

Phenotypic selection on leaf functional traits of two congeneric species in a temperate rainforest is consistent with their shade tolerance

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Abstract Several studies across species have linked leaf functional traits with shade tolerance. Because evolution by natural selection occurs within populations, in order to explain those interspecific patterns it is crucial to examine variation of traits associated with shade tolerance and plant fitness at an intraspecific scale. In a southern temperate rainforest, two climbing plant species coexist but differ in shade tolerance. Whereas *Luzuriaga radicans* is most abundant in the shaded understory, *L. polyphylla* typically occurs in intermediate light environments. We carried out an intraspecific approach to test the hypothesis of differential selection patterns in relation to shade tolerance in these congeneric species. The probability of showing reproductive structures increased with specific leaf area (SLA) in *L. polyphylla*, and decreased with dark respiration in *L. radicans*. When reproductive output of fertile individuals was the fitness variable, we detected positive directional selection on SLA in *L. polyphylla*, and negative directional selection on dark respiration and positive directional selection on leaf size in *L. radicans*. Total light radiation differed between the microsites where the *Luzuriaga* species were sampled in the old-growth forest understory. Accordingly, *L. radicans* had a lower minimum light requirement and showed fertile individuals in darker microsites. *L. radicans*

showed lower dark respiration, higher chlorophyll content, and greater leaf size and SLA than *L. polyphylla*. Results suggest that in more shade-tolerant species, established in the darker microsites, selection would favor functional traits minimizing carbon losses, while in less shade-tolerant species, plants displaying leaf traits enhancing light capture would be selected.

Keywords Climbing plants · Distribution · Ecophysiology · Light niche · Natural selection · Shade · Valdivian forest

Introduction

Shade tolerance, broadly understood as the ability of a given plant species (or population) to tolerate low light levels, is a major driver of forest structure and dynamics (Givnish 1988; Valladares and Niinemets 2008). Interspecific variation in shade tolerance has been assessed monitoring survival rates in low light (Kitajima 1994; Lusk 2002), comparing the range of light environments occupied by juveniles (Davies 1998; Lusk and Reich 2000), or comparing their minimum light requirement, i.e., the lower part of the distribution of each species in relation to light availability (Lusk et al. 2008).

Several leaf functional traits, including gas exchange traits and biomass allocation patterns, have been associated with shade tolerance (reviewed in Valladares and Niinemets 2008). Most of this evidence has been obtained from comparative studies across species (Abrams and Kubiske 1990; Kitajima 1994; Walters and Reich 1996; Reich et al. 1998; Lusk 2004; Saldaña et al. 2005; Baltzer and Thomas 2007; Janse-ten Klooster et al. 2007; Poorter 2009). Overall, these interspecific patterns provide hypotheses about the

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adaptive role of leaf functional traits (Arntz and Delph 2001), but do not provide evidence that particular shade tolerance strategies at the species level originated (and are maintained) by natural selection. It has been argued that, because of their apparent constancy over evolutionary time, natural selection has kept functional traits in their current level of expression (Reich et al. 2003), and that these traits should be under stabilizing selection (Ackerly 2004). Still, because evolution by natural selection takes place within populations (Endler 1986), it is crucial to examine variation of traits associated with shade tolerance and plant fitness at an intraspecific scale in order to detect the selective processes behind the interspecific patterns of shade tolerance. Phenotypic selection analysis estimates the link between individual relative fitness (reproduction and/or survival) and trait values, assessing the strength and direction of selection on particular phenotypic traits taking into account character correlations (Lande and Arnold 1983; Brodie et al. 1995). Surprisingly, to our knowledge, no study has explicitly addressed selection on functional traits in natural populations of plant species differing in shade tolerance.

Leaf functional traits influencing shade tolerance have been extensively studied in both tropical and temperate ecosystems, but there is a long-standing debate over the relative importance of traits maximizing net carbon gain and growth versus those enhancing survival in low light, the latter being related to the role of biotic and abiotic agents of foliar damage (Givnish 1988; Kitajima 1994; Walters and Reich 1999; Lusk 2002; Reich et al. 2003; Craine and Reich 2005; Baltzer and Thomas 2007; Valladares and Niinemets 2008). The prevailing view has been that plants in low light display leaf traits enhancing light capture or carbon gain per unit biomass invested, e.g., increased leaf area ratio, specific leaf area, and chlorophyll content, and/or minimizing carbon losses, e.g., increased leaf toughness (via larger cell wall fractions; see Lusk et al. 2010) and decreased photosynthesis and dark respiration rates (Lusk 2002; Valladares and Niinemets 2008). Whether these functional patterns at the individual level translate into similar or opposite patterns at the interspecific level is still a matter of discussion (Lusk 2002; Reich et al. 2003; Lusk et al. 2010). Overall, and considering the heterogeneity in light availability observed even in mature forest understories (Bazzaz 1996; Montgomery and Chazdon 2002), it is expected that, in those plant species able to establish in the darker microsites, natural selection should favor individuals with a conservative strategy of resource use, i.e., minimizing carbon losses, while in those species distributed in the less-dim microsites, individuals displaying leaf traits enhancing light capture should be selected. This hypothesis is based on the assumption that highly shade-tolerant species, often long-lived plants, should rely on a long-term, conservative strategy that

favors endurance over increased resource exploitation, while less shade-tolerant species, having a better ability to exploit light availability, should rely on a less conservative strategy of resource use.

Climbing plants have been traditionally considered pioneer-like species preferentially distributed in well-lit microsites (Putz 1984; Schnitzer and Bongers 2002). However, significant evidence shows that a number of climbing plant species are able to thrive along the whole light gradient (Baars et al. 1998; Mascaro et al. 2004; Carrasco-Urra and Gianoli 2009; Gianoli et al. 2010). In fact, not all climbing plants in mature forests reach the canopy and so several species must survive in the shaded understory during their early stages (Aide and Zimmerman 1990; Ray 1992; Nabe-Nielsen 2002) or throughout their entire life cycle (Putz 1984; Valladares et al. 2011a). Woody vines growing in the mature forest understory show a shade tolerance syndrome very similar to that of trees (Valladares et al. 2011a; Gianoli et al. 2012). Likewise, the classical life history trade-off between juvenile growth and survival, which has been hypothesized to explain species' partition of light environments (Wright 2002), is equally observed for trees and woody climbers (Gilbert et al. 2006).

The temperate evergreen rainforest in southern Chile exhibits significant abundance of climbing plants (Gentry 1991; Gianoli et al. 2010). Two of the most abundant climbers in this forest belong to the genus *Luzuriaga*: *L. polyphylla* and *L. radicans* (Gianoli et al. 2010). These species are common in the forest understory and use adhesive roots to climb onto trees, but are almost always confined to the first 1.5 m of the vertical gradient. Both species coexist in the old-growth forest understory, but seemingly differ in their shade tolerance. While *L. radicans* shows its greatest abundance in the shaded forest understory, *L. polyphylla* is preferentially distributed in intermediate light environments such as forest borders and secondary forest stands (Gianoli et al. 2010). The two *Luzuriaga* species constitute an appropriate study system to evaluate the hypothesis of differential selection patterns in relation to species' shade tolerance because the probability of occurrence of confounding factors, at least those associated with phylogenetic distance, is significantly reduced (see Harvey et al. 1995; Ackerly et al. 2000; Reich et al. 2003).

In this study, carried out in the evergreen temperate rainforest in southern Chile, we tested the following hypotheses: (1) *L. polyphylla* and *L. radicans* will show distinct distribution in light microsites and different minimum light requirements in the old-growth forest understory, (2) leaf functional traits related to light capture and carbon balance will differ between these species in accordance with their shade tolerance, and (3) phenotypic

selection in the shaded forest understory will favor leaf functional traits optimizing light capture in the less shade-tolerant species (*L. polyphylla*) and traits minimizing carbon losses in the more shade-tolerant species (*L. radicans*).

Materials and methods

Study species and site

Luzuriaga R. & P. (formerly Luzuriagaceae, currently Luzuriagaceae within Alstroemeriaceae; Stevens 2011) is a small Gondwanean genus with three of its four species occurring in the temperate rainforests of southern South America. The two study species, *L. radicans* R. & P. and *L. polyphylla* (Hooker) Macbride, are climbing plants endemic to these forests (Rodríguez and Marticorena 1987). Both species are self-incompatible (Riveros et al. 1996; Arroyo and Humaña 1999), show an entomophilous pollination syndrome (Smith-Ramírez and Armesto 1994), and their fleshy fruits are dispersed by birds and mammals (Armesto et al. 1987; Smith-Ramírez and Armesto 1994). Plant height is usually between 0.5 and 1 m for the two species and stem diameter is equally similar. We studied *L. polyphylla* and *L. radicans* populations located in shaded forest understories in the old-growth temperate rainforest at Parque Nacional Puyehue (40°39'S, 72°11'W; 350 m a.s.l.), in the western piedmont of the Andes in southern Chile. The study site (Anticura) has a mean annual precipitation of 2,800 mm and a mean temperature of 9.8 °C (Dorsch 2003). The old-growth forest is composed exclusively of broad-leaved evergreen species (Lusk 2002; Saldaña and Lusk 2003) and woody vines are fairly common (Gianoli et al. 2010). A total of 14 climbing plant species belonging to 10 families were reported for the study site, with *L. radicans* being the second most abundant climber in the shaded understory; *L. polyphylla* was more abundant in microsites of intermediate light availability but co-occurred with *L. radicans* in the mature forest understory (Gianoli et al. 2010).

Field data

Plant sampling was carried out in the old-growth forest where the “El Indio” trail is embedded, within an area of ca. 8 ha. For the phenotypic selection analysis, 75 individuals from each *Luzuriaga* species were chosen and tagged in the shaded mature forest understory (0.005–0.250 of total light radiation, measured as global site factor; see below) after random walks into the forest from both sides of the trail. All plants found were considered for the analysis unless they were too close to another marked individual (less than 10 m), had an unhealthy appearance

(massive pathogen infection or substantial herbivory), or were outliers in terms of height of leaf display (above 2 m). We only sampled plants that were actually attached to the trunk of host trees because support availability influences climber fitness (Putz 1984; Gianoli 2002). In each individual plant, we selected for morphological and physiological measurements a section of the shoot including three complete internodes and avoiding both distal and basal parts. The following leaf functional traits were measured in situ in all tagged individuals at the beginning of the study: area-based dark respiration rate (R_d), leaf size, specific leaf area (SLA), and relative chlorophyll content. All sampled leaves were mature leaves. We carried out gas exchange measurements between January and March 2008 using a CIRAS II portable infrared gas analyser and leaf chamber (PP Systems, Hitchin, UK). Dark respiration was measured at PAR 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$, between 9:00 a.m. and 3:00 p.m. Cuvette temperatures were at 18–20 °C. Dark respiration is considered as one of the most reliable predictors of plant performance in shade, based on the long-held notion that shade tolerance is closely and inversely related to tissue respiratory costs (Grime and Jeffery 1965; Givnish 1988; Baltzer and Thomas 2007; Valladares and Niinemets 2008). Leaf size (cm^2) was estimated by means of digital photography and later analysis with Sigma-Scan Pro5 software (SPSS, Chicago, IL, USA). Leaves were then oven-dried at 70 °C for 48 h and weighed. SLA ($\text{cm}^2 \text{g}^{-1}$) was determined for each sample. The above-mentioned traits were measured in three leaves per plant and the average of these measurements was used as an individual value. The relative chlorophyll content was estimated with a hand-held chlorophyll meter (CCM-200, ADC; Opti-Sciences, Hudson, NH, USA). The CCM-200 calculates an index in ‘CCI units’ based on absorbance at 650 and 940 nm. This non-destructive optical method provides reliable estimates of relative leaf chlorophyll content (Richardson et al. 2002). In this case, five separate measurements on one completely full-expanded leaf of each individual were performed and later averaged. One year after the functional traits were measured, fecundity was estimated in each individual as the number of reproductive structures displayed (flowers plus fruits). This aimed at assessing the relationship between plant traits and reproductive fitness (phenotypic selection, see below). Reproductive output was estimated counting fruits and flowers per m^2 of climbing plant cover in the host trunk. Since not all tagged individuals displayed reproductive structures, plants were classified as ‘fertile’ and ‘non-fertile’. This allowed an additional analysis of the relationship between leaf traits and plant fitness, this time considering the probability of reproduction as the response variable (see below). We measured the stem diameter of the climbing plants to assess possible age differences between

fertile and non-fertile individuals. A total of 12 *L. polyphylla* plants and 10 *L. radicans* plants lost their number tags during the study and hence were omitted from the analyses.

Light availability over each plant was quantified by hemispherical photography, which is considered an accurate method for the description of understory light availability (Bellow and Nair 2003). Photographs were taken using a horizontally-leveled digital camera (CoolPix 995; Nikon, Tokyo, Japan) mounted on a tripod and aimed at the zenith, using a fish-eye lens of 180° field of view (FCE8; Nikon). Photographs were analyzed for canopy openness using Hemiview canopy analysis software (version 2.1, 1999; Delta-T Devices, UK), which is based on the CANOPY program (Rich 1990). Photographs were taken under homogenous sky conditions (overcast). Hemispherical photographs were taken in each individual plant over the part of the crown where leaf functional traits and fecundity were measured. The global site factor (GSF, the fraction of total radiation expected to reach the spot where the photograph was taken) was computed by Hemiview, considering the geographic features associated with the coordinates of the study site. A total of 160 sky sectors were considered resulting from 8 azimuth \times 20 zenith divisions. The light environment in this forest is described in detail by Lusk et al. (2006) and Valladares et al. (2011b).

Data analysis

We compared means of light availability (GSF), leaf functional traits, stem diameter, and fecundity between the two *Luzuriaga* species using *t* tests. We also compared the minimum light requirement (MLR), estimated as the 10th percentile of the distribution of each species in relation to light availability (GSF), and used this field-based parameter as an approximation of the lowest light levels tolerated by each species (Lusk et al. 2008). The relationship between the reproductive status (fertile/non-fertile) and stem diameter was estimated for each species using logistic regressions.

To test adaptive hypotheses, for each *Luzuriaga* species we calculated phenotypic selection gradients (Lande and Arnold 1983), which assessed the relationship between plant relative fitness (reproductive output and reproduction probability) and the standardized leaf functional traits (mean = 0; SD = 1) using multiple regression analyses. Phenotypic selection gradients estimate the selection acting directly upon the focal trait, independent of selection on correlated traits that have also been included in the analysis. In particular, linear selection gradients (β) assess the magnitude of directional selection and non-linear selection gradients (γ) assess the form (curvature) of the selection

function (Lande and Arnold 1983; Brodie et al. 1995). Directional and stabilizing/disruptive selection gradients were obtained from linear and quadratic coefficients of the regressions between reproductive output and functional traits, respectively (GLM; Statistica 6.0; StatSoft, Tulsa, OK, USA). We estimated selection gradients for R_d , leaf size, SLA, and Chl. Because the probability of reproduction is a dichotomous variable (fertile/non-fertile) and may violate parametric assumptions of normality, we used multivariate nonparametric logistic regressions (Janzen and Stern 1998) to evaluate whether leaf traits were related to reproduction probability. We estimated coefficients of phenotypic correlations among traits obtained from Pearson correlation matrix to rule out collinearity between traits.

Results

The average total light radiation (GSF) differed between the microsites where the two *Luzuriaga* species were sampled, with light availability being greater for *L. polyphylla* than for *L. radicans* (Table 1; Fig. 1). Accordingly, *L. radicans* had a lower minimum light requirement (MLR) than that of *L. polyphylla* (Fig. 1), and showed fertile individuals in darker microsites (Fig. 2). The four leaf functional traits measured showed significant differences between the study species (Table 1). *L. radicans* individuals showed lower dark respiration rate, higher chlorophyll content and greater leaf size and SLA than *L. polyphylla* individuals (Table 1). For both species, the average GSF differed between the microsites where fertile and non-fertile individuals occurred (*L. polyphylla*, $F_{1,61} = 8.66$, $P < 0.01$; *L. radicans*, $F_{1,63} = 4.04$, $P < 0.05$), with fewer plants showing reproductive structures in the darkest sites (Fig. 2). Logistic regressions showed that in *L. polyphylla* fertile individuals had thicker stems ($\chi^2 = 6.23$; $R^2 = 0.07$; $P = 0.013$; $n = 63$), while in *L. radicans* there was no significant relationship between reproductive status and stem diameter ($\chi^2 = 0.12$; $R^2 = 0.01$; $P = 0.73$; $n = 65$).

Regarding directional selection patterns, the multivariate logistic analysis showed that in *L. polyphylla* the probability of showing reproductive structures was greater for plants having leaves with greater specific area (SLA), a functional trait related to enhanced light capture. In contrast, the display of reproductive structures in *L. radicans* was associated with lower dark respiration rate, a functional trait minimizing carbon losses (Table 2). The overall model for each climbing plant species was significant (*L. polyphylla*: $\chi^2 = 32.20$, $P < 0.001$, $n = 63$; *L. radicans*: $\chi^2 = 31.68$, $P < 0.001$, $n = 65$). When the phenotypic selection analysis was focused on fertile individuals only, taking reproductive output as the fitness variable, we

Table 1 Mean values (\pm SE) of total light radiation (GSF), leaf functional traits, and proportion of fertile individuals (those displaying reproductive structures: flowers or fruits) of *Luzuriaga polyphylla* ($n = 63$) and *L. radicans* ($n = 65$) in forest understory

	<i>L. polyphylla</i>	<i>L. radicans</i>
Global site factor (GSF)	0.077 \pm 0.005	0.039 \pm 0.004**
Dark respiration ($\mu\text{mol CO}_2\text{m}^{-2}\text{s}^{-1}$)	0.26 \pm 0.02	0.20 \pm 0.02*
Leaf size (cm^2)	1.36 \pm 0.13	4.91 \pm 0.13***
Specific leaf area (cm^2g^{-1})	55.30 \pm 7.49	168.01 \pm 7.37***
Relative chlorophyll content (CCI)	18.61 \pm 1.02	24.21 \pm 1.00***
Proportion of fertile individuals	0.62	0.75

Significant differences between species: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (t test); non-significant differences ($\chi^2 = 2.71$; $P = 0.10$)

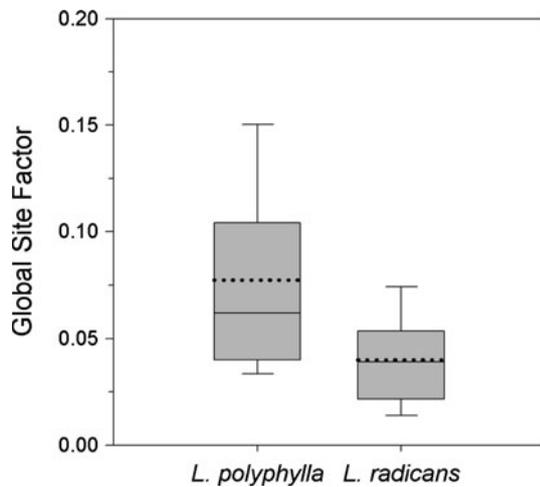


Fig. 1 Box plots of the distribution of individuals in relation to light availability (Global Site Factor) for *Luzuriaga polyphylla* and *L. radicans* populations. Error bar below box 10th percentile; lower limit of box 25th percentile; dotted line within box mean; solid line within box median; upper limit of box 75th percentile; error bar above box 90th percentile. Thus, the MLR (minimum light requirement of species = 10th percentile, following Lusk et al. 2008) was 0.033 and 0.014 for *L. polyphylla* and *L. radicans*, respectively

also detected positive and significant directional selection on SLA in *L. polyphylla* (Table 2). In the case of *L. radicans*, there was negative directional selection on dark respiration rate, as found above, and also positive directional selection on leaf size (Table 2). The overall model for each plant species was significant (*L. polyphylla*: $R^2 = 0.89$, $P < 0.001$, $n = 39$; *L. radicans*: $R^2 = 0.62$, $P < 0.001$, $n = 49$). We calculated both linear (β) and quadratic (γ) coefficients of the regression to estimate directional and stabilizing/disruptive selection gradients, respectively. However, quadratic coefficients are not shown because none of them were statistically significant,

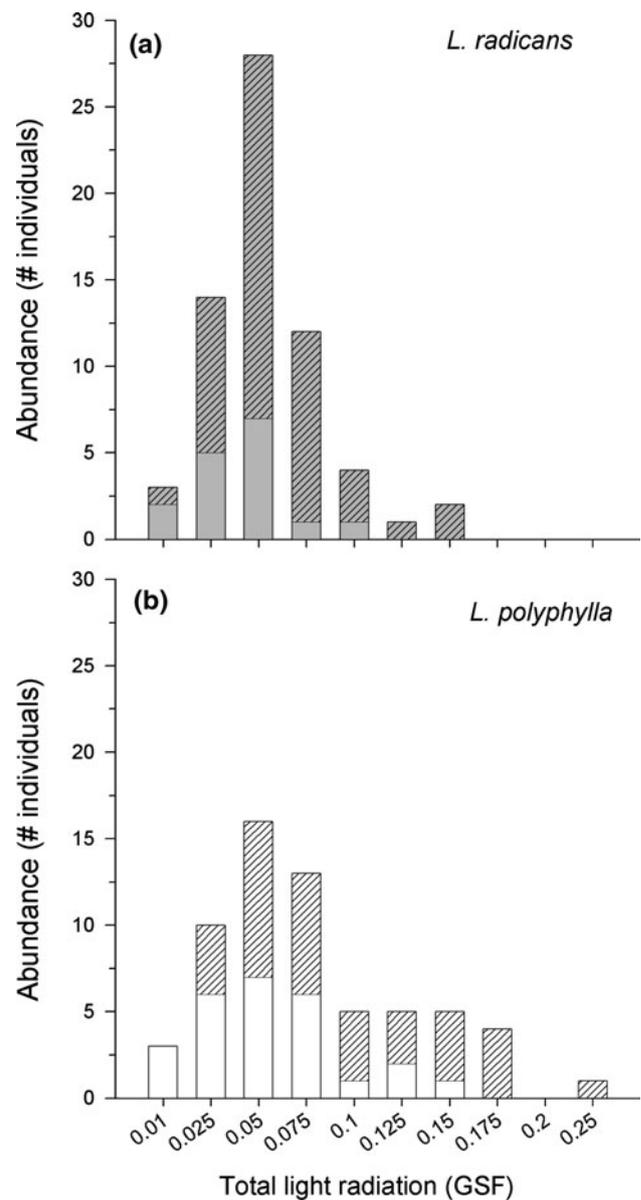


Fig. 2 Frequency distribution of *Luzuriaga* plants across the light gradient within an old-growth forest understory in a southern temperate rainforest. The hatched sections of bars correspond to fertile individuals (those showing flowers or fruits); $n = 63$ and 65 for *L. polyphylla* and *L. radicans*, respectively

i.e., there was no evidence of stabilizing or disruptive selection.

The phenotypic correlations among traits showed that dark respiration was negatively correlated to leaf size in both species, and that leaf size was positively correlated to SLA in *L. radicans* (Table 3). Because the correlation coefficients were, in any case, lower than 0.50 (Table 3), we ruled out the occurrence of collinearity (i.e., correlation coefficients >0.80) and hence all traits were included in the phenotypic selection analyses reported above. Stem

Table 2 Partial coefficients of multiple logistic regressions of the probability of showing reproductive structures against leaf functional traits (b), and linear standardized selection gradients ($\beta \pm SE$) of leaf functional traits for reproductive output, in *Luzuriaga polyphylla* and *L. radicans* populations in a forest understory

Trait	<i>L. polyphylla</i>		<i>L. radicans</i>	
	b	$\beta \pm SE$	b	$\beta \pm SE$
Dark respiration rate	0.77	-0.26 ± 0.12	-2.66^{***}	$-1.31 \pm 0.22^{***}$
Leaf size	0.27	-0.04 ± 0.08	0.01	$0.48 \pm 0.16^{**}$
Specific leaf area	2.27^{**}	$1.87 \pm 0.12^{***}$	-0.17	-0.25 ± 0.15
Relative chlorophyll content	0.19	-0.23 ± 0.08	-0.11	-0.21 ± 0.14

** $P < 0.01$, *** $P < 0.001$

Table 3 Phenotypic correlations among traits of the study species in a shaded forest understory

	Dark respiration	Leaf size	Specific leaf area	Chl	Stem diameter
Dark respiration	–	-0.11^{**}	0.03	-0.05	-0.01
Leaf size	-0.11^{**}	–	0.01	-0.02	<-0.01
Specific leaf area	-0.04	0.45^{***}	–	0.01	<0.01
Chl	-0.01	0.01	-0.03	–	0.02
Stem diameter	<-0.01	<0.01	<-0.01	0.12^{***}	–

The Pearson coefficients of correlation are shown for *Luzuriaga polyphylla* (above the diagonal) and *L. radicans* (below the diagonal)

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

diameter, our proxy for plant age, was not correlated to any of the functional traits shown to be under selection.

Discussion

The multivariate regression approach to phenotypic selection of Lande and Arnold (1983) was early questioned because the selection gradient estimates may be biased due to the probable covariation between fitness and individual traits driven by microenvironmental differences (Mitchell-Olds and Shaw 1987; Rausher 1992). In view of our results, such a bias is very unlikely. Plants in darker microsites tended to show lower fitness, while those traits under selection showed the opposite variation. Thus, we found that individual plants with high SLA and low Rd had greater fitness, but plants in brighter microsites tend to show low SLA and high Rd due to the ecophysiological adjustment to resource exploitation (Valladares and Niinemets 2008). In other words, from a biologically meaningful perspective, any environmental covariance between traits and fitness would have led to selection on decreased SLA and increased Rd, which is opposite to what we found.

The debate on the phenotypic basis of plant adaptation to shade (see Lusk 2002; Valladares and Niinemets 2008) started with Givnish's early assertion that natural selection should favor plants whose form and physiology tend to

maximize their net rate of energy capture (Givnish 1988). Surprisingly, although several studies have documented the selective value of plant traits related to carbon economy (Farris and Lechowicz 1990; Dudley 1996; Ackerly et al. 2000; Arntz and Delph 2001; Geber and Griffen 2003; Heschel et al. 2004; Saldaña et al. 2007; Agrawal et al. 2008), to our knowledge this is the first study addressing phenotypic selection on plant functional traits in the field in the context of comparisons of shade tolerance between species.

In our study system, two congeneric climbing plants coexisted in the dark understory of an old-growth forest stand located in the evergreen temperate rainforest of southern Chile, where light availability can be lower than 0.5 % of full sunlight (Gianoli, unpublished data). The two species, however, showed different levels of both shade tolerance, estimated from their light microsite distribution, and minimum light requirements for the occurrence and display of reproductive structures. Thus, *Luzuriaga radicans* was more shade-tolerant than *L. polyphylla*. Results are in accordance with patterns of distribution and abundance across the entire light gradient reported earlier for these climbers, where *L. radicans* showed its greatest abundance in the shaded forest understory, while *L. polyphylla* was preferentially distributed in intermediate light environments (Gianoli et al. 2010). In both species, fertile individuals were less likely to be found in darker microsites of the forest understory, which may suggest resource

limitation for reproduction. In the case of *L. polyphylla*, the probability of reproduction was positively associated with plant age (as estimated by stem diameter). Thus, in the less shade-tolerant species, the reproductive stage would only be attained by long-lived individuals, and provided that they are located in relatively lit forest spots.

When plant functional traits were compared between the study species, individuals from the more shade-tolerant species, *L. radicans*, showed lower dark respiration rate, higher chlorophyll content and greater leaf size and specific leaf area than *L. polyphylla* plants. Whereas the decreased metabolic rate supports the idea of conservative use of resources for shade species, the increased expression of leaf size, SLA and chlorophyll content instead agrees with an energy capture strategy (see Valladares and Niinemets 2008), and is also congruent with typical phenotypic responses of plants to shading at the within-species level (Salgado-Luarte and Gianoli 2011, 2012, and references therein). Overall, results support a shade tolerance strategy relying on the maximization of light capture and use in photosynthesis together with the minimization of respiration costs for maintenance (Givnish 1988). These climbing plants, therefore, match with the classic shade tolerance syndrome described for tree saplings (Valladares and Niinemets 2008), as has been shown by a study carried out in the same forest and with several woody vine species (Valladares et al. 2011a). These results somewhat challenge the view of climbers as light-demanding species (Schnitzer and Bongers 2002). Importantly, in order to determine the processes that generated and/or maintain the observed characteristics of leaf trait expression in *Luzuriaga* species, it is essential to examine the patterns of natural selection acting on leaf traits associated with shade tolerance. Consequently, the association between leaf functional traits and plant fitness must be evaluated at the intraspecific scale.

The two *Luzuriaga* species constitute an appropriate study system to evaluate the hypothesis of differential selection patterns in relation to species' shade tolerance because the occurrence of confounding factors associated with phylogenetic distance is unlikely (see Harvey et al. 1995; Ackerly et al. 2000; Reich et al. 2003). In general, the phenotypic selection analyses, based either on the probability of display of reproductive structures or on their number, rendered similar patterns. Thus, we found evidence of natural selection promoting reduced dark respiration in *L. radicans* and increased specific leaf area in *L. polyphylla*. Therefore, results support the hypothesis that in the more shade-tolerant species, established in the darker microsites, selection would favor functional traits minimizing carbon losses, while in the less shade-tolerant species, established in the lighter microsites of the forest understory, plants displaying leaf traits enhancing light

capture would be selected. Both dark respiration rate and specific leaf area (SLA) are key traits for plant performance in low light. Field studies have shown that dark respiration is the best predictor of shade tolerance, estimated as the whole-plant light compensation point (Baltzer and Thomas 2007) or as the minimum light requirement of species (Lusk and Reich 2000). It has been reported that increased SLA is associated with enhanced carbon gain, particularly in low light environments (Evans and Poorter 2001; Sims et al. 1994). Interestingly, despite being closely related species with similar phenotypes and the same growth habit (Rodríguez and Marticorena 1987), the two *Luzuriaga* species, when coexisting in the dark forest understory, are subject to different selection pressures by the light environment: reduced dark respiration versus increased SLA. In other words, it is not the case that the less shade-tolerant *L. polyphylla* tends to follow the evolutionary path of eco-physiological adjustment of the more shade-tolerant *L. radicans* by minimizing metabolic losses, i.e., reducing dark respiration. It would be of interest to discern the relative importance of intrinsic constraints (the dark respiration rate cannot be lowered) and competitive interactions (*L. radicans* dominates the darkest microsites) in explaining the selective patterns for *L. polyphylla*.

Field-based measurements of plant traits and fitness do not allow the distinction between selection on mean trait values and on their phenotypic plasticity (Pigliucci 2001). Although phenotypic plasticity may evolve independently of the mean trait value (Matesanz et al. 2010 and cited references), the ultimate target of selection is the phenotypic value displayed in the field, regardless of the extent to which it is due to plastic responses (Gianoli and Valladares 2012). Phenotypic plasticity is a major mechanism of plant adaptation to changing environmental conditions (Pigliucci 2001; Valladares et al. 2007; Matesanz et al. 2010; Gianoli and Valladares 2012), but its occurrence does not challenge the conclusions of a phenotypic selection experiment. Further experiments under controlled conditions would allow the estimation of the relative contribution of plastic responses to the observed adaptive patterns in the field (e.g., Weinig 2000; González-Teuber and Gianoli 2008). Interestingly, both *Luzuriaga* species show similar patterns of variation in the expression of leaf functional traits when growing in different light environments in this forest (canopy gaps vs. mature forest): little change in leaf size, SLA, and photosynthetic rate, and significant differences in dark respiration rate (Gianoli et al. 2012).

A recent study with the seven most important climbers in this temperate rainforest, including the two *Luzuriaga* species, reported that SLA was not associated with species' abundance in the shaded understory (Valladares et al. 2011a). We found that there was positive directional selection on SLA for *L. polyphylla*. The apparent

contradiction between intraspecific and interspecific patterns of variation in functional traits related to light use has been amply discussed (Lusk 2002; Reich et al. 2003; Lusk et al. 2010), and no consensus has yet been reached. We stress that the appropriate scale at which to test adaptive explanations for a given phenotypic pattern, at least from a Darwinian approach, is the within-population level. Evolution by natural selection occurs—or not—in populations in which individuals show differential survival or reproduction associated with their phenotypes (Endler 1986). We think that evidence of natural selection on plant functional traits in contrasting light environments is much needed to enrich the discussion on the relationship between intraspecific and interspecific patterns of trait variation with light availability.

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References

- Abrams MD, Kubiske ME (1990) Leaf structural characteristics of 31 hardwood and conifer tree species in central Wisconsin: influence of light regime and shade tolerance rank. *For Ecol Manag* 31:245–253
- Ackerly DD (2004) Adaptation, niche conservatism, and convergence: comparative studies of leaf evolution in the California Chaparral. *Am Nat* 163:654–671
- Ackerly DD, Dudley SA, Sultan SE, Schmitt J, Coleman JS, Linder CR, Sandquist DR, Geber MA, Evans AS, Dawson TE, Lechowicz MJ (2000) The evolution of plant ecophysiological traits: recent advances and future directions. *Bioscience* 50:979–995
- Agrawal AA, Erwin AC, Cook SC (2008) Natural selection on and predicted responses of ecophysiological traits of swamp milkweed (*Asclepias incarnata*). *J Ecol* 96:536–542
- Aide TM, Zimmerman JK (1990) Patterns of insect herbivory, growth and survivorship in juveniles of a neotropical liana. *Ecology* 71:1412–1421
- Armesto JJ, Rozzi R, Miranda P, Sabag C (1987) Plant/frugivore interactions in South American temperate forests. *Rev Chil Hist Nat* 60:321–336
- Arntz AM, Delph LF (2001) Pattern and process: evidence for the evolution of photosynthetic traits in natural populations. *Oecologia* 127:455–467
- Arroyo MTK, Humaña AM (1999) Breeding systems of two endemic rainforest species in Southern Chile: *Amomyrtus meli* (Phil.) Legr. et Kaus. (Myrtaceae) and *Luzuriaga polyphylla* (Hook.) Macbr. (Philesiaceae). *Gayana Bot* 56:31–37
- Baars R, Kelly D, Sparrow AD (1998) Liana distribution within native forest remnants in two regions of the South Island New Zealand. *NZ J Ecol* 22:71–85
- Baltzer JL, Thomas SC (2007) Determinants of whole-plant light requirements in Bornean rain forest tree saplings. *J Ecol* 95:1208–1221
- Bazzaz FA (1996) Plants in changing environments. Linking physiological, population, and community ecology. Cambridge University Press, Cambridge
- Bellow JG, Nair PKR (2003) Comparing common methods for assessing understory light availability in shaded-perennial agroforestry systems. *Agric For Meteorol* 114:197–211
- Brodie ED, Moore AJ, Janzen FJ (1995) Visualizing and quantifying natural-selection. *Trends Ecol Evol* 10:313–318
- Carrasco-Urra F, Gianoli E (2009) Abundance of climbing plants in a southern temperate rainforest: host-tree characteristics or light availability? *J Veg Sci* 20:1155–1162
- Craine JM, Reich PB (2005) Leaf-level light compensation points in shade-tolerant woody seedlings. *New Phytol* 166:710–713
- Davies SJ (1998) Photosynthetic characteristics of nine pioneer *Macaranga* species from Borneo in relation to life-history traits. *Ecology* 79:2292–2308
- Dorsch K (2003) Hydrogeologische Untersuchungen der Geothermalfelder von Puyehue und Cordón Caulle, Chile. PhD dissertation, Ludwig-Maximilians-Universität, München, Germany
- Dudley SA (1996) Differing selection on plant physiological traits to environmental water availability: a test of adaptive hypotheses. *Evolution* 50:92–102
- Endler JA (1986) Natural selection in the wild. Princeton University Press, Princeton
- Evans JR, Poorter H (2001) Photosynthetic acclimation of plants to growth irradiance: the relative importance of specific leaf area and nitrogen partitioning in maximizing carbon gain. *Plant Cell Environ* 24:755–767
- Farris MA, Lechowicz MJ (1990) Functional interactions among traits that determine reproductive success in a native annual plant. *Ecology* 71:548–557
- Geber MA, Griffen LR (2003) Inheritance and natural selection on functional traits. *Int J Plant Sci* 164:S21–S42
- Gentry AH (1991) The distribution and evolution of climbing plants. In: Putz FE, Mooney HA (eds) The biology of vines. Cambridge University Press, Cambridge, pp 3–49
- Gianoli E (2002) Maternal environmental effects on the phenotypic responses of the twining vine *Ipomoea purpurea* to support availability. *Oikos* 99:324–330
- Gianoli E, Valladares F (2012) Studying phenotypic plasticity: the advantages of a broad approach. *Biol J Linn Soc* 105:1–7
- Gianoli E, Saldaña A, Jiménez-Castillo M, Valladares F (2010) Distribution and abundance of vines along the light gradient in a southern temperate rainforest. *J Veg Sci* 21:66–73
- Gianoli E, Saldaña A, Jiménez-Castillo M (2012) Ecophysiological traits may explain the abundance of climbing plant species across the light gradient in a temperate rainforest. *PLoS ONE* 7(6):e38831
- Gilbert B, Wright SJ, Muller-Landau HC, Kitajima K, Hernández A (2006) Life history trade-offs in tropical trees and lianas. *Ecology* 87:1281–1288
- Givnish TJ (1988) Adaptation to sun and shade—a whole-plant perspective. *Aust J Plant Physiol* 15:63–92
- González-Teuber M, Gianoli E (2008) Damage and shade enhance climbing and promote associational resistance in a climbing plant. *J Ecol* 96:122–126
- Grime JP, Jeffery DW (1965) Seedling establishment in vertical gradients of sunlight. *J Ecol* 53:621–642
- Harvey PH, Read AF, Nee S (1995) Why ecologists need to be phylogenetically challenged. *J Ecol* 83:535–536
- Heschel MS, Stinchcombe JR, Holsinger KE, Schmitt J (2004) Natural selection on light response curve parameters in the herbaceous annual, *Impatiens capensis*. *Oecologia* 139:487–494
- Janse-ten Klooster SH, Thomas EJP, Sterck FJ (2007) Explaining interspecific differences in sapling growth and shade tolerance in temperate forests. *J Ecol* 95:1250–1260

- Janzen FJ, Stern HS (1998) Logistic regression for empirical studies of multivariate selection. *Evolution* 52:1564–1571
- Kitajima K (1994) Relative importance of photosynthetic traits and allocation patterns as correlates of seedling shade tolerance of 13 tropical trees. *Oecologia* 98:419–428
- Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. *Evolution* 37:1210–1226
- Lusk CH (2002) Leaf area accumulation helps juvenile evergreen trees tolerate shade in a temperate rainforest. *Oecologia* 132:188–196
- Lusk CH (2004) Leaf area and growth of juvenile temperate evergreens in low light: species of contrasting shade tolerance change rank during ontogeny. *Funct Ecol* 18:820–828
- Lusk CH, Reich PB (2000) Relationships of leaf dark respiration with light environment and tissue nitrogen content in juveniles of 11 cold-temperate tree species. *Oecologia* 123:318–329
- Lusk CH, Chazdon RL, Hofmann G (2006) A bounded null model explains juvenile tree community structure along light availability gradients in a temperate rain forest. *Oikos* 112:131–137
- Lusk CH, Falster DS, Jara-Vergara CK, Jimenez-Castillo M, Saldaña A (2008) Ontogenetic variation in light requirements of juvenile rainforest evergreens. *Funct Ecol* 22:454–459
- Lusk CH, Onoda Y, Kooyman R, Gutiérrez-Girón A (2010) Reconciling species-level vs. plastic responses of evergreen leaf structure to light gradients: shade leaves punch above their weight. *New Phytol* 186:429–438
- Mascaro J, Schnitzer SA, Carson WP (2004) Liana diversity, abundance and mortality in a tropical wet forest in Costa Rica. *For Ecol Manag* 190:3–14
- Matesanz S, Gianoli E, Valladares F (2010) Global change and the evolution of phenotypic plasticity in plants. *Ann NY Acad Sci* 1206:35–55
- Mitchell-Olds T, Shaw RG (1987) Regression analysis of natural selection: statistical inference and biological interpretation. *Evolution* 41:1149–1161
- Montgomery RA, Chazdon RL (2002) Light gradient partitioning by tropical tree seedlings in the absence of canopy gaps. *Oecologia* 131:165–174
- Nabe-Nielsen J (2002) Growth and mortality rates of the liana *Machaerium cuspidatum* in relation to light and topographic position. *Biotropica* 34:319–322
- Pigliucci M (2001) Phenotypic plasticity: beyond nature and nurture. Johns Hopkins University Press, Baltimore
- Poorter L (2009) Leaf traits show different relationships with shade tolerance in moist versus dry tropical forests. *New Phytol* 181:890–900
- Putz FE (1984) The natural history of lianas on Barro Colorado Island, Panama. *Ecology* 65:1713–1724
- Rausher MD (1992) The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. *Evolution* 46:616–626
- Ray TS (1992) Foraging behaviour in tropical herbaceous climbers (Araceae). *J Ecol* 80:189–203
- Reich PB, Tjoelker MG, Walters MB, Vanderklein DW, Buschena C (1998) Close association of RGR, leaf and root morphology, seed mass and shade tolerance in seedlings of nine boreal tree species grown in high and low light. *Funct Ecol* 12:327–338
- Reich PB, Wright IJ, Cavender-Bares J, Craine JM, Oleksyn J, Westoby M, Walters MB (2003) The evolution of plant functional variation: traits, spectra, and strategies. *Int J Plant Sci* 164:S143–S164
- Rich PM (1990) Characterizing plant canopies with hemispherical photographs. *Rem Sens Rev* 5:13–29
- Richardson AD, Duigan SP, Berlyn GP (2002) An evaluation of noninvasive methods to estimate foliar chlorophyll content. *New Phytol* 153:185–194
- Riveros M, Humaña AM, Arroyo MTK (1996) Sistemas de reproducción en especies del bosque valdiviano (40° Latitud Sur). *Phyton* 58:167–176
- Rodríguez R, Marticorena C (1987) Las especies del genero *Luzuriaga* R. et P. Gayana Bot 44:3–15
- Saldaña A, Lusk CH (2003) Influence of overstorey species identity on resource availability and variation in composition of advanced regeneration in a temperate rainforest in southern Chile. *Rev Chil Hist Nat* 76:639–650
- Saldaña A, Gianoli E, Lusk CH (2005) Ecophysiological responses to light availability in three *Blechnum* species (Pteridophyta, Blechnaceae) of different ecological breadth. *Oecologia* 145:252–257
- Saldaña A, Lusk CH, Gonzáles WL, Gianoli E (2007) Natural selection on ecophysiological traits of a fern species in a temperate rainforest. *Evol Ecol* 21:651–662
- Salgado-Luarte C, Gianoli E (2011) Herbivory may modify functional responses to shade in seedlings of a light-demanding tree species. *Funct Ecol* 25:492–499
- Salgado-Luarte C, Gianoli E (2012) Herbivores modify selection on plant functional traits in a temperate rainforest understory. *Am Nat* 180:E42–E53
- Schnitzer SA, Bongers F (2002) The ecology of lianas and their role in forests. *Trends Ecol Evol* 17:223–230
- Sims DA, Gebauer RLE, Pearcy RW (1994) Scaling sun and shade photosynthetic acclimation of *Alocasia macrorrhiza* to whole-plant performance. 2. Simulation of carbon balance and growth at different photon flux densities. *Plant Cell Environ* 17:889–900
- Smith-Ramírez C, Armesto JJ (1994) Flowering and fruiting patterns in the temperate rainforest of Chiloé, Chile—ecologies and climatic constraints. *J Ecol* 82:353–365
- Stevens PF (2011) Angiosperm phylogeny website. Version 11 (WWW document). <http://www.mobot.org/MOBOT/research/APweb/>. Accessed 22 May 2011
- Valladares F, Niinemets Ü (2008) Shade tolerance, a key plant feature of complex nature and consequences. *Annu Rev Ecol Evol Syst* 39:237–257
- Valladares F, Gianoli E, Gómez JM (2007) Ecological limits to plant phenotypic plasticity. *New Phytol* 176:749–763
- Valladares F, Gianoli E, Saldaña A (2011a) Climbing plants in a temperate rainforest understory: searching for high light or coping with deep shade? *Ann Bot* 108:231–239
- Valladares F, Saldaña A, Gianoli E (2011b) Costs versus risks: architectural changes with changing light quantity and quality in saplings of temperate rainforest trees of different shade tolerance. *Aust Ecol* 37:35–43
- Walters MB, Reich PB (1996) Are shade tolerance, survival, and growth linked? low light and nitrogen effects on hardwood seedlings. *Ecology* 77:841–853
- Walters MB, Reich PB (1999) Low-light carbon balance and shade tolerance in the seedlings of woody plants: do winter deciduous and broad-leaved evergreen species differ? *New Phytol* 143:143–154
- Weinig C (2000) Differing selection in alternative competitive environments: shade-avoidance responses and germination timing. *Evolution* 54:124–136
- Wright SJ (2002) Plant diversity in tropical forests: a review of mechanisms of species coexistence. *Oecologia* 130:1–14