

Forests are not immune to plant invasions: phenotypic plasticity and local adaptation allow *Prunella vulgaris* to colonize a temperate evergreen rainforest

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Abstract In the South American temperate evergreen rainforest (Valdivian forest), invasive plants are mainly restricted to open sites, being rare in the shaded understory. This is consistent with the notion of closed-canopy forests as communities relatively resistant to plant invasions. However, alien plants able to develop shade tolerance could be a threat to this unique forest. Phenotypic plasticity and local adaptation are two mechanisms enhancing invasiveness. Phenotypic plasticity can promote local adaptation by facilitating the establishment and persistence of invasive species in novel environments. We investigated the role of these processes in the recent colonization of Valdivian forest understory by the perennial alien herb *Prunella*

vulgaris from nearby populations in open sites. Using reciprocal transplants, we found local adaptation between populations. Field data showed that the shade environment selected for taller plants and greater specific leaf areas. We found population differentiation and within-population genetic variation in both mean values and reaction norms to light variation of several ecophysiological traits in common gardens from seeds collected in sun and shade populations. The colonization of the forest resulted in a reduction of plastic responses to light variation, which is consistent with the occurrence of genetic assimilation and suggests that *P. vulgaris* individuals adapted to the shade have reduced probabilities to return to open

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sites. All results taken together confirm the potential for rapid evolution of shade tolerance in *P. vulgaris* and suggest that this alien species may pose a threat to the native understory flora of Valdivian forest.

Keywords Adaptive divergence · Evolutionary response · Genetic assimilation · Invasive plants · Temperate rainforest

Introduction

Southern South America has been widely invaded by alien plant species introduced mainly from Europe and North America (Arroyo et al. 2000; Ugarte et al. 2010). The South American evergreen temperate rainforest (Valdivian forest) is identified as a hotspot of world biodiversity due to its remarkable levels of endemism and biogeographical isolation (Myers et al. 2000). While invasive plants have been detected in protected areas within the Valdivian forest, they are mainly restricted to roadsides and open sites where human activities take place, being very rare in the shaded understory (Pauchard and Alaback 2004). This pattern of distribution is consistent with the widespread notion of closed-canopy forests as communities relatively resistant to plant invasions (Martin et al. 2009). This assumption is based on the low shade tolerance of alien plants, which are predominantly early-successional species. Exotic plants with the potential to exhibit—or develop—shade tolerance may thus be a threat to this unique forest ecosystem. Understanding the mechanisms by which alien plant species may be able to successfully colonize the Valdivian forest understory is crucial to develop ecological protocols for its conservation and foresee future biological invasions.

One possible mechanism involved in plant invasion success is phenotypic plasticity (Hulme 2008; Pyšek and Richardson 2007; Richards et al. 2006; Matesanz et al. 2010). Ecological niche breadth of an alien plant species, and therefore invasion success, may be enhanced by the ability to express advantageous phenotypes under a great variety of environmental conditions (Pigliucci 2001). Adaptive phenotypic plasticity may first grant access to the novel environment and further allow the occurrence of rapid

adaptive evolution by exposing plants to new selective pressures, thus facilitating colonization of habitats in the introduced range (Bossdorf et al. 2005; Sakai et al. 2001; Sexton et al. 2002). Because not all genotypes of the invasive plant species would be successful colonizers, adaptation to the novel environment may involve a decrease in the invasive population size and at the same time an increase in genetic differences with the source population (Dlugosch and Parker 2008; Lavergne and Molofsky 2007). This process may be fostered by genetic drift and inbreeding in the newly adapted population (Allendorf and Lundquist 2003; Kawecki and Ebert 2004; Sexton et al. 2002). Associated to this genetic differentiation, evolution of phenotypic plasticity may also occur provided that there is genetic variation for plasticity of traits under selection. Several studies suggest that the evolution of phenotypic plasticity may be beneficial for alien species (Agrawal 2001; Bossdorf et al. 2008; Etterson 2004). However, in a low resources scenario (e.g. low light) this evolution tends to lower values of phenotypic plasticity (Valladares and Niinemets 2008; Valladares et al. 2000), presumably due to associated fitness costs (see Crispo 2007; Pigliucci et al. 2006), thus reducing the capacity of the evolved population to colonize again its original environment.

The present study was carried out in Puyehue National Park, which is a protected area within the Valdivian forest. Although we found several alien species occurring in disturbed areas and forest gaps, e.g., *Ranunculus repens* (Ranunculaceae), *Anthemis cotula*, *Taraxacum officinale* (Asteraceae), and *Lotus corniculatus* (Fabaceae), we decided to investigate *Prunella vulgaris* (Lamiaceae) because it is the only exotic species invading the mature forest understory (Saldaña et al. personal observations). *Prunella vulgaris* is a short lived, perennial herb native from Europe that is widely distributed in South America and along Chile (Matthei 1995). It was first introduced in Chile 130 years ago for gardening purposes (Philippi 1881) and now it can be found from 31°30'S to 42°53'S, and from sea level to 1,500 m (CONC Herbarium, 2005). Previous studies have documented substantial genotypic and phenotypic variation in growth, morphology, physiology and life history among populations from different habitats and geographic regions (Winn and Evans 1991; Winn and Werner 1987). Specifically, *P. vulgaris* showed significant phenotypic plasticity to contrasting light

environments (Winn and Evans 1991). This species is self-compatible, being capable of producing numerous seeds in the absence of pollinators (Winn and Werner 1987). Thus, a hypothetical abiotic filter for some pollinators exerted by the shade, as has been observed for another Lamiaceae species (Herrera 1995), should not hamper the naturalization of *P. vulgaris* in the forest understory.

We investigated the role of phenotypic plasticity, genetic differentiation and local adaptation in the invasion of the forest understory (shade) by *P. vulgaris* from nearby populations in open sites (sun). We evaluated in the field whether there is phenotypic differentiation between sun and shade populations and examined the adaptive value of plant traits in each environment using selection analysis. We further tested for the occurrence of local adaptation using reciprocal transplants. Working with seeds of *P. vulgaris* collected in sun and shade populations, we evaluated in greenhouse common gardens whether there is genetic differentiation between populations in both mean phenotypic values and phenotypic plasticity to shading for several plant characters functionally involved in light harvesting and exploitation. This was done using several genetic families per light environment in order to further determine whether there is genetic variation for plant traits and their phenotypic plasticity. If there is genetic variation for the characters shown to be under selection in the field, we then would prove that evolutionary change in these traits could occur in the invasive populations of *P. vulgaris* (see Geber and Griffen 2003).

Materials and methods

Study site

The lowland forest of Puyehue National Park (40°39'S, 72°11'W; 350–400 m a.s.l.), located in the western foothills of the Andes in south-central Chile, was the site selected for the present study. The climate is maritime temperate, with an average annual precipitation of 2,800 mm and a mean temperature of 9.8°C (Dorsch et al. 2003). This old-growth temperate rainforest is dominated by broad-leaved evergreen trees including *Laureliopsis philippiana* (Atherospermataceae), *Aextoxicon punctatum* (Aextoxicaceae),

Nothofagus dombeyi (Fagaceae) and *Eucryphia cordifolia* (Cunoniaceae; Saldaña and Lusk 2003), and woody vines are fairly abundant (Gianoli et al. 2010).

Field experiments

To characterize the patterns of abundance of *P. vulgaris* across light environments in the forest, six random transects of 50 meters (three in shade and three in sun conditions) were established to evaluate cover and density of the study species in October 2008. To locate each transect, we selected six areas representative of both sun and shade conditions. In each area, we randomly selected an angle direction to conduct the survey. North was equivalent to 0 and South to 180. We avoided transects crossing rivers and cliffs. Randomization selection was performed using Research Randomizer Program (RRP; www.randomizer.org). Every 5 m a quadrat of 1 m² was set up to count *P. vulgaris* individuals and estimate % cover area of: bare soil, litter, herbs and *P. vulgaris*. Additionally, in each point, light environmental conditions (direct and diffuse radiation) were characterized with hemispherical photographs taken with a digital camera (Coolpix 900, Nikon, Tokio, Japan), using a fish-eye lens of 180° field of view (FCE8, Nikon), and processed with HemiView 2.1 (Delta-T Devices, Cambridge, U.K.).

To test for the occurrence of local adaptation to light conditions in populations of *P. vulgaris*, a reciprocal transplant experiment was carried out in the forest. In November 2008, 50 seedlings were transplanted to a sun common garden (100% canopy openness; maximum radiation ca. 2,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and another 50 seedlings were transplanted to a shade common garden (8% canopy openness; maximum radiation ca. 160 $\mu\text{mol m}^{-2} \text{s}^{-1}$). In both cases, half of the plants (25 seedlings) were from a sun population (100% canopy openness) and the other half (25 seedlings) from a shade population (10% canopy openness). Following the criteria of Schlaepfer et al. (2010), we defined a population within an area of ca. 10 km². Seedlings to be planted in sun and shade common gardens were selected from multiples sites within one sun and one shade population. This sampling design minimized the possibility that observed patterns were due to reduced genetic variation from individuals of one single small location or to unmeasured micro-climatic characteristics varying across multiple

common gardens. To collect seedlings randomly, we first located 10 widely spaced, small plots (4 m^2) for each light population (20 in total). We then selected 5 plots to pick up 10 seedlings for each light population, using RRP. We collected sun plants from open gaps prairies within the forest matrix and not from roadside or path edges, hence avoiding outliers with extreme phenotypes.

We measured several plant traits prior to transplanting: plant height (cm), plant size (projected cover area from a vertical view, cm^2), number of leaves per individual, and SLA (specific leaf area) in one fully expanded leaf per individual ($\text{cm}^2 \text{ g}^{-1}$). These measurements provided data to determine initial phenotypic differentiation (subset of plants transplanted into their original environment) and also allowed the evaluation of phenotypic selection (Lande and Arnold 1983) by conducting multiple regression analysis considering fitness traits (survival, number of flower heads) at the end of the experiment—6 months later—as dependent variables and plant traits as independent variables. We also measured at the end of the experiment the same plant traits—with the exception of SLA—to better understand selective pressures involved in local adaptation patterns. An earlier study reported that the number of flowers per inflorescence (flower head) in *P. vulgaris* can be highly variable (Winn 1988). Consequently, we checked whether that was the case in our study system: we counted the number of flowers produced per inflorescence within and between sun and shade environments. We found that in both light environments the number of flowers per inflorescence showed relatively little variation (from 8 to 12). Therefore, the number of inflorescences is a reliable predictor of the total number of flowers.

Greenhouse experiments

Seeds of *P. vulgaris* were collected in Puyehue National Park during late austral summer 2007 (February–March). We sampled 10 widely spaced plants at each light environment, forest understory (4–8% canopy openness) and large gaps (>35% canopy openness), and collected 20–30 seeds per plant, hereafter termed “family”. In September 2007, seeds were placed on moistened paper in the dark at room temperature to allow germination. Seeds failed to germinate in three understory families and three gap

families. Ten days after emergence, 14 individuals of each family (seven families per original light environment) were transplanted to a greenhouse at Universidad de Concepción ($36^{\circ}49' \text{ S}$, $73^{\circ}03' \text{ W}$), where a factorial experiment was carried out crossing origin (understory/gap) with light treatment (shade/sun). Sun treatment was 55% of full sunlight (daily range: $600\text{--}1,200 \text{ PAR } \mu\text{mol m}^{-2} \text{ s}^{-1}$), which are the light conditions within the greenhouse. Shade treatment was 6% full sunlight ($35\text{--}200 \text{ PAR } \mu\text{mol m}^{-2} \text{ s}^{-1}$), which was obtained by placing plants under a double-layer dark mesh hung 1.5 m above the greenhouse benches. Measurements of photosynthetic active radiation (PAR) were done with a LI-250 Light Meter (LICOR, Lincoln, Nebraska, USA). Seven individuals from each family and origin were assigned to each light treatment (total initial $N = 7$ replicates $\times 7$ families $\times 2$ origins $\times 2$ treatments = 196 seedlings). Plants were watered every 3 days in all treatments. After 5 months, for each individual plant, maximum photosynthetic rate (A_{max}), stomatal conductance (gs) and instantaneous water use efficiency ($\text{WUE} = A_{\text{max}}/\text{gs}$) were measured at a saturating not inhibitory light ($\text{PAR } 1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$) using a CIRAS II portable infrared gas analyzer and leaf chamber (PP Systems, Hitchin, England). Additionally, in five individuals of five sun and shade families, total chlorophyll content (Chl) and chlorophyll a: chlorophyll b ratio (Chl a:b) was measured with a hand-held chlorophyll meter (SPAD-502, Minolta Camera Co., Osaka, Japan). Five separate measurements on one completely full-expanded leaf of each individual were performed. The SPAD-502 calculates an index in ‘SPAD units’ based on absorbance at 650 and 940 nm. We used the arithmetic mean of these measurements for all subsequent analyses. The non-invasive optical method provided reliable estimates of relative leaf Chl (Richardson et al. 2002). Afterwards, in two mature leaves per plant, leaf size (area) was estimated by means of digital photography and later analysis with Sigma-Scan Pro 5 software (SPSS Inc, Chicago, IL, USA). Leaves were then oven-dried at 70°C for 48 h and weighed. Specific leaf area (SLA) was determined for each sample.

Statistical analyses

A one-way ANOVA was used to compare the mean phenotype of study populations in the field before the

reciprocal transplant. To test for local adaptation in the field, a two-way ANOVA was used to detect significant Origin \times Light interactions in fitness estimators (survival and reproduction). To identify plant traits that could explain local adaptation, we calculated selection differentials and selection gradients (Lande and Arnold 1983) in each light environment regressing plant fitness against those traits measured at the end of the field experiment. Selection differentials (S) were calculated as the covariance between each standardized trait and relative fitness. Linear selection gradients (β) were obtained from multiple regression analysis using a general linear model (GLM). These metrics assessed the relationship between relative fitness and the standardized measured traits, taking into account the effect of correlated characters. In the case of plant survival, being a dichotomous response variable and because it may violate parametric assumptions of normality (Janzen and Stern 1998), multivariate nonparametric logistic regressions were used instead of linear regressions. To test for genetic differentiation between sun and shade populations and for genetic variation (differences between maternal families) in both mean values and reaction norms of ecophysiological traits measured in the greenhouse, a GLM was applied. In greenhouse analyses, Origin and Light were fixed factors and Family nested in Origin was a random factor.

A potential limitation of these experiments is that for both lab and field common garden experiments we did not obtain phenotypes from the second generation grown under similar environmental conditions. Thus, the results may include a carry-over effect from the maternal environment. All statistical analyses were performed with Statistica 7.0 (StatSoft Inc., Tulsa, OK, USA).

Results

Patterns of abundance

Although *P. vulgaris* has the capacity to invade the forest understory, its abundance was significantly higher in forest gaps (Cover (%): Shade = 6.5 ± 2.7 , Sun = 18.7 ± 4.8 ; $F_{1,60} = 5.6$, $P < 0.05$; Mean density per quadrat (# individuals m^{-2}): Shade = 5.3 ± 2.2 , Sun = 12.3 ± 1.25 , $F_{1,60} = 6.4$, $P < 0.05$). The

abundance of *P. vulgaris* was positively correlated with % bare soil and direct radiation, and negatively correlated with % soil surface covered by litter (Table 1).

Field experiments

Individuals from shade populations were taller ($F_{1,49} = 4.7$, $P < 0.05$, One-way ANOVA) and displayed greater SLA ($F_{1,49} = 84.4$, $P < 0.001$, One-way ANOVA) than individuals from sun populations. No significant differences were found for diameter plant size and number of leaves. After reciprocal transplants, individuals from shade origin were—overall—larger, and significant Origin \times Light environment interactions were found for most traits (Table 2, Fig. 1). For instance, plants from the shade were taller than sun plants only in the shade environment and plants from the sun produced more leaves than shade plants only in the sun environment (Table 2, Fig. 1). More importantly, a pattern of local adaptation was evident since survival of both sun and shade plants were greater in their respective environments (Table 2, Fig. 1). Interestingly, no evidence of local adaptation was found when fitness was estimated in terms of reproduction, with sun plants consistently producing more flowers than shade plants although these differences were more marked in the sun environment (Table 2, Fig. 1).

No differences in selection patterns were observed when we compared selection differentials (S) and selection gradients (β coefficients). Thus, trait selection in the field was basically direct (Table 3). In the sun, a significant selection gradient indicated that

Table 1 Multiple regression model estimating the relationship between *Prunella vulgaris* abundance, estimated both as plant cover (%) and plant density (# individuals m^{-2}), and habitat characteristics in a southern temperate rainforest: bare soil cover (%), litter cover (%), herbaceous cover (%), and direct and diffuse radiation ($\text{mol } m^{-2} \text{ year}^{-1}$) estimated from the analysis of hemispherical pictures

	R ²	Bare soil	Litter	Herbs	Direct Light	Diffuse Light
Cover	0.42	0.78***	-0.46*	0.27	0.27	0.32
Density	0.35	0.65***	-0.21	0.16	0.44*	0.01

The model fit is shown (R²) together with partial regression coefficients

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

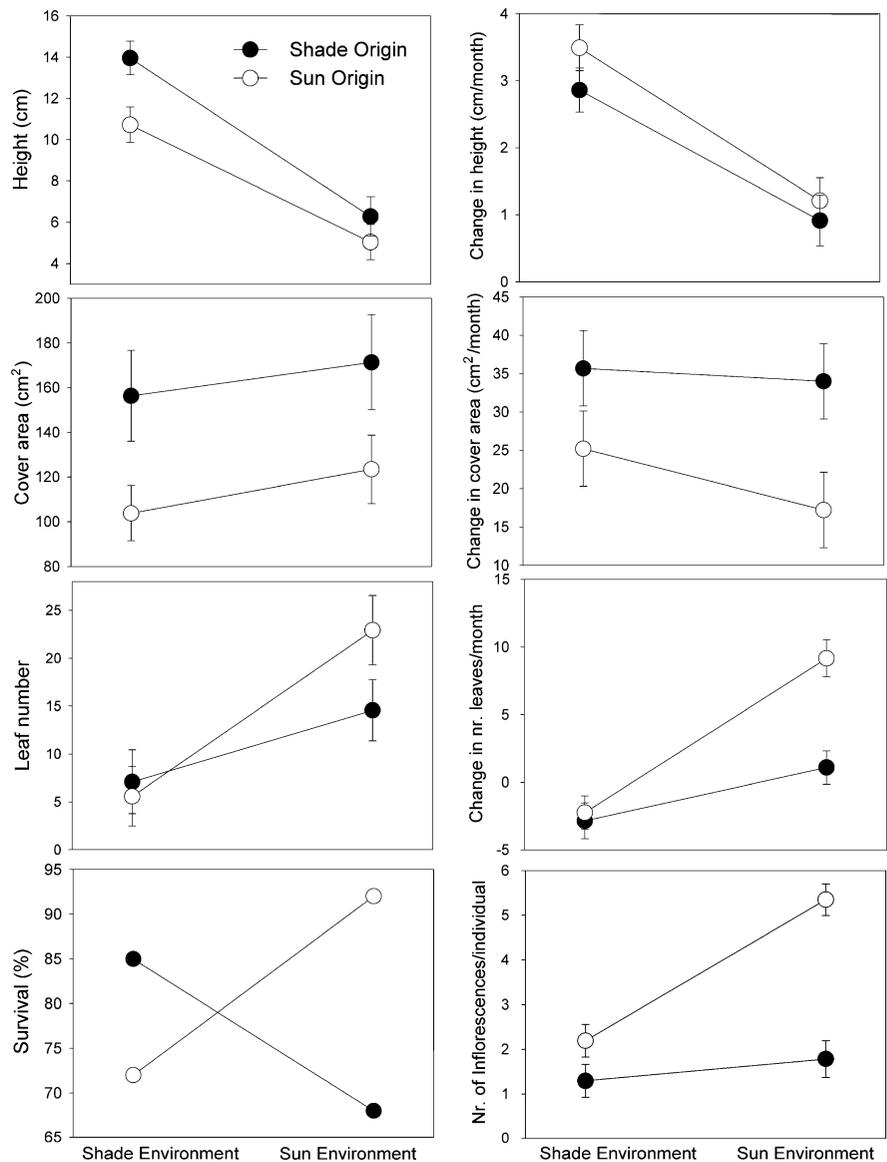
Table 2 Two-way ANOVA of *P. vulgaris* traits at the end of the reciprocal transplant in the field

	Height	# leaves	Size	Δ Height	Δ # leaves	Δ Size	Survival	Flowers
Origin (O)	15.7***	4.6*	36.2***	3.7	9.1***	37.6 ***	14.5**	15.3**
Light (L)	84.7***	0.6	1.9	52.1***	52.2***	3.4	76.4***	22.5***
O × L	13.8***	9.1**	0.7	2.5	6.6**	5.5*	45.2***	15.3**

Main Factors: Origin (gaps or understory) and Light (common garden in sun or shade environments). Data include the mean value of the traits, the change (Δ) in these traits during the experiment (compared to the initial measurements conducted immediately after the transplant), and fitness traits. F-ratios are shown (*df* for O and L = 97; *df* for O × L = 96), with the exception of Survival, where values of χ^2 are shown

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

Fig. 1 Results of a reciprocal transplant experiment between shade (forest understory) and sun (canopy gaps) populations of *Prunella vulgaris* in Puyehue National Park, Valdivian forest. Mean trait values, their change during the experiment, and fitness estimates are shown. Statistical analyses are given in Table 2



plants with lower height and displaying leaves with lower SLA were favored in terms of reproductive fitness, and increased plant survival was associated with reduced size and lower SLA (Table 3). In contrast, plants with higher SLA and greater height produced more flowers in the shade, and survival was enhanced when plants were taller (Table 3).

Greenhouse experiments

Prunella vulgaris phenotype was affected by origin (forest understory or canopy gap), light treatment in the greenhouse (sun or shade), the maternal family and the interaction among them for all five traits taken together (Table 4). Specifically, all measured traits showed significant differences according to

origin, which indicates the occurrence of population differentiation, and were significantly affected by the experimental treatment, which indicates plasticity to light availability (Table 4). Furthermore, there was a significant Origin \times Light effect for all traits but SLA, which indicates differential plasticity to shading in plants from the forest understory and from canopy gaps. The mean value of SLA, WUE, Chl and Chl a:b showed differences among families and there was genetic variation within populations for plasticity to shading in Amax, WUE, Chl and Chl a:b (Table 4). Overall, plants subjected to shading exhibited higher SLA and Chl, and lower Amax, WUE, and Chl a:b (Figs. 2, 3). *Prunella vulgaris* individuals from shade origin showed higher SLA and lower Amax, WUE, Chl and Chl a:b ratio than plants from

Table 3 Linear standardized selection differentials (S) and selection gradients (β) of reproduction (# flowers) and partial coefficients of multiple logistic regressions (B) of survival of

	Sun		Survival B	Shade		Survival B
	Reproduction			Reproduction		
	S	β		S	β	
R ²		0.24*	0.75***		0.21*	0.40*
Plant height	-0.30*	-0.24	0.11	0.38*	0.37*	1.36*
# leaves	0.16	0.10	2.27	-0.12	-0.23	0.01
Plant size	0.26	0.25	-4.27*	0.21	0.23	0.01
SLA	-0.47*	-0.43*	-7.70***	0.44*	0.47*	-0.02

R² indicates model fit

An overall R² does not apply for S coefficients, which are calculated as the covariance between each standardized trait and relative fitness

*** P < 0.001, * P < 0.05

Prunella vulgaris against plant traits in the sun and shade environments of a southern temperate rainforest

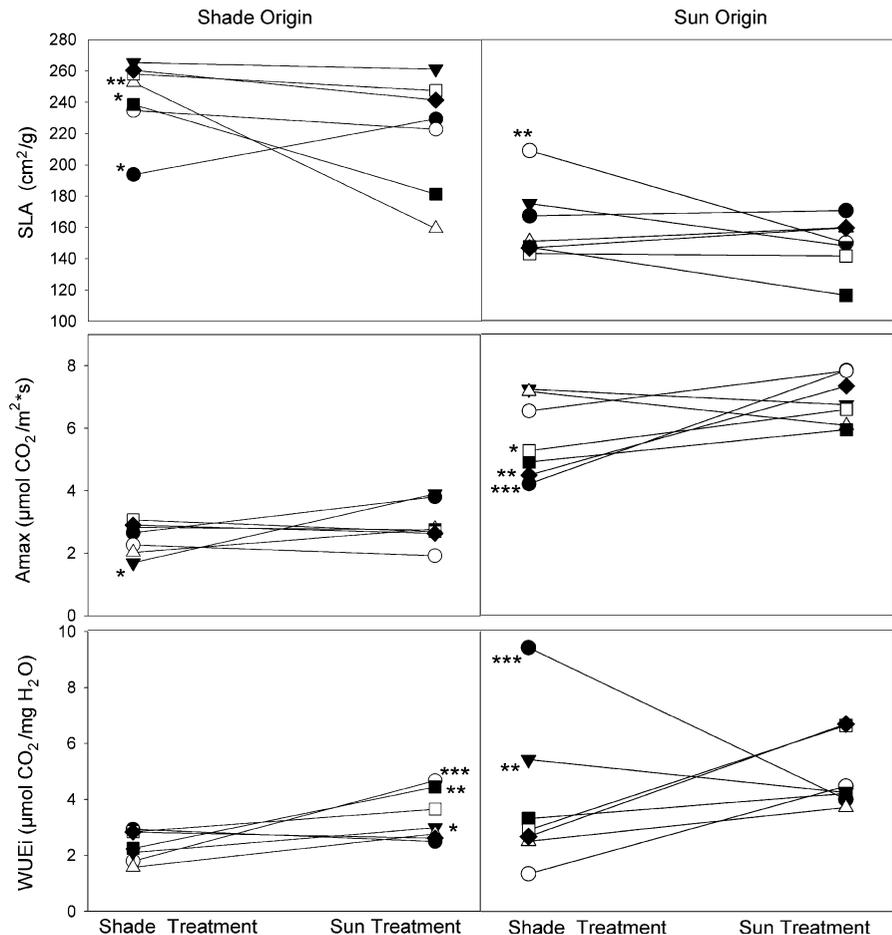
Table 4 GLM of variation in *P. vulgaris* traits in greenhouse common gardens

Traits	SLA	Amax	WUE	Chl	Chl a:b	
Origin (O)	134.4 (5)***	102.1 (1)***	252.1 (1)***	33.4 (1)***	108.5 (1)***	198.9 (1)***
Family (F in O)	8.2 (40)***	2.3 (12)*	1.7 (12)	5.5 (12)***	38.7 (8)***	7.5 (8)***
Light (L)	64.8 (5)***	6.6 (1)**	15.9 (1)***	35.0 (1)***	254.1 (1)***	80.2 (1)***
O \times L	50.9 (5)***	0.2 (1)	5.2 (1)***	5.1 (1)***	212.2 (1)***	19.7 (1)***
(F in O) \times L	5.1 (40)**	1.6 (12)	3.2 (12)**	3.4 (12)***	11.3 (8)***	4.9 (8)***

Main Factors: Origin (gaps or understory), Family nested in Origin (maternal family sampled in the field), and Light (sun or shade treatment). Analyses include the whole plant phenotype, with all traits taken together (Traits) and each individual trait: specific leaf area (SLA), maximum photosynthetic rate (Amax), instantaneous water use efficiency (WUE), total chlorophyll content (Chl), and chlorophyll a:chlorophyll b ratio (Chl a:b). Wilk's lambda (degrees of freedom) and F-ratios (df) are shown for Traits and each individual trait respectively

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

Fig. 2 Phenotypic plasticity of Specific Leaf Area (SLA), Maximum Photosynthetic Rate (A_{\max}) and instantaneous Water Use Efficiency (WUE) to shading in *Prunella vulgaris* seedlings as affected by origin of seeds (shade forest understory; sun canopy gaps) and maternal family. Light treatments in the greenhouse were 55 and 6% of full daylight for sun and shade, respectively. Maternal families (within origin) are identified by different symbols and fills. Differences between sun/shade treatment within families of the same origin were calculated with Fisher LSD post hoc and are represented with **** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. Further statistical analyses are provided in Table 4



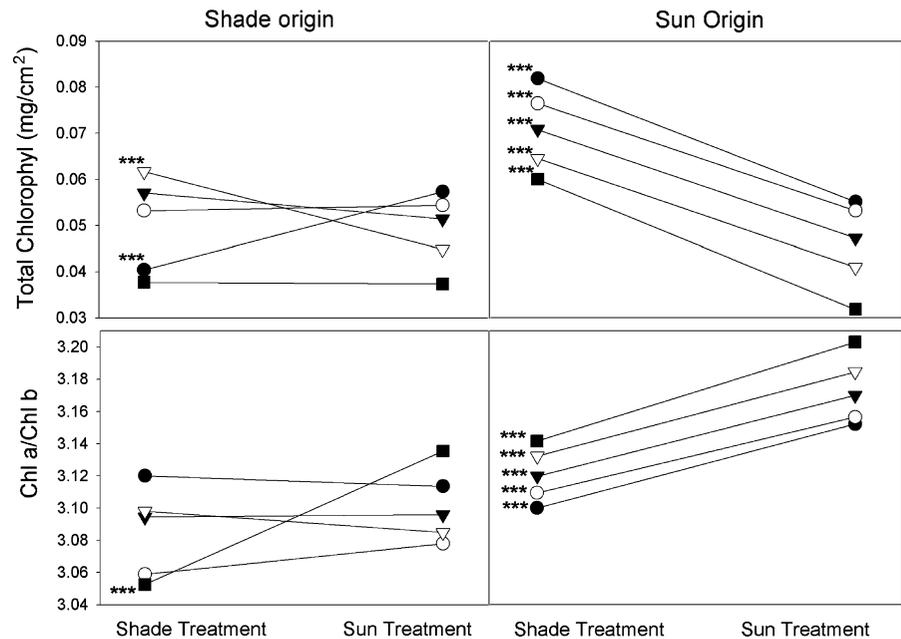
sun origin (Figs. 2, 3). Plants from the shade showed lower plasticity in WUE, Chl and Chl a:b ratio than plants from the sun (Figs. 2, 3).

Discussion

Invasive plant species spread over novel environments mainly by two mechanisms that are not mutually exclusive: local adaptation and phenotypic plasticity (Ross et al. 2009; Bossdorf et al. 2008; Parker et al. 2003). Our results indicate that both mechanisms are involved in the successful invasion of the Valdivian forest understory by *P. vulgaris*. On one hand, reciprocal transplant experiments demonstrated that phenotypic differentiation between sun and shade populations has an adaptive value in each light environment. On the other hand, phenotypic plasticity to shading in sun populations of *P. vulgaris*

most likely allowed the initial establishment of *P. vulgaris* individuals in the shaded understory, which was probably followed by—still detectable—selective processes that shaped the observed shade phenotypes (see Ghalambor et al. 2007; Sexton et al. 2002). Furthermore, we detected genetic variation for the same ecophysiological traits that are targets of selection, hence demonstrating the potential for evolutionary responses to occur in the respective selective scenarios. Examples of adaptive responses to environmental change during the spread of biological invasions are numerous (Blair and Wolfe 2004; Bossdorf et al. 2005; Sakai et al. 2001; Sexton et al. 2002; Strauss et al. 2006). However, most of them have focused on exotic plant species invading areas with some degree of disturbance. Our results show that adaptive evolution of invasive plants can also occur in relatively pristine, well-conserved forests and adds to the debate of whether mature

Fig. 3 Phenotypic plasticity of Total Chlorophylls and ratio Chlorophyll a/Chlorophyll b (Chla/Chlb) to shading in *Prunella vulgaris* seedlings as affected by origin of seeds (*shade* forest understory; *sun* canopy gaps) and maternal family. Light treatments in the greenhouse were 55 and 6% of full daylight for sun and shade, respectively. Figure description is the same as Fig. 2



forests are particularly resistant to plant invasion (Martin et al. 2009).

We found population differentiation in plasticity to shading, with reduced plasticity exhibited by individuals from the forest understory. Considering that open, sunny sites is the habitat from which *P. vulgaris* individuals invaded the understory, the reduction in plastic responses of shade individuals suggests that adaptation to deep shade conditions entails a relative canalization of these phenotypes by a genetic assimilation process (see Pigliucci et al. 2006). The genetic assimilation hypothesis posits that the novel phenotype resulting from plasticity becomes genetically fixed following directional selection on the optimum phenotype in the novel habitat, and the magnitude of phenotypic plasticity might be overall reduced because of fitness costs of plasticity (Crispo 2007). A reduction in the plastic responses of ecophysiological traits that may confer performance advantages in the sun but not in the shade, such as WUE, is somewhat expected. There are reports of excessive plasticity being maladaptive for plants, especially under relatively homogeneous environments (Valladares et al. 2007; van Kleunen and Fischer 2005). For instance, woody plant species typical of forest understory tend to show low values of phenotypic plasticity compared to their counterparts in the nearby open (Valladares et al. 2000).

There are early reports of population differentiation in ecophysiological responses to light availability in *P. vulgaris*, but comparing very distant populations (Winn and Evans 1991). In the present study, we detected small-scale population differentiation in both mean value and plasticity of traits that are relevant to performance under low light. This phenomenon may have been favored by the small population size in the forest understory, where only a subset of the pool of sun genotypes is represented, the likely decrease in outcrossing rate due to decreased pollination in the cool forest shade, and the ensuing population genetics phenomena (Allendorf and Lundquist 2003; Dlugosch and Parker 2008; Kawecki and Ebert 2004). Moreover, reproduction type was rather contrasting between sun and shade populations. While sun individuals mainly exhibited sexual reproduction by profuse flowering, shade individuals barely produced inflorescences but showed greater vegetative growth. It is important to recall that *P. vulgaris* is able to display clonal behavior (Macek and Lepš 2003).

Prunella vulgaris individuals showed functional phenotypic adjustments in response to the low light environment. Both populations initially differed in plant height and SLA. Accordingly, variation in these morphological traits was associated with local adaptation in each population. After the reciprocal

transplant, plants in the shade showed increased height and SLA, and reduced number of leaves. Thus, the invasion of the understory by this perennial herb has imposed variations in plant architecture in order to optimize light harvesting, which is a typical feature of the shade-avoidance syndrome (Smith and Witelam 1997). Results also suggest that *P. vulgaris* suffers a limitation of carbon acquisition in the shade because only those individuals with higher SLA and height—and hence with greater capabilities of carbon acquisition—were able to produce expensive plant tissues such as inflorescences. It is important to note that those traits favoring fitness in the shade had the opposite effect in the sun. For instance, higher SLA promotes flowering and clonal reproduction (size) in the shade but both traits (SLA and size) are negatively related to survival in the sun. Likewise, adaptation to the shade implies being taller and producing fewer leaves, while in open sites being taller reduces the number of inflorescences formed. These patterns would prevent a successful return to sun conditions of the individuals adapted to shade.

Although *P. vulgaris* is one of the few exotic plant species able to invade the deep shade understory of the undisturbed Valdivian temperate rain forest, its level of invasion is still low. Furthermore, the species abundance is positively related to the percentage of bare soil and negatively related to the percentage of soil surface covered by litter, which suggests some dependence on fine-scale disturbance. However, some preventive actions are already necessary at this first invasion stage in order to reduce the costs and augment the success of controlling this species later on (Hulme 2006). At the forest ecosystem scale, we recommend to avoid the creation of new forest gaps, which may act as reservoirs for *P. vulgaris* from where some individuals can “jump” into the forest understories. At the local scale, we recommend to maintain or recover the litter in the forest understory because it is negatively associated with *P. vulgaris* cover. We emphasize that this species has already invaded an enormous variety of environments along 4,000 km in Chile within the last 100 years (Matthei 1995). Although the lag-time to invade forests is generally long and invasions are not as dramatic as in other ecosystems (Martin and Marks 2006), evolutionary changes may occur rapidly (Maron et al. 2004) and forest understories are vulnerable to exotic species that rapidly adapt to low light. The present

study has documented the potential for rapid evolution of shade tolerance in *P. vulgaris* and hence this alien species may pose a threat to native understory flora of Valdivian forest. Further work should evaluate whether the mechanisms of phenotypic plasticity and local adaptation allowing *P. vulgaris* to invade the understory of this relatively pristine, well conserved forest can be also valid for other forest types or perhaps for other exotic species invading limited-light ecosystems.

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