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Herbivores Modify Selection on Plant Functional Traits in a Temperate Rainforest Understory

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ABSTRACT: There is limited evidence regarding the adaptive value of plant functional traits in contrasting light environments. It has been suggested that changes in these traits in response to light availability can increase herbivore susceptibility. We tested the adaptive value of plant functional traits linked with carbon gain in contrasting light environments and also evaluated whether herbivores can modify selection on these traits in each light environment. In a temperate rainforest, we examined phenotypic selection on functional traits in seedlings of the pioneer tree *Aristotelia chilensis* growing in sun (canopy gap) and shade (forest understory) and subjected to either natural herbivory or herbivore exclusion. We found differential selection on functional traits depending on light environment. In sun, there was positive directional selection on photosynthetic rate and relative growth rate (RGR), indicating that selection favors competitive ability in a high-resource environment. Seedlings with high specific leaf area (SLA) and intermediate RGR were selected in shade, suggesting that light capture and conservative resource use are favored in the understory. Herbivores reduced the strength of positive directional selection acting on SLA in shade. We provide the first demonstration that natural herbivory rates can change the strength of selection on plant ecophysiological traits, that is, attributes whose main function is resource uptake. Research addressing the evolution of shade tolerance should incorporate the selective role of herbivores.

Keywords: natural selection, ecophysiological traits, specific leaf area (SLA), herbivory, *Aristotelia chilensis*, shade.

Introduction

Light is an essential resource for plants. A significant decrease in available photosynthetically active radiation reduces plant performance and fitness (Valladares and Niinemets 2008). In most natural ecosystems, light availability is temporally and spatially heterogeneous (Bazzaz 1996). In this context, the phenotypic expression of

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functional traits that improve plant carbon balance in a given light environment has been interpreted from an adaptive perspective (Pearcy et al. 1987; Givnish 1988; Ackerly et al. 2000). Plants in the shade express phenotypic traits that maximize light capture per unit biomass invested (greater specific leaf area [SLA] and chlorophyll content) and reduce metabolic costs (lower photosynthesis and dark respiration rates), whereas in sun environments, plants exhibit functional traits that enhance resource exploitation and competitive ability, such as higher photosynthetic capacity and increased growth rate (Givnish 1988; Kitajima 1994; Evans and Poorter 2001; Lusk and Del Pozo 2002; Saldaña et al. 2005; Valladares and Niinemets 2008). Although it is widely thought that plant ecophysiological traits related to carbon economy have an impact on plant fitness, there is still limited empirical evidence on the adaptive value of these functional traits and the selective forces acting on them in natural populations (Farris and Lechowicz 1990; Ackerly et al. 2000; Arntz and Delph 2001; Geber and Griffen 2003; Heschel et al. 2004; Saldaña et al. 2007; Agrawal et al. 2008).

Plant functional traits may influence patterns of herbivory in forest communities (Coley and Barone 1996). Thus, expression of phenotypic traits that enhance carbon balance could also increase the probability of consumption by herbivores (Givnish 1988; Herms and Mattson 1992; Poorter et al. 2004). There is evidence that herbivores prefer plants with high photosynthetic performance and growth rate (Price 1991; Herms and Mattson 1992; Roberts and Paul 2006), which would be closely related to the nutrient content of plant tissues (Field and Mooney 1986; Reich et al. 1994). These plants are typically found in open habitats. Conversely, optimization of the surface area for light capture in the shade, achieved via increased SLA, may result in greater vulnerability to leaf damage by herbivores (or physical agents) because high-SLA leaves are generally softer and less tough than low-SLA leaves (Lei and Lech-

owicz 1990; Choong 1996; Reich et al. 1999; Hanley et al. 2007). Therefore, individual plants that adjust the expression of functional traits according to the light environment may suffer fitness costs because of the associated increase in susceptibility to herbivore damage. It is reasonable to expect that herbivores may be involved in the selection and evolution of plant traits related to light use.

The role of herbivores in generating selection on plant defensive characters has been previously demonstrated (Rausher and Simms 1989; Marquis 1992; Mauricio and Rausher 1997; Shonle and Bergelson 2000; Valverde et al. 2001; Geber and Griffen 2003; Agrawal 2005), and several studies have shown variation in herbivory rates and/or plant defensive characters with the light environment (e.g., Dudt and Shure 1994; Folgarait and Davidson 1994; Louda and Rodman 1996; Molina-Montenegro et al. 2006; Muth et al. 2008). However, the hypothesis that herbivores exert selective pressures on functional traits involved in carbon balance has not been tested explicitly. Arguments in favor of this idea have been discussed, particularly in the case of interspecific comparisons between shade-tolerant and light-demanding tree species (Kitajima 1994; Walters and Reich 1999; Lusk et al. 2010). It has been hypothesized that robust, low-SLA leaves have been selectively favored in the shade because they confer protection against herbivores and physical damage, thus avoiding the loss of valuable tissue in an environment where resources are limited (Kitajima 1994; Kobe 1997; Valladares and Niinemets 2008). Interspecific differences in shade-tolerance strategies originate as a result of historical differences in selection on populations growing in distinct light environments. To discern the role of selection in generating phenotypic differences between plants found in sun and shade environments, independent of other interspecific differences in trait expression, it is necessary to examine traits associated with sun-shade adaptation at an intraspecific scale (within populations).

Phenotypic selection analysis estimates the strength and direction of selection on multiple correlated traits by quantifying the relationship between individual relative fitness and trait values (Lande and Arnold 1983; Brodie et al. 1995). In plants, phenotypic selection analysis has been used to estimate patterns of selection on defensive, floral, and ecophysiological traits (Mauricio et al. 1997; Shonle and Bergelson 2000; Arntz and Delph 2001; Kingsolver et al. 2001; Geber and Griffen 2003; Saldaña et al. 2007; Agrawal 2005; Agrawal et al. 2008; Wise and Hébert 2010). To show that a given factor is exerting selection on a trait, it is necessary to estimate and compare selection patterns when the putative selective agent is present and absent using a manipulative approach (e.g., Mauricio and Rausher 1997; Shonle and Bergelson 2000).

In this field study, we tested for (i) the adaptive value of variation in plant ecophysiological traits in contrasting light environments and (ii) selection exerted by herbivores on these traits. Specifically, we estimated patterns of phenotypic selection on ecophysiological traits related to carbon economy in seedlings of the light-demanding tree *Aristotelia chilensis* (hereafter *Aristotelia*). This was done in sun (treefall canopy gap) and shade (mature forest understory) sites in a temperate rainforest in southern Chile. We also experimentally excluded herbivores from *Aristotelia*, which shows high levels of herbivore damage in the field (Salgado-Luarte and Gianoli 2011), and evaluated the effect of this exclusion on patterns of phenotypic selection on functional traits in both light environments.

Material and Methods

Species and Study Site

Aristotelia chilensis (Mol.) Stuntz (Elaeocarpaceae) is a fast-growing evergreen tree endemic to South American austral forests (Muñoz and González 2006). Although this species is primarily distributed in canopy gaps and forest edges and is thus considered a pioneer or light-demanding species (Lusk et al. 2006), seedlings and saplings are also found in the dark forest understory (Lusk 2002; Lusk and Del Pozo 2002). Experimentally shaded seedlings of *Aristotelia* exhibit a number of functional responses, including an increase in SLA, leaf area ratio (relative to plant mass) and shoot biomass allocation, and reduced photosynthetic rate (Lusk and Del Pozo 2002; Salgado-Luarte and Gianoli 2011). *Aristotelia* sustains significant damage by insects, slugs and snails (Guerra et al. 2010; Salgado-Luarte and Gianoli 2011), and foliar damage modifies seedling functional responses to shade (Salgado-Luarte and Gianoli 2011).

The experiment was carried out in a temperate rainforest located within Puyehue National Park (40°39′S, 72°11′W; 350–400 m a.s.l.), in the western foothills of the Andes, southern Chile. The study site (Anticura) has an annual precipitation of 2,800 mm and a mean annual temperature of 9.8°C, with potential evapotranspiration only 1/8 of precipitation rates (Dorsch 2003). The old-growth lowland forest is composed of evergreen tree species and woody vines (Lusk 2002; Lusk and Del Pozo 2002; Gianoli et al. 2010). In this forest 43% of microsites occur at approximately 5% canopy openness, while microsites with canopy openness greater than 25% are rare (Lusk et al. 2006). Soils, derived from volcanic ash, are deep and well-drained and have low nutrient availability due to low mineralization and high immobilization (Saldaña and Lusk 2003). Light is the most limiting resource in the understory and changes in light availability across the forest are not associated with changes in nutrient availability

(Saldaña and Lusk 2003). The main *Aristotelia* herbivores in the study site are small slugs, weevils, and leaf beetles, and are evenly distributed across open and shaded microsites (C. Salgado-Luarte and E. Gianoli 2010 and unpublished data). Vertebrate herbivores in this forest are very rare (C. Salgado-Luarte and E. Gianoli, personal observations).

Experimental Design and Measurements

In September 2007, we collected small seedlings of *Aristotelia* in the same patch of rainforest (~15 ha) where this field trial was conducted. Seedlings were taken from sites of intermediate light availability between the dark mature forest understory and the sunlit treefall canopy gaps. Seedling height (cm) was measured with a ruler. Thus, September 2007 was t_0 for the calculation of relative growth rate (RGR; see below). Plants were then assigned to one of two common gardens that were located in two contrasting light environments: a large treefall canopy gap (sun: canopy openness = 25.6% ± 2.3%; 15 m × 20 m) and a closed-canopy forest understory (shade: canopy openness = 5.1% ± 0.9%; 10 m × 18 m). The light environment was characterized using hemispherical canopy photographs taken directly above each plant. To estimate canopy openness, images were analyzed using Winphot software (H. ter Steege, 2005, http://web.science.uu.nl/Amazon/winphot/wp_index.htm). The distance between common gardens was ~90 m (gap: 40°39′59.59″S–72°10′33.07″W; understory: 40°39′56.78″S–72°10′32.26″W). Because previous observations in this forest suggested greater herbivore pressure in the gaps, we initially planted 350 and 210 seedlings in the sun and shade common gardens, respectively. Both sun and shade seedlings were planted 35 cm apart to replicate natural densities. After transplantation seedlings were watered and allowed to acclimate during 2 months for the development of sun and shade phenotypes. During the acclimation period, all seedlings were treated monthly with systemic insecticides (Dimethoate Plus and Fastac) and a molluscicide (Metarex) to remove the possibility of damage due to natural herbivory. At the end of the transplant, unidentified sources of mortality reduced the number of seedlings in sun and shade to 300 and 180, respectively. Seedlings planted into the two light environments did not differ in height (overall mean ± SE = 15.25 ± 0.23 cm; t value = 0.243, df = 478, P = .44) or number of leaves (overall mean ± SE = 5 ± 0.2; t value = −0.761, df = 478, P = .47) at the beginning of the experiment.

In December 2007, half of the seedlings in each light environment were chemically protected from herbivores via individual foliage fumigation (no-herbivory treatment). Plants were sprayed monthly with two systemic insecticides, Dimethoate plus and Fastac (10 mL L^{-1} in each case), and a molluscicide (Metarex; 0.25 g L^{-1}). This insecticide and molluscicide treatment is effective against invertebrate herbivores (Salgado-Luarte and Gianoli 2011) and has no effect on the phenotype of *Aristotelia* (greenhouse trials conducted in both full light and neutral shade conditions; C. Salgado-Luarte, unpublished data). The other half of the seedlings were sprayed with a similar volume of water (herbivory treatment). Because we alternated seedlings in the two treatments within the common gardens, we were extremely careful during the spraying procedure to ensure that pesticides applied on a target plant would not affect the neighboring plants. In total, we had four experimental groups: sun/no-herbivory, sun/herbivory, shade/no-herbivory, and shade/herbivory.

Immediately after the onset of treatments, in December 2007, the following ecophysiological traits were measured: A_{max}, E, internode length, petiole length, and SLA. The CO_2 assimilation (A_{max}; μmol m^{-2} s^{-1}) and leaf transpiration (E; mmol m^{-2} s^{-1}) were estimated under 1,000 PAR and 25°C with a portable leaf chamber and infrared gas analyzer (LCi portable photosynthesis system; ADC). These measurements were taken between 0930 and 1230 hours in fully expanded leaves at midshoot. The length of the last internodes and petioles (cm) was measured with a ruler. We estimated SLA (leaf area per unit leaf mass, cm^2 g^{-1}) in the field in a nondestructive manner. First, leaf area was determined in situ from digital pictures analyzed with Sigma-Scan (SPSS, Chicago), and leaf thickness was measured with a digital calliper (Mitutoyo, Kanagawa, Japan; 0.01-mm resolution). We conducted measurements on another group of *Aristotelia* seedlings to develop a nondestructive method to estimate leaf mass. For this group we measured leaf thickness and leaf area in *Aristotelia* leaves taken from canopy gaps (sun) and forest understory (shade) in the same forest patch, oven-dried the leaves at 70°C for 48 h to obtain dry mass, and conducted a regression analysis. Separate regression analyses to estimate leaf mass from leaf area and leaf thickness for sun leaves and shade leaves were conducted. Sun: [dry mass] = 0.0038 + 1.3605 × ln [leaf area] + 0.2312 × [leaf thickness] (R^2 = 0.95; n = 65); shade: [dry mass] = 0.0064 + 0.8714 × ln [leaf area] + 0.1046 × [leaf thickness] (R^2 = 0.93; n = 65). Using these equations and field measurements of leaf area and leaf thickness in experimental plants, we estimated the SLA of experimental plants as leaf area divided by "estimated" leaf dry mass. All leaf traits were measured on two fully expanded leaves per plant, and the average of these two measurements was used as the individual value. In January 2008, 4 months after the initial measurement, seedling height was measured again (t_1), and we estimated relative growth rate from the increase in plant height as follows: RGR = [ln (height in t_1)− ln (height in t_0)]/(number of months).

Seedling survival at the end of experiment, in April 2009, was used as a proxy for plant fitness.

Herbivory Damage

To verify the effectiveness of the chemical exclusion of herbivores and to assess natural levels of herbivory in both light environments, in February 2009 we calculated an herbivory index (Dirzo and Domínguez 1995) for each experimental seedling. Herbivory was visually estimated for each leaf using five categories referring to percentage of leaf area damaged: 0 = no damage; 1 = damage less than 25%; 2 = from 25% to 50%; 3 = from 50% to 75%; and 4 = above 75%. The scores of all leaves were used to calculate an individual plant's herbivory index, HI = $\Sigma(n_i \times C_i)N^{-1}$, where C_i is the category of damage (with $i = 0$–4), n is the number of leaves in the ith category, and N is the total number of leaves in the plant (Dirzo and Domínguez 1995).

Data Analysis

Early comparisons of functional traits between seedlings grown in sun and shade were done with t-tests. These tests evaluated whether the 2-month acclimation period was sufficient for plants to show distinct sun/shade phenotypes. A factorial ANOVA (main effects: light environment and herbivory) was used to compare the herbivory index across treatments.

We inspected coefficients of phenotypic correlation between traits in a Pearson correlation matrix to ensure that traits measured were not collinear (correlation coefficients greater than 0.8; table A1). Consequently, all traits were included in the phenotypic selection analysis. All trait distributions were standardized to mean 0 and unit standard deviation before the analysis. Differences in seedling survival in the field among treatments were first detected using a binomial linear model with logit link (factorial ANOVA-GLZ). Afterwards, we used a test of proportions to conduct pairwise comparisons between treatments.

To estimate selection on plant functional traits we calculated phenotypic selection gradients, which assessed the association between plant relative fitness and standardized plant traits. These metrics estimate the selection acting directly on the focal trait, taking into account the effect of correlated characters. Linear selection gradients (β) assess the magnitude of directional selection; nonlinear selection gradients (γ) assess the form (curvature) of the selection function (Lande and Arnold 1983). Seedling fitness was scored as a binary trait with dead and surviving seedlings receiving fitness scores of 0 and 1, respectively. Because the fitness variable was dichotomous and thus might violate parametric assumptions of normality, selec-

tion gradients were estimated using logistic regression analyses (GLZ procedure). Logistic coefficients were converted into linear gradients using the average gradient of the estimated selection surface (i.e., the probability of survival; Janzen and Stern 1998). Converting logistic coefficients into linear gradients yields selection coefficients (β_{avggrad}; hereafter β_{av}) that are interpreted similar to those obtained from multiple linear regressions for analyzing directional selection (β; Lande and Arnold 1983; Janzen and Stern 1998).

We used a generalized linear model (GLM) with logit link function to test for significant differences in the fitness function of functional traits (slope or curvature) in presence and absence of herbivores. Significant herbivory × trait interactions in the GLM indicate differences in selection gradients between herbivory treatments and can be interpreted as evidence that herbivores impose selection on a given trait (Mauricio and Rausher 1997; Shonle and Bergelson 2000). We used a backward stepwise procedure for these analyses, and we report the simplest model, which did not differ from the full model (likelihood ratio test, LRT; χ^2 $P > .1$). The significance of the effects in the models was tested using LRT.

Results

At the beginning of the experiment, after the acclimation period, five of the six functional traits measured differed between sun and shade seedlings. Individuals in sun showed higher photosynthetic capacity (A_{max}) and RGR, while SLA, petiole length, and internodes length were greater in shade seedlings (table A2). No differences in leaf transpiration (E) were detected.

Chemical exclusion of herbivores dramatically reduced herbivory damage to HI ~ 0 (fig. 1). Seedlings in sun (canopy gap) received slightly higher herbivory than seedlings in shade (forest understory), but this difference was not statistically significant (fig. 1). Overall survival of *Aristotelia*, a light-demanding species, was lower in the dark forest understory (fig. 2). Herbivore exclusion had a positive effect on plant fitness in shade but not in sun (significant light × herbivory interaction in the GLM; fig. 2).

In sun, we detected phenotypic selection for higher A_{max} and RGR for both herbivory and no-herbivory treatments (significant and positive β_{av} gradients; table 1), indicating that the probability of seedling survival, our fitness estimate, increases with the expression of these traits. In shade, *Aristotelia* seedlings experienced positive directional selection on SLA ($\beta_{\text{av}} > 0$) and stabilizing selection on RGR ($\gamma < 0$) in both herbivory and no-herbivory treatments (table 1).

Herbivores did not affect trait selection in sun (table 2; fig. 3A, 3B). In contrast, there were significant differences
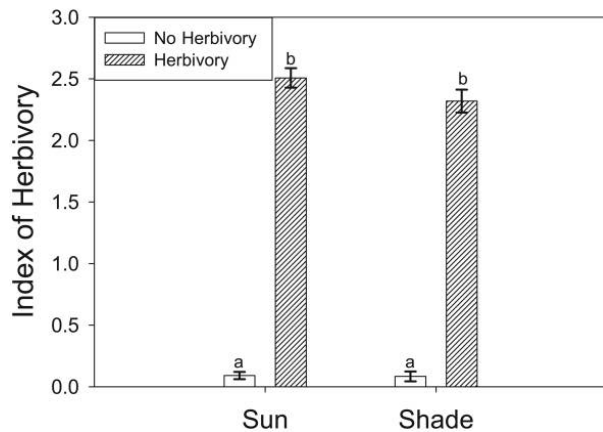
Figure 1: Herbivory damage on *Aristotelia chilensis* seedlings in sun (treefall canopy gap) and shade (mature forest understory) in a southern temperate rainforest. Leaf damage by herbivores was estimated by a categorical herbivory index (HI; $\pm$ SE; see "Methods"). Herbivory (shaded bars) = plants exposed to herbivores; No Herbivory (white bars) = herbivores excluded. Results of a factorial ANOVA: light: $F_{1,356} = 2.73$, $P > .05$; herbivory: $F_{1,356} = 516.4$, $P < .001$; light $\times$ herbivory: $F_{1,356} = 2.319$, $P > .05$. Bars sharing a letter are not significantly different ($P > .05$, Tukey HSD test)

in the pattern of directional selection on SLA in the presence and absence of herbivores in shade (table 3; fig. 3*C*, 3*D*), indicating that herbivores modified the selection on SLA in low light. Specifically, the strength of positive directional selection on SLA in the shade was relaxed when herbivores were present (fig. 3*C*, 3*D*).

Discussion

Light Environment and Patterns of Phenotypic Selection

Variation in plant functional traits has been extensively documented and is often assumed to be adaptive because of the link between functional traits and resource harvest and use (Pugnaire and Valladares 2007). However, there is comparatively little evidence demonstrating their adaptive value and evolutionary trends in natural populations (Farris and Lechowicz 1990; Ackerly et al. 2000; Arntz and Delph 2001; Heschel et al. 2004; Saldaña et al. 2007; Agrawal et al. 2008). In this field study, we found a close association between functional traits related to light use and plant fitness in *Aristotelia*, a light-demanding temperate rainforest tree species. Moreover, we found differential selection on plant functional traits in sun and shade habitats.

Populations of broadly distributed plant species frequently show increased SLA in shade environments. This reflects an optimization of the area devoted to light capture through the production of leaves with a large surface area

relative to the allocated biomass (Givnish 1988; Valladares and Niinemets 2008). Some models have shown that increased SLA is associated with enhanced relative growth rate and carbon gain, particularly in low light environments (Sims et al. 1994; Evans and Poorter 2001). Accordingly, we found strong and positive directional selection on SLA in shade but not in sun, clearly indicating that natural selection favors high SLA when *Aristotelia* seedlings grow in low-light environments. A similar result was reported earlier for the herb *Sinapis arvensis* subjected to neutral shade in a greenhouse experiment, where total selection favoring high SLA values was detected only under low-light conditions (Steinger et al. 2003). We found positive directional selection on A_{max} only in sun. High photosynthetic rates are advantageous in environments with high resource availability, where an elevated capacity of carbon uptake would translate into improved plant performance and competitive ability (Givnish 1988; Kitajima 1994). Arntz et al. (2000) demonstrated that increased photosynthetic rates are adaptive in high-light but not in low-light conditions in *Amaranthus hybridus*. Conversely, in the shade, where light availability (not carbon fixation) is the limiting factor, a high metabolic potential is disfavored because the biochemical machinery necessary to sustain elevated photosynthetic rates incurs high energetic costs (Amthor 1984; Walters and Reich 2000). A study carried out in the same temperate rainforest with the fern *Blechnum chilense* demonstrated a negative relationship
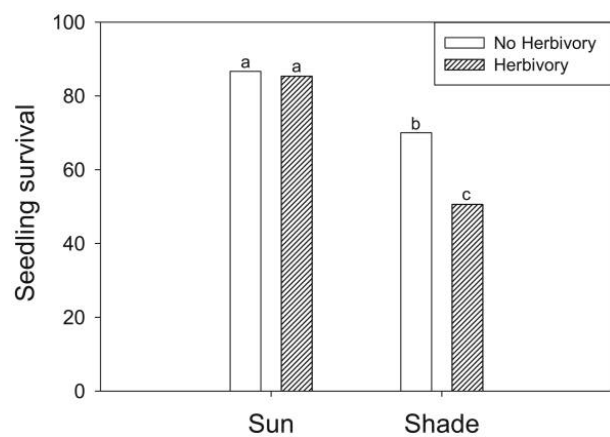
Figure 2: Effects of herbivory on *Aristotelia chilensis* seedling survival (%) in sun and shade environments in a temperate rainforest. Herbivory (shaded bars) = plants exposed to herbivores; No Herbivory (white bars) = herbivores excluded. Results of the binomial linear model with logit link were as follows. Light: log likelihood = -245.2, $\chi^2(1) = 49.98$, $P < .001$; herbivory: log likelihood = -241.4, $\chi^2(1) = 7.463$, $P < .001$; light $\times$ herbivory: log likelihood = -239.4; $\chi^2(1) = 4.111$; $P < .05$. Bars sharing a letter are not significantly different ($P > .05$, paired proportion tests).

Table 1: Selection gradients ($\pm$ SE) on functional traits of *Aristotelia chilensis* seedlings in a temperate rainforest

	Herbivory		No herbivory	
	β_{av}	γ	β_{av}	γ
Sun:				
A_{max}	**.086 $\pm$ .022****	$-.313 \pm .280$	**.137 $\pm$ .037*****	$-.177 \pm .277$
E	$-.050 \pm .036$	$-.539 \pm .801$	$.046 \pm .031$	$.814 \pm .771$
SLA	$-.014 \pm .039$	$.043 \pm .067$	$.022 \pm .049$	$-.499 \pm .394$
Petiole length	$.045 \pm .071$	$-.165 \pm .332$	$-.024 \pm .020$	$.059 \pm .050$
Internode length	$-.040 \pm .026$	$-.043 \pm .064$	$.062 \pm .044$	$-.280 \pm .414$
RGR	**.097 $\pm$ .029****	$-.533 \pm .612$	**.078 $\pm$ .027****	$-.226 \pm .164$
Shade:				
A_{max}	$-.026 \pm .020$	$.518 \pm .431$	$.002 \pm .002$	$.170 \pm .136$
E	$.013 \pm .016$	$-.142 \pm .094$	$-.013 \pm .024$	$-.132 \pm .152$
SLA	**.120 $\pm$ .026****	$.469 \pm .445$	**.240 $\pm$ .031*****	$.467 \pm .421$
Petiole length	$-.022 \pm .021$	$.426 \pm .360$	$-.003 \pm .002$	$.166 \pm .149$
Internode length	$.057 \pm .081$	$.158 \pm .145$	$.017 \pm .017$	$-.275 \pm .379$
RGR	$-.050 \pm .046$	**$-.994 \pm$ .413***	$-.040 \pm .035$	**$-.860 \pm$ .391***

Note: Sun = treefall canopy gap; shade = mature forest understory. Herbivory = plants were exposed to local herbivores; no herbivory = herbivores were chemically excluded. A_{max} = CO_2 assimilation; E = leaf transpiration; SLA = specific leaf area; RGR = relative growth rate. Significant (bold) values of β_{av} indicate directional selection. Significant values (bold) of γ indicate stabilizing (if <0) or disruptive (if >0) selection. Selection gradients were estimated using logistic regression analyses and logistic coefficients were converted into linear gradients using the average gradient of the estimated selection surface (Janzen and Stern 1998).

* $P < .05$.
** $P < .01$.
*** $P < .001$.

between metabolic rate (estimated from the dark respiration rate) and reproductive fitness in the shaded forest understory (Saldaña et al. 2007). Consistent with the above findings, we found that high RGR increased seedling survival in the sun, while intermediate values of RGR were favored in the shade.

The pattern of stabilizing selection on RGR found in the shade, both in presence and absence of herbivores, deserves further discussion. Variation in RGR has traditionally been related to variation in leaf net assimilation rate (NAR, g C per cm^2 per time), SLA, and leaf mass ratio (LMR, the ratio of total leaf mass to plant mass; Shipley 2006; Enquist et al. 2007). In *Aristotelia*, our data show that selection acts on RGR independently of variation in SLA and A_{max}, which is closely related to NAR. LMR was not included in the selection analysis because estimation requires plant destruction (early measurements are needed to link them with final estimates of fitness), but this trait is inversely associated with SLA (Salgado-Luarte and Gianoli 2011). If selection on RGR is also independent of selection on LMR, it would suggest that there is probably concurrent selection on other, unmeasured component of plant growth, such as carbon use efficiency (the net proportion of fixed carbon converted into biomass; Enquist et al. 2007). Specifically, the pattern of stabilizing selection we observed in the shade could reflect that the fastest

Table 2: Generalized linear model for survival of *Aristotelia chilensis* seedlings grown in sun (treefall canopy gap) in a temperate rainforest

Source of variation	Log likelihood	χ^2	P
Directional selection:			
Herbivory	-93.80	.139	.708
A_{max}	-98.81	40.32	**<.001**
RGR	-93.87	9.846	**.001**
Herbivory $\times$ A_{max}	-94.19	3.665	.160
Herbivory $\times$ RGR	-119.58	3.812	.148
Stabilizing selection:			
Herbivory	-121.4	.110	.739
A_{max}	-96.60	42.75	**<.001**
RGR	-77.11	10.87	**<.001**
A_{max}^2	-82.54	.563	.452
RGR^2	-75.10	3.006	.065
Herbivory $\times$ A_{max}	-66.63	2.073	.149
Herbivory $\times$ RGR	-60.58	1.218	.269
Herbivory $\times$ A_{max}^2	-115.6	.990	.609
Herbivory $\times$ RGR^2	-118.6	2.009	.156

Note: Results from a 16-month field experiment, where plants were either exposed to natural herbivory or protected from herbivores using pesticides. The simplest model is reported (df = 1 in all cases). Significant herbivory $\times$ [trait] and herbivory $\times$ [trait]^2 interactions indicate that herbivores change the patterns of directional selection and stabilizing selection, respectively. A_{max} = CO_2 assimilation; RGR = relative growth rate.
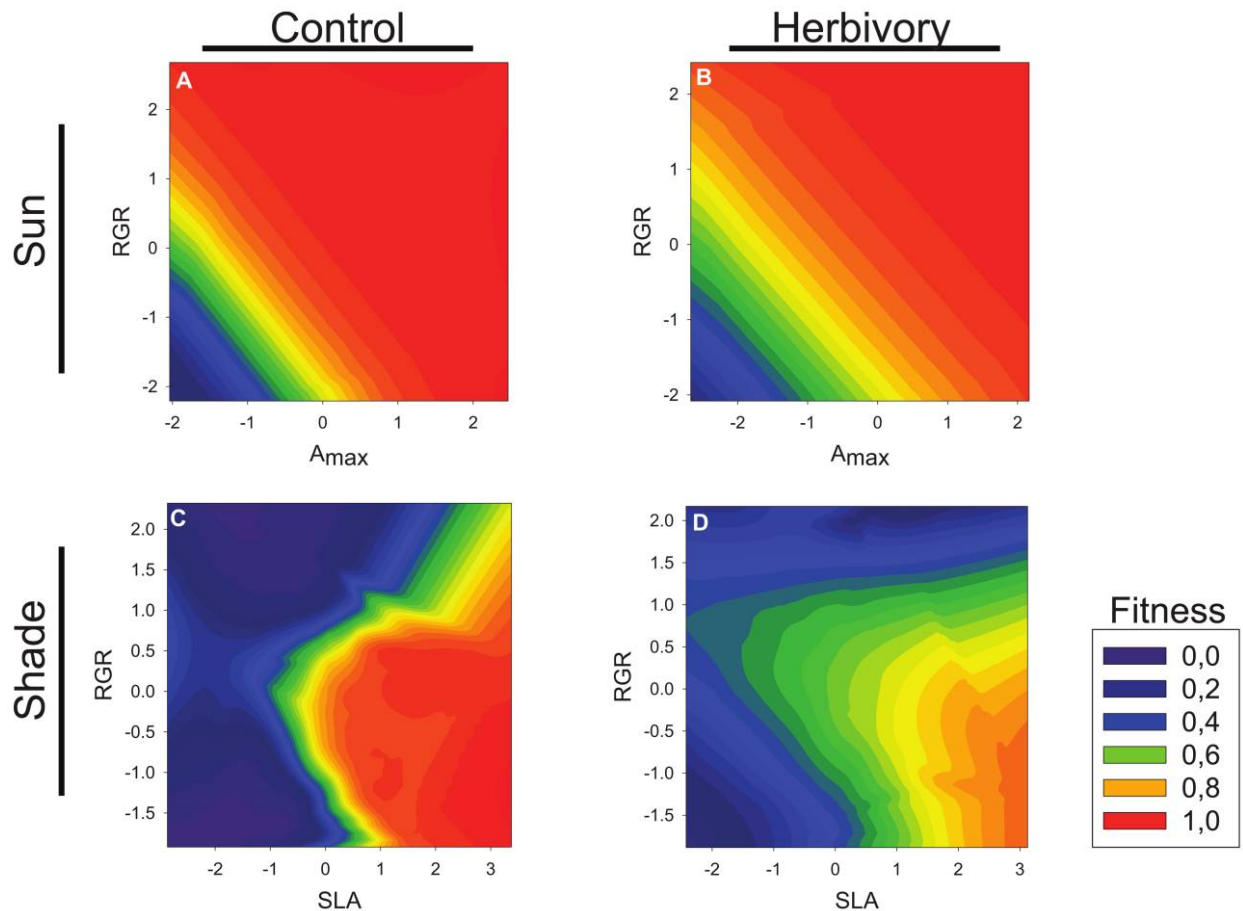
Figure 3: Fitness contours (seedling survival probability: from 0 to 1) for the traits experiencing selection in the sun or shade environment (see tables 2 and 3). *Top row*, sun environment: RGR (relative growth rate) and A_{max} (photosynthetic rate) of *Aristotelia chilensis* seedlings grown in absence (*A*) and presence (*B*) of herbivores. Bottom row: shade environment: RGR and SLA (specific leaf area) and RGR of seedlings grown in absence (*C*) and presence (*D*) of herbivores. These fitness contours, which show selection patterns, reveal reductions in both the slope and height of fitness peaks associated with increased SLA with herbivory only in the shade environment (*bottom row*). No such herbivory effect was seen in the sun environment (*top row*).

growing *Aristotelia* plants are the less carbon efficient, as has been shown at the interspecific level (Enquist et al. 2007). The slowest growing individuals are those already showing symptoms of light starvation that would eventually lead to seedling death.

We did not find evidence of selection acting on elongation of internodes or petioles. Although several studies have demonstrated the adaptive value of these traits in conditions of low light or competition with neighbors (Dudley and Schmitt 1996; Weinig 2000; Steinger et al. 2003), elongation of *Aristotelia* seedlings would be ineffective in shade avoidance because shading is caused by the closed forest canopy that is located several meters above the seedlings (Valladares et al. 2007).

Strictly speaking, we did not measure phenotypic plas-

ticity in *Aristotelia* seedlings or selection acting on plasticity. Nonetheless, it is undeniable that plastic responses played a role in determining plant phenotype in each light environment. All trait variation detected between sun and shade habitats was observed after similar seedlings were taken from an intermediate light environment, planted in sun and shade sites, and then measured months later. Moreover, we know that the study species is able to display significant phenotypic plasticity in response to changes in light availability (Salgado-Luarte and Gianoli 2011). Therefore, with some caution, our results can be extrapolated to improve our understanding of the expression of adaptive phenotypic plasticity (Ghalambor et al. 2007; Valladares et al. 2007; Auld et al. 2010). Plant adaptation to contrasting light environments has long been associated

Table 3: Generalized linear model for survival of *Aristotelia chilensis* seedlings grown in shade (mature forest understory) in a temperate rainforest

Source of variation	Log likelihood	χ^2	P
Directional selection:			
Herbivory	−103.2	3.948	**.046**
SLA	−107.2	32.94	**<.001**
Herbivory × SLA	−96.40	16.10	**.008**
Stabilizing selection:			
Herbivory	−77.03	6.536	**.010**
SLA	−99.75	46.41	**<.001**
RGR	−88.64	3.013	.082
SLA^2	−98.00	3.504	.072
RGR^2	−77.14	23.01	**<.001**
Herbivory × SLA	−96.40	53.10	**<.001**
Herbivory × RGR	−83.83	3.630	.162
Herbivory × SLA^2	−94.88	3.037	.218
Herbivory × RGR^2	−93.86	4.999	.082

Note: Results from a 16-month field experiment, where plants were either exposed to natural herbivory or protected from herbivores using pesticides. The simplest model is reported (df = 1 in all cases). Significant herbivory × [trait] and herbivory × [trait]^2 interactions indicate that herbivores change the patterns of directional selection and stabilizing selection, respectively. SLA = specific leaf area; RGR = relative growth rate.

with the expression of ecophysiological traits (Ackerly et al. 2000; Valladares and Niinemets, 2008), and it was early affirmed that natural selection should favor plants whose form and physiology tend to maximize net energy capture (Givnish 1988). Surprisingly, very few studies have demonstrated the adaptive value of light-use ecophysiological traits in natural populations (Arntz and Delph 2001; Saldaña et al. 2007). Our study provides field evidence of adaptation to light availability driven by selection on trait means (or trait plasticity).

Natural Selection by Herbivores

Results showed that natural herbivory decreased seedling survival only in shade (mature forest understory). Similar outcomes have been found in this temperate rainforest when simulated herbivory was applied to both *Aristotelia* and *Embothrium coccineum*, another light-demanding tree species (Salgado-Luarte and Gianoli 2010, 2011). These results support the idea that the detrimental effects of herbivory are strongest in the shade, because the recovery of plant tissue lost to herbivores is more difficult in low-resource environments (Wise and Abrahamson 2007).

Although A_{max} (in sun), SLA (in shade), and RGR (in sun and shade) were under selection in both herbivory treatments, only SLA in shade showed a change in its selection pattern due to the presence of herbivores. This differential selection between sun and shade cannot be

ascribed to a higher herbivore pressure in the shade because herbivory levels were similar in both light environments. Therefore, herbivores are selective agents on plant ecophysiological traits only in the dark forest understory. Specifically, selection by herbivores partially counteracted the selective pressure exerted by shade on increased expression of SLA.

Thin leaves with a high SLA have reduced mechanical strength and are thought to be more vulnerable to herbivore attack (Givnish 1988; Lei and Lechowicz 1990; Coley and Barone 1996; Hanley et al. 2007). However, this pattern is not always supported. For instance, data from 13 species in an Australian rainforest suggest that shade leaves with large SLA are unattractive to some herbivores because they offer lower nutritional benefits per ingestion effort (Lusk et al. 2010). In contrast, intraspecific studies have shown that SLA, or some of its components, is negatively related to leaf toughness (Choong 1996; Vile et al. 2005; Onoda et al. 2008). A recent study showed that herbivory damage on *Aristotelia* saplings in the field was negatively associated with leaf thickness (one component of SLA), which in turn was positively correlated with leaf toughness (Guerra et al. 2010). No-choice palatability assays with invertebrate herbivores showed that shade leaves of *Aristotelia* were consumed more than sun leaves (Guerra et al. 2010; C. Salgado-Luarte, unpublished data). Therefore, in view of the likely negative relationship between SLA and leaf structural components, increased SLA might enhance the palatability of *Aristotelia* seedlings to herbivores in our study system. It has been shown that herbivores may play a significant role in the selection and evolution of plant defensive characters (Rausher and Simms 1989; Marquis 1992; Mauricio and Rausher 1997; Shonle and Bergelson 2000; Agrawal 2005). Similarly, our study shows that the strength of directional selection acting on SLA is significantly reduced in the presence of herbivores, revealing an ecological cost of increased SLA (or decreased leaf toughness) most likely due to greater herbivory risk. Nonetheless, SLA was still positively associated with seedling survival in the shade when herbivores were present. This suggests that, on the whole, the capture of the limiting resource (light) was favored over leaf mechanical protection in the dark forest understory.

Identifying traits that can be measured accurately in the field and reflect variation in lifetime fitness in studies of long-lived plant species is a daunting task. We report results of a 1.5-year experiment. *Aristotelia* is a moderately long-lived species (<100 years; Muñoz and González 2006), and both SLA and RGR—the selected traits in the shade—vary with ontogeny (Lusk 2004). However, the relevant selective scenarios for a given plant population/species in low light do not necessarily occur when individuals are mature (Lusk 2004). In our study system, the seedling

and young sapling stages are particularly vulnerable in low light because of energy starvation (Lusk 2002, 2004). Therefore, the adaptive display of functional traits in this early stage is essential for the survival of this light-demanding species. Furthermore, herbivores may alter plant functional strategies to cope with low light in the forest understory. Because of their role as selective agents on seedling functional traits, herbivores may modify the phenotypes expressed while *Aristotelia* populations persist in a seedling bank, waiting for a canopy opening (Blundell and Peart 2001).

The involvement of herbivores in trait evolution within plant populations has been discussed extensively (Haukioja 1991; Marquis 1992; Thompson 1994; Herrera and Pellmyr 2002). Experimental studies have primarily addressed the role of herbivores as agents of selection in the evolution of plant resistance traits (Rausher and Simms 1989; Mauricio and Rausher 1997; Shonle and Bergelson 2000; Agrawal 2005) or plant tolerance traits (Stinchcombe and Rausher 2002; Fornoni 2011). There is also evidence that herbivore pressure may modify pollinator-mediated selection on plant traits (Ashman 2002; Gómez 2003; Wise and Hébert 2010). To our knowledge this is the first experimental demonstration that herbivores change the patterns of selection on a plant ecophysiological trait, that is, a character primarily involved in resource uptake and use (SLA).

Conclusions

We showed distinct patterns of selection on plant functional traits in different light environments in a temperate rainforest, with natural selection overall favoring phenotypes that improved the plant's net carbon gain. In the sun (treefall canopy gap), selection favored an increase in metabolic rates (A_{max}) and increased growth (RGR), which would increase the competitive ability of *Aristotelia* seedlings. In contrast, selection in the shade (mature forest understory) favored both the optimization of light capture (increased SLA) and a conservative use of resources (intermediate RGR).

In a classic article, Wade and Kalisz (1990) highlighted the usefulness of an experimental approach—as a complement to the correlational approach of multivariate selection analysis—to identify agents of selection. The experimental exclusion of herbivores from *Aristotelia* allowed us to demonstrate that their presence modifies the patterns of selection acting on plant ecophysiological traits. We found that the strength of positive directional selection on SLA in low light is reduced when herbivores—mainly insects, slugs, and snails—are present. Our study provides the first demonstration that natural herbivores can change the strength of selection on plant ecophysiological traits, that is, attributes whose main function is resource uptake. If there is sufficient genetic variation in the ecophysiological traits we measured, as is commonly the case (Geber and Griffen 2003), herbivory might modify the evolutionary trajectory of plant functional traits, resulting in a deviation from the "optimal" phenotype that enhances light capture in the shaded forest understory. This finding, together with the fact that leaf damage constrains plastic responses to shading in *Aristotelia* seedlings (Salgado-Luarte and Gianoli 2011), strongly suggests that research addressing the evolution of shade tolerance in plants should incorporate the selective role of herbivores.

Acknowledgments

APPENDIX

Tables showing mean values of functional traits and pairwise trait correlations in both sun and shade environments.

Table A1: Phenotypic correlations between functional traits of *Aristotelia chilensis* in two light environments in a temperate rainforest

	A_{max}	E	SLA	Petioles	Internodes	RGR
A_{max}	...	.32*	− .24	−.09	−.10	.30**
E	.29*	...	−.10	.04	.11	.14
SLA	−.32***	.24*	...	−.13	−.12	−.20
Petioles	−.05	.10	−.10	...	.15	−.10

Table A1 (*Continued*)

	$A_{\max}$	E	SLA	Petioles	Internodes	RGR
Internodes	.09	$-.13$	$-.05$	.11	...	$-.07$
RGR	.23	.07	.22	.10	.40***	...

Note: $A_{\max} = CO_2$ assimilation; $E =$ leaf transpiration; SLA $=$ specific leaf area; RGR $=$ relative growth rate. Petioles and internodes refer to lengths. The Pearson coefficients of correlation are shown the treefall canopy gap (sun) population (above the diagonal) and for the mature forest understory (shade) population (below the diagonal).

  * $P < .05$.

  ** $P < .01$.

  *** $P < .001$.

Table A2: Mean value ($\pm$ SE) of functional traits of *Aristotelia chilensis* in treefall canopy gap (sun) and mature forest understory (shade)

	Sun	Shade	t value
$A_{\max}$ (μmol CO_2 m^{-2} s^{-1})	$10.65 \pm .183$	$6.356 \pm .221$	14.69 ***
E (mmol H$_2$O m^{-2} s^{-1})	$2.338 \pm .030$	$2.451 \pm .035$	-2.498
SLA (cm^2 g^{-1})	185.5 ± 10.35	231.3 ± 12.35	3.989^*
Petiole length (cm)	$.890 \pm .024$	$1.167 \pm .026$	-8.660^{***}
Internode length (cm)	$.954 \pm .030$	$1.848 \pm .052$	-15.88^{***}
RGR (cm cm^{-1} month^{-1})	$.043 \pm .003$	$.034 \pm .002$	4.502^{***}

Note: $A_{\max} = CO_2$ assimilation; $E =$ leaf transpiration; SLA $=$ specific leaf area; RGR $=$ relative growth rate. Sun: $n = 300$ plants; shade: $n = 180$ plants.

  * $P < .05$.

  ** $P < .01$.

  *** $P < .001$; t-test.

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