual variances). We calculated the standard errors of the heritabilities ( $SE_{h^2}$ ) with the formula derived from Falconer and Mackay (1996).  $V_A$  was calculated as:

$$V_A = \frac{1}{2\theta} V_{FAM},$$

where  $\theta$  is the coefficient of co-ancestry, which is half the coefficient of relatedness ( $r$ ) for diploids (Lynch and Walsh 1998), and  $V_{FAM}$  is the between-family variance component. Since heritability is a population and site-specific parameter, we extracted  $V_{FAM}$  components from mixed models fitted to each trial and population separately. We used  $\theta=0.137$ , a value derived from isozyme genotypes obtained for a set of populations that included PL, E and CA (Pastorino M, Marchelli P, Azpilicueta M, unpublished publication). This value is consistent with the reproductive system of *N. obliqua*, a monoecious species with potential full-sib progeny by self-pollination (Squillace 1974).

**Results**

Phenotypic variation of resprouting-related traits and between-trait association

*Root – shoot (R/S) early biomass partition*

The seedlings used for determining early root–shoot biomass partition had average height and basal diameter of  $sh=64.8\pm 45.7$  mm and  $sbd=1.60\pm 0.68$  mm. Their average root and shoot dry weight fractions were  $Rdw=0.286\pm 0.364$  g and  $Sdw=0.095\pm 0.108$  g respectively, with an average ratio of  $R/S=2.94\pm 1.10$ . The variance components analysis for the R/S fraction showed a significant population effect: except for the Y–CA contrast, all the post hoc comparisons of means were significant (Table 2). The effect of family on the variance of R/S was nil.

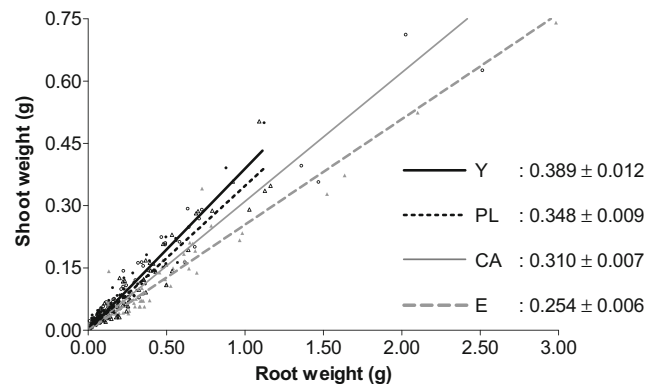
The slope of the regression of shoot on root dry weight (Fig. 1) was lower than 1 for all the populations; the pairwise contrasts between the slopes were all significant.

*Pre-clipping seedling size (Sh)*

Prior to clipping, the seedlings grew to  $99.2\pm 32.9$  cm, on average (Table 3). Due to significant Site×Population ( $S\times P$ ) interaction, the main explanatory effects for Sh cannot be interpreted clearly. This interaction was mainly explained by the between-site contrasting performance of Epulauquen (E) population (Table 4); seedling performance in E was poorer in both trials, but in Golondrinás average seedling height growth was much lower than in the other populations (Fig. 2).

*Initial resprouting capacity (IRC)*

Seven weeks after clipping, most seedlings had resprouted in both trials ( $IRC=97.6$  % in Bariloche and  $IRC=90.7$  % in Golondrinás). Overall, IRC was very weakly associated with Sh ( $r_{pb}=0.082$ ;  $p<0.001$ ); the correlation almost doubled



**Fig. 1** Shoot dry weight as a function of root dry weight of 1-year-old seedlings from four *Nothofagus obliqua* populations. The slopes of the regressions (slope parameters±standard errors) were all significantly different from each other

( $r_{pb}=0.13$ ;  $p<0.001$ ) in Golondrinás, where the non-resprouted plants were shorter (mean  $Sh=87.1\pm 34.8$  cm) than the resprouted ones (mean  $Sh=103.4\pm 32.4$  cm) ( $t=-4.50$ ;  $p<0.001$ ). Despite the high incidence of resprouting, the variance components model detected a significant  $S\times P$  interaction effect (Table 4), reflecting the lower proportion of resprouted seedlings of E population in Golondrinás (Table 3).

*Post-resprouting environmental constraints (SFD and AFD)*

In Bariloche, one episodic early-summer frost caused moderate to severe apex and leaf damage ( $SFD=37.5$  % of tissues damaged, on average); in addition, seasonal autumn cold caused apical damage (AFD) in 64 % of resprouted plants. Both types of damage were correlated ( $r_{pb}=0.40$ ;  $p<0.001$ ) and negatively associated with resprouting vigour (RV) ( $r=-0.29$ ,  $p<0.001$  for RV–SFD, and  $r_{pb}=-0.35$ ,  $p<0.001$  for RV–AFD). The effect of the episodic frost was significantly lower in E and PL populations; their predicted proportions of damage were ca. 20 and 10 % lower than the model intercept, i.e. the estimate for CA population (Table 4). Autumn frost also had a lower incidence in PL population (ca. 6 % lower than the AFD predicted for CA population).

**Table 2** Variation of root and shoot dry weight fractions of 1-year-old seedlings from four *N. obliqua* populations cultivated under nursery conditions

Population	R/S fraction	p-MCMC	CA	E	PL	Y
CA	2.54 (2.28–2.82)	<0.001	–	$t=6.32$ ; $p<0.001$	$t=-3.31$ ; $p=0.001$	$t=-5.56$ ; $p<0.001$
E	3.51 (2.90–4.10)	<0.001	$z=5.90$ ; $p<0.001$	–	$t=-8.79$ ; $p<0.001$	$t=-9.90$ ; $p<0.001$
PL	3.03 (2.40–3.64)	0.008	$z=2.83$ ; $p=0.024$	$z=-2.88$ ; $p=0.020$	–	$t=-2.70$ ; $p=0.007$
Y	2.59 (1.97–2.66)	0.802	$z=0.25$ ; $p=0.994$	$z=-5.72$ ; $p<0.001$	$z=-2.62$ ; $p=0.044$	–

Columns 2–3: Fixed effect population parameters (MCMC-mean with highest posterior distribution intervals in brackets) and associated  $p$  values obtained with MCMC sampling on the posterior distribution of the mixed model Eq. 1 parameters. Columns 4–7: Above the diagonal, the statistics of the pairwise contrasts between the allometric slopes of the shoot–root regressions; below the diagonal, the statistics of the post hoc multiple comparisons of means of the R/S fraction. The p-MCMC values indicate the significance of the population parameters with regard to the model’s intercept, which corresponds to CA population.

**Table 3** Summary statistics of seedling resprouting traits in four *N. obliqua* populations planted in two common garden field trials (Bariloche and Golondrinas)

Population	Bariloche							Golondrinas				
	Sh	IRC	RV	RP	SFD	AFD	RS	Sh	IRC	RV	RP	RS
CA	99.9±32.3	97.1	68.9±24.1	5 (2.0)	45.2±23.3	67.6	89.2	116.4±34.4	93.3	92.5±29.9	2 (0.5)	100
E	87.4±27.3	98.3	59.8±20.2	7 (2.0)	25.6±16.9	70.6	98.3	79.3±28.4	85.0	93.7±30.5	3 (1.0)	100
PL	96.9±28.6	97.0	69.5±27.5	4 (1.5)	35.7±22.0	49.3	90.4	103.9±31.5	89.3	89.3±29.5	2 (0.5)	99.5
Y	101.3±31.6	97.9	69.0±26.3	4 (1.5)	43.6±21.6	68.0	92.3	108.2±33.8	93.3	93.6±32.4	2 (0.5)	100
Average	96.4±30.4	97.6	66.7±24.9	5 (2.0)	37.5±22.4	64.0	92.5	101.9±34.9	90.7	92.3±30.7	2 (1.0)	99.8

For the continuous traits (Sh, RV and SFD), we show the mean±one standard deviation; for the binary traits (IRC, AFD and RS), we show the incidence (%) and for counts (RP), the median with the quartile deviation (Zar 1999) are enclosed in parentheses

Sh pre-clipping seedling height (cm), IRC initial resprouting capacity (binary), RV resprouting vigour (% of Sh), RP resprouting profusion (count), SFD summer frost damage (% of foliage affected), AFD autumn frost damage (binary), RS survival of the resprouts (%).

### Resprouting vigour and profusion (RV and RP)

The height of the dominant resprout per plant was correlated ( $r=0.48$ ,  $p<0.001$ ) with initial seedling height (Sh) (data not shown). Resprouting vigour (RV), which scaled between 7 and 258 %, was negatively correlated with Sh in both trials (Bariloche:  $r=-0.44$ ,  $p<0.001$ ; Golondrinas:  $r=-0.57$ ,  $p<0.001$ ). The regression slopes of the allometric  $\ln$  Sh– $\ln$  RV relationships<sup>2</sup> did not differ between trials ( $F=0.63$ ,  $p=0.427$ ) for the data pooled across populations; instead, the intercepts were different ( $F=601.86$ ,  $p<0.001$ ) (Fig. 3-a). When comparing the linear fits by population, we found that for Epulauquen, both the intercepts ( $F=13.8$ ,  $p<0.001$ ) and the slopes ( $F=6.14$ ,  $p=0.014$ ) differed between trials (Fig. 3-b). For the other populations, the allometric slopes did not differ between trials.

On average, resprouting vigour (RV) was ca. 25 % higher in Golondrinas (Fig. 2). There was a significant  $S\times P$  interaction, which was mainly explained by the between-site performance of E population (Table 4). Overall, there was a moderate, negative correlation between RV and resprouting profusion (RP) ( $r_S=-0.48$ ,  $p<0.001$ ); this association was higher in Bariloche ( $r_S=-0.39$ ,  $p<0.001$ ) than in Golondrinas ( $r_S=-0.13$ ,  $p<0.001$ ).

Resprouting profusion (RP) was much higher in Bariloche, with plants that produced a maximum of RP=30 co-dominant resprouts, while in Golondrinas, the maximum was RP=11 (Table 3). In Golondrinas, RP had almost nil association with

Sh ( $r_S=-0.07$ ,  $p=0.004$ ). In Bariloche, RP was not associated with Sh or with SFD.

The variance components model fitted to RP showed a significant  $S\times P$  interaction, which was explained by the effect of E population: it was, in general, the most profuse resprouter, although in Golondrinas, E seedlings resprouted less profusely than expected in the absence of interaction.

### First resprouting success (RS)

Survival after the first post-clipping growing and dormant seasons was very high (Table 3). RS had low (although significant) association with Sh ( $r_{pb}=-0.19$ ,  $p<0.001$ ) and RV ( $r_{pb}=0.25$ ,  $p<0.001$ ). In Bariloche, the individual survival scores were negatively correlated with the injury levels caused by both episodic and seasonal cold ( $r_{pb}=-0.36$ ,  $p<0.001$  for RS–SFD;  $r_{\varphi}=-0.16$ ,  $p<0.001$  for RS–AFD). The variance analysis detected as significant the slightly higher proportion of resprouts from E population that finally survived, as well as the small difference between trials (Table 4).

### Resilience under successive disturbances (MRC and CR)

Both maintenance of resprouting capacity after the second clipping (MRC:  $z=3.753$ ;  $p<0.001$ ) and cumulative resilience (CR:  $z=2.951$ ;  $p=0.003$ ) were significantly higher in Golondrinas (MRC=92.7 %; CR=84 %) than in Bariloche (MRC=71.6 %; CR=68 %). For MRC, we found no differences between population means or a significant ‘family’ effect. For CR, there was a significant  $S\times P$  interaction ( $z=-2.695$ ;  $p=0.007$ ), which was nevertheless explained mainly by the effect of the lower initial resprouting of E population in Golondrinas (see Table 4).

<sup>2</sup> The high, unexplained variances of the  $\ln$  Sh– $\ln$  RV relationships can be attributed to variation in traits that are important for resprouting but not measured here, such as starch and nutrient concentration (e.g. Moreira et al. 2012), which usually span orders of magnitude within tree classes. The highest determination ( $r^2=0.58$ ) corresponded to the allometric fit of Epulauquen in Golondrinas test (Fig. 3b), suggesting lower variance for the above-mentioned or other unmeasured explanatory traits.

**Table 4** Fixed effect parameters from mixed model analyses of variance of seedling resprouting traits in two common garden tests with four *N. obliqua* populations

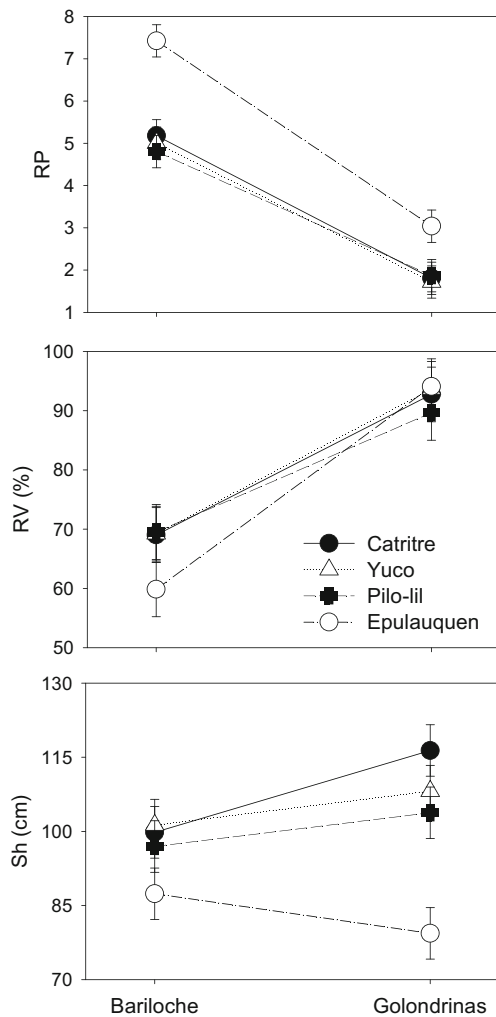
Fixed effects	Sh	IRC	RV	RP	SFD	AFD	RS
Intercept*	99.85 (85.20–115.84)	3.65 (SE=0.46)	69.01 (58.76–79.58)	5.19 (4.17–6.19)	45.2 (34.73–55.67)	0.82 (SE=0.42)	2.36 (SE=0.41)
Trial site (S)	G: 16.54 (11.27–21.93)	ns	G: 23.66 (8.65–37.41)	G: -3.40 (-3.79 to -2.99)	-	-	G: 4.33 ( $z=4.21$ ; $p<0.001$ )
Population (P)	E: -12.43 (-20.60 to -4.46)	ns	E: -9.15 (-14.87 to -3.21)	E: 2.24 (1.78 to 2.71)	E: -19.63 (-24.9 to -14.59) PL: -9.10 (-14.47 to -4.30)	PL: -0.89 ( $z=-3.53$ ; $p<0.001$ )	E: 2.00 ( $z=3.35$ ; $p<0.001$ )
S×P	G×E: -24.63 (-32.15 to -17.28) G×PL: -9.65 (-17.30 to -2.23) G×Y: -9.69 (-17.10 to -2.09)	G×E: -1.51 ( $z=-2.11$ ; $p=0.035$ )	G×E: 10.48 (3.16–16.76)	G×E: -0.99 (-1.57 to -0.40)	-	-	ns

For the linear models fitted to Sh, RV, RP and SFD, we show in parentheses the HPD interval of each significant parameter. For the logistic models fitted to IRC, AFD and RS, we provide the standard errors of model intercepts and the  $z$  scores and associated  $p$  values as measures of parameter significance. The parameters are in the logit scale (back-transformations to proportions are used within the text when necessary)

Sh pre-clipping seedling height, IRC initial resprouting capacity, RV resprouting vigour, RP resprouting profusion, SFD summer frost damage, AFD autumn frost damage, RS survival after the first post-clipping growing and dormant seasons, HPD 95% highest posterior density intervals obtained with MCMC sampling from the posterior distribution of the parameters of the linear mixed effects models (see 'Materials and methods' for details), ns not significant, - the fixed effect in the header column was not used in model fit

\* The model intercept corresponds to Catritre population (CA) in Bariloche Trial (B); the remaining significant parameters are expressed relative to the intercept, with Golondrinas Trial (G) and Epulauquen (E), Pilo-lil (PL) and Yuco (Y) populations

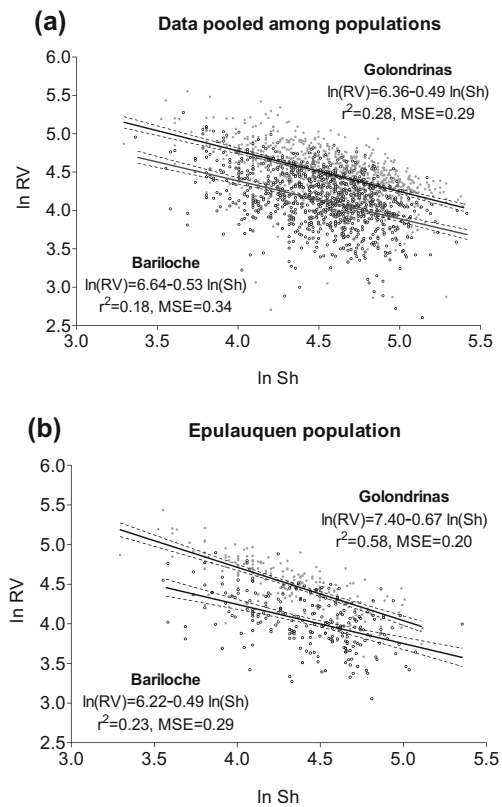




**Fig. 2** Site  $\times$  Population interactions for pre-clipping seedling height (*Sh*), resprouting vigour (*RV*) and resprouting profusion (*RP*) of four *N. obliqua* populations in two common garden trials (Bariloche and Golondrinas). The symbols are the mean effects conditional on the GLMM fits, with standard errors represented by whiskers

Genetic differentiation between populations in resprouting traits

Point  $Q_{ST}$  estimates were higher than the mean  $F_{ST}=0.074$  (0.024–0.127) in five out of eight traits (Table S1). As expected for a low number of sampled populations, the confidence intervals of the  $Q_{ST}$  estimates were large (O’Hara and Merilä 2005), with two cases of bad coverage, which also had the highest point estimates (the R/S fraction and RP in Bariloche). Adopting a cautious approach, only for *Sh* and RP in Golondrinas trial can we be quite confident that the  $Q_{ST}$  estimates were significantly higher than the  $F_{ST}$ , since the IC95 intervals of both parameters did not overlap, and the bootstrapped  $Q_{ST}$  distributions also had acceptable coverage (Fig. 4).

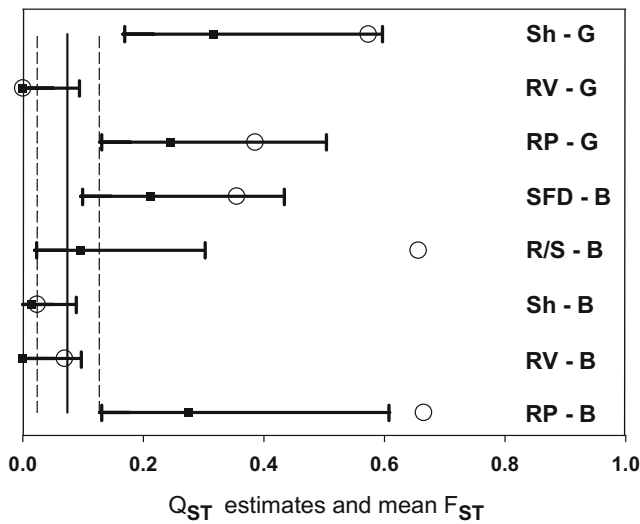


**Fig. 3** Resprouting vigour (*RV*) as a function of pre-clipping seedling height (*Sh*) in two common garden trials (Bariloche and Golondrinas) with *N. obliqua*. For the data pooled among populations, **a** the slope of the linear fit could be considered common between trials ( $\beta=-0.51 \pm 0.022$ ) when analysed by population, and **b** Epulauquen fitted a steeper line in Golondrinas trial. The *dashed lines* are the 95 % confidence intervals of the linear fits

Heritability of resprouting traits

The ‘family’ variance components extracted from the complete models Eq. 2 were low, although significant for most traits (Table 5). When we estimated the heritability of those traits in each trial, we found that in Golondrinas only seedling height (*Sh*) in Yuco (Y) population had significant heritability ( $h^2=0.29 \pm 0.19$ ).

In Bariloche, the heritability of *Sh* was moderate to high in all the populations: Y and Catritre (CA) had equal heritabilities ( $h^2=0.45 \pm 0.25$ ); Pilo-lil (PL) had the lowest value ( $h^2=0.37 \pm 0.22$ ) and E the highest one ( $h^2=0.61 \pm 0.29$ ). Also, summer frost damage (SFD) was moderately heritable in populations CA and E ( $h^2=0.32 \pm 0.19$ ), and autumn frost damage (AFD) in PL ( $h^2=0.32 \pm 0.20$ ). The heritability of resprouting vigour (*RV*) (which had no significant  $Q_{ST}$ ) was low and only significant in E ( $h^2=0.20 \pm 0.16$ ) and CA ( $h^2=0.22 \pm 0.17$ ) populations. Resprouting profusion (*RP*) had no significant heritability.



**Fig. 4** Between-population genetic differentiation  $Q_{ST}$  (open circles are point estimates) for traits associated with resprouting in *N. obliqua* seedlings in two common garden trials, Golondrinas (G) and Bariloche (B). The traits are pre-clipping seedling height (Sh), resprouting vigour (RV), resprouting profusion (RP), root–shoot dry weight fraction (R/S) and degree of summer frost damage (SFD); the vertical solid line is the between-population neutral differentiation  $F_{ST}$  with its 95 % confidence interval (dashed line). The horizontal error bars are 95 % confidence intervals around the medians (black squares) of  $Q_{ST}$  empirical distributions obtained with nonparametric bootstrap

**Discussion**

Our quantitative study yielded three main results: (1) within-population additive genetic variances were low relative to environmental variances; (2) the genetic variance could be attributed largely to between-population differentiation, which proved significantly higher than neutral differentiation, especially in our warmer test site; and (3) genotype×environment interactions at population level were mainly explained by the behaviour of the high-altitude population. We will discuss our findings in the context of seedling qualities and sequential events that determined the success of the resprouts between and within populations, and their implications for the conservation and management of the species’ genetic resources in a warming climate and in the broad context of population divergence.

Phenotypic variation and association between resprouting traits

*Early root – shoot biomass partition*

Our four *N. obliqua* populations differed from each other in their shoot on root partition/size trajectories. Epulauquen, the highest northernmost population, had the lowest slope. Although the slope of the xeric Pilo-lil population was intermediate between Yuco and Catritre (Fig. 1), the between-

**Table 5** Phenotypic variance partitioning (in %) among random effects, from mixed model analyses of variance of seedling resprouting traits in two common garden tests with four *N. obliqua* populations

Random factors	Sh	IRC	RV	RP	SFD	AFD	RS
Family	4.90 (4.68–8.96)	2.33 ns	1.91 (1.96–5.98)	1.61 (0.08–0.46)	4.22 (2.13–6.04)	4.45 ( $\chi^2=7.64$ ; $p=0.006$ )	5.32 ns
Row	1.02 (1.06–9.05)	4.46 ( $\chi^2=8.16$ ; $p=0.004$ )	7.21 (4.39–11.71)	3.60 (0.23–1.04)	10.79 (3.65–14.9)	11.18 ( $\chi^2=65.95$ ; $p<0.001$ )	5.32 ns
Column	5.04 (3.36–27.45)	2.80 ( $\chi^2=3.94$ ; $p=0.047$ )	5.53 (3.07–11.70)	2.43 (0.15–1.72)	1.28 ns	5.65 ( $\chi^2=28.83$ ; $p<0.001$ )	6.43 ns
Residual error	89.04	90.41	85.34	92.35	83.71	78.72	82.93

For the linear models fitted to Sh, RV, RP and SFD, we show in parentheses the HPD interval of the standard deviation attributed to each significant factor, and for the logistic models fitted to IRC, AFD and RS, we show the  $\chi^2$  and  $p$  value of likelihood ratio tests as measures of factor significance  
 Sh pre-clipping seedling height, IRC initial resprouting capacity, RV resprouting vigour, RP resprouting profusion, SFD summer frost damage, AFD autumn frost damage, RS survival after the first post-clipping growing and dormant seasons, HPD 95 % highest posterior density intervals obtained with MCMC sampling from the posterior distribution of the parameters of the linear mixed effects models (refer to Materials and Methods for details), ns not significant

population contrasts for the root–shoot (R/S) fraction showed that seedlings from these two humid mixed populations invested, on average, more in shoot per unit of root biomass (Table 2). Within populations, we did not find significant additive genetic effects for R/S. These results suggest that *N. obliqua* may have evolved population specific root–shoot functional equilibria at seedling stage, which may, in part, reflect adaptation to regional (ancient and/or current) disturbance regimes (Bellingham and Sparrow 2000; Clarke et al. 2013) and also to local factors such as water, nutrient and light availability (Reich 2002). Based on the nursery results, we expected that in our field tests, the seedlings from Epulauquen and Pilo-lil would display higher resprouting capacity, vigour and survival than those from the populations of Lake Lácar. The hypothesis behind this expectation was that by investing more in the root biomass fraction, the first two populations fund future resprouting capacities at the expense of current aerial growth and thus the ability to compete spatially, which could be more necessary (potentially adaptive) in the mixed forests of Lake Lácar. This hypothesis has been widely tested at an interspecific level (e.g. Schwilk and Ackerly 2005).

#### Initial resprouting abilities

Previous studies emphasize the importance of plant size (and stored reserves, not measured here) for the vigorous initiation of resprouting (e.g. Moreira et al. 2012). Here, pre-clipping seedling size (Sh) was associated with our vigour measure (RV), but not with resprouting profusion (RP), which, as we will discuss, could be an important trait for the resprouting strategy of some populations. Also, the difference in mean heights between the resprouting (90 % of seedlings) and non-resprouting plant groups in Golondrinas suggests that the initial probability of resprouting may depend, in part, on certain size-related traits; this may have implications if disturbances occur at earlier stages (smaller plant size) of seedling recruitment.

The negative correlation ( $r=-0.44$ ) between seedling height (Sh) and resprouting vigour (RV) reflects, in part, biological limits to growth, i.e. the probability of recovering in one growing season the size reached over the previous 2 years should decrease with that size. Growth to storage (not measured) trade-off may also explain this correlation. At a meta-population level, the fact that the slope of the relationship between resprouting vigour and seedling height did not differ between trials suggests that changes with size in the allocation of resources to resprouting capacities could be under low environmental control. In contrast, the site-specific intercepts of the allometric relationship reflect different levels of potential resprouting vigour (Fig. 3a).

When we compared the size–vigour allometry by population, we found that Epulauquen followed a steeper slope in

Golondrinas, our warmer, more productive test site (Fig. 3b). One explanation could be that prevailing stressful native conditions in association with high altitude (1,500 m.a.s.l.) determine higher organ maintenance costs (Laureano et al. 2008); the fact that the pre-clipping height growth of Epulauquen seedlings was lower than the other populations in both trials supports this hypothesis. It is also likely that with the increment in growth of aerial parts, storage potential might have diminished more markedly in Epulauquen due to higher costs of obtaining and using carbon. Even if prior to clipping, Epulauquen had adjusted to the same allocation equilibria in both sites, the absolute of root respiration, which augments non-linearly with soil temperature (Burton and Pregitzer 2003), may have increased at a higher rate with plant size (root volume) in Golondrinas, thus lowering the rate of size recovery.

Our prediction based on biomass allocation results, i.e. that plants from Epulauquen and Pilo-lil would resprout more vigorously, was not fulfilled. Among the explanatory factors, the experimental location had the largest effect on resprouting vigour and profusion, the interactions with population being attributable to the relative performance of Epulauquen. In Bariloche, our plants resprouted much more profusely, while they were less able to recover their initial height than in Golondrinas; Epulauquen had the lowest capacity for height recovery (RV), probably as a correlated response ( $r_S=-0.39$ ) to multiple resprouting (RP). Multiple stems can limit maximum height because most stems emerge at an angle and are less securely attached to the roots (Falster and Westoby 2005), but also the remobilization of a fixed amount of reserves to multiple buds implies a trade-off. In Golondrinas, seedlings from Epulauquen produced many fewer shoots, and its mean vigour was similar to the other three populations, reflecting this height–profusion trade-off. Overall, Epulauquen displayed a more plastic interplay capacity between resprouting profusely and resprouting tall, depending on the environmental conditions. If we used the product of height and number of co-dominant resprouts as a biomass proxy (instead of RV, which reflects the capacity of recovering height), on average, resprouting would turn out to be much more vigorous in Bariloche (MCMC- $p=0.0012$ ), and Epulauquen would become, slightly, the population with highest vigour (MCMC- $p=0.04$ ), not interacting with site. This highlights the importance of profusion (RP) as a component of the resprouting syndrome of Epulauquen, probably more sensitive to environmental cues that determine whether resprouting profusely versus tall was the output. The fact that this high-altitude population resprouted more profusely in both experiments implies an intrinsic, genetic tendency to produce multiple stems to recover lost aerial biomass.

### *Environmental limitations to first resprouting success and resilience under successive disturbances*

The difference in resprouting profusion between trials was observed very early in the post-clipping growing season and was not associated with any site-specific factor or event. Neither was it correlated with the degree of summer frost damage (SFD) in Bariloche. It remains unclear which environmental factors determined the difference between sites in total post-clipping availability and/or resourcing of collar buds. Regarding the possible effect of apical frost damage on vigour, it has been demonstrated that in *N. obliqua*, the death of the apex of an annual extension unit does not necessarily imply a reduction in the final length of the annual shoot; the formation of second extension units can result in even longer shoots (Puntieri et al. 2006). Nevertheless, the moderate negative correlation ( $r=-0.29$ ) between the degree of summer-frost damage (SFD) and vigour (RV) indicates that foliar injuries may have reduced potential size recovery. Our results suggest that *N. obliqua* may have evolved under population-specific interactions between disturbance and temperature (frost) regimes. The higher degree of summer frost tolerance found for the resprouts of the altitudinal (Epulauquen) and xeric (Pilo-lil) margins of the species suggest their adaptation to a higher risk of episodic frosts.

Both survival and resilience (MRC) after the first and second clippings were higher in Golondrinas. Except for this site effect, the remaining (genetic) factors showed subtle variations, e.g. the slightly higher proportion of seedlings from Epulauquen surviving the first year. In Bariloche, the negative association ( $r_{pb}=-0.36$ ) between individual survival scores (RS) and frost injury levels (SFD) suggests that the final success of the resprouts could be determined by environmental limitations to the plants' initial capacity for recovering stored reserves.

### Between-population genetic differentiation and heritability of resprouting traits

In this work, we firstly aimed to explore whether differences in resprouting ability and success during seedling establishment exist between a specific set of environmentally contrasting populations of *N. obliqua*. We found that the genetic effect of population was significant in determining: (1) pre-clipping seedling state, i.e. seedling size and biomass partition, (2) initial resprouting abilities, i.e. initiation, vigour and profusion, (3) tolerance to episodic and seasonal frosts and (4) the final success of the resprouts. Within the populations, the additive genetic effects were, in general, much lower than the environmental variances (i.e. low or nil heritabilities). Only for seedling height (Sh) did we find a consistent pattern of heritable variation in Bariloche (average  $h^2=0.47$ ), which does not agree with the general expectation of higher

heritability in more productive environments (Visscher et al. 2008). A hypothesis for this may be that the residual variance of seedling height was higher in Golondrinas due to the cumulative effects of neo-formation as a response to warmer, extended growing seasons. Regarding cold tolerance during resprouting, only Pilo-lil population had moderate heritability ( $h^2=0.32\pm 0.20$ ) for seasonal frost damage (AFD), suggesting a certain micro-evolutionary capacity at this marginal xeric location. Overall, most of the genetic variance could be attributed to differentiation between populations (i.e. putative divergence).

Our approximation for testing the hypothesis of trait divergence, i.e. the overlap between  $Q_{ST}$  and  $F_{ST}$  confidence intervals, has been previously used (i.e. Sahli et al. 2008) and can be regarded as cautious with respect to comparing point  $Q_{ST}$  estimates to a mean  $F_{ST}$  or to a distribution of  $F_{ST}$  values. Despite our small number of populations, which may imply methodological problems associated with  $Q_{ST}$  properties (e.g. bad coverage of the confidence intervals; O'Hara and Merilä 2005), it seems that divergent selection could be a major process in determining the resprouting syndrome of *N. obliqua*. The  $Q_{ST}$  estimates varied depending on the test site, reflecting phenotypic plasticity. In Golondrinas, located ca. 300 m below the core range of the species (1,000 m below Epulauquen population), genetic differentiation was clearly expressed for seedling size (point  $Q_{ST}=0.57$ ) and resprouting profusion (point  $Q_{ST}=0.39$ ). In Bariloche, although the results of differentiation must be considered with caution because of methodological caveats, there were signs of divergence.

### Implications for conservation and management in a warming context

For forestry practices such as the selection of seed sources, the determination of seed transfer zones or genetic resource conservation programs, both molecular and quantitative (potentially adaptive) information must be considered. Our results can contribute to refining previous clustering of *N. obliqua* populations based on neutral variance, with information that can be crucial in a warming context with associated changes in disturbance regimes. For example, we have shown that Epulauquen and Pilo-lil populations, which belong to a single northern genetic zone as defined by cpDNA and nSSR information (Azpilicueta et al. 2013), differed in their resprouting ability. Together with other quantitative information (Azpilicueta et al. 2014), our results suggest that the northern genetic zone should be subdivided for management purposes.

Final success of the resprouts (RS) was higher in Golondrinas trial, located 300 m below the altitude at which most of *N. obliqua* distributional range lies in Argentina. The better performance of the resprouts in Golondrinas could be attributed to more rapid size (and probably reserves) recovery after clipping (RV was 25 % higher) and to the absence of frost

damage, which limited resprouting vigour and survival in Bariloche. Overall, the results suggest that *N. obliqua* is capable of plastic responses in seedling resprouting traits if ongoing climate changes generate warmer conditions along the elevation gradients the species inhabits. Directional changes in climate may well involve habitat modification due to increased disturbance levels (e.g. severe droughts, adult tree mortality, fires); this may imply complex interactions between *N. obliqua* and its congeners, *N. alpina* (resprouter) and *N. dombeysi* (non-resprouter) (Veblen et al. 1996), involving mixed persistence strategies. Uphill migration, the expected and most frequently observed response to climate warming (e.g. Kelly and Goulden 2008), is plausible for *N. obliqua*. Therefore, for the planning of assisted migration to keep abreast with progressive warming, interspecific interactions need to be carefully tested, with the appropriate experimental designs, including multi-specific genetic experiments.

*N. obliqua* is a valuable timber species and the object of a domestication programme. Our results show the potential for planting certain genetic sources of the species well outside its natural range. For example, the lower levels of both episodic (SFD) and seasonal (AFD) frost damage types, as well as the significant heritability of AFD ( $h^2=0.32$ ) in the xeric marginal Pilo-lil population, suggest that it could be a useful seed source for forestry within and even outside its genetic zone. Recent distribution-modelling studies suggest that *N. obliqua* may find suitable conditions at much higher than current latitudes. We might plan assisted migrations to southern locations, both for ex situ conservation and forestry. Seedling resprouting will play a crucial role when facing not only the expected increase in environmental variance and associated disturbances but also the pressure of biotic factors, such as introduced herbivores that cause the loss of above-ground biomass.

#### Data archiving statement

The phenotypic databases from our common garden experiments will be supplied through the revision process to the TreeGenes Database. This will be completed prior to final acceptance of the manuscript. The accession numbers will be supplied when available.

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