

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

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1 **Abstract**

2 In the South American temperate evergreen rainforest (Valdivian forest), invasive plants
3 are mainly restricted to open sites, being rare in the shaded understory. This is
4 consistent with the notion of closed-canopy forests as communities relatively resistant
5 to plant invasions. However, alien plants able to develop shade tolerance could be a
6 threat to this unique forest. Phenotypic plasticity and local adaptation are two
7 mechanisms enhancing invasiveness. Phenotypic plasticity can promote local adaptation
8 by facilitating the establishment and persistence of invasive species in novel
9 environments. We investigated the role of these processes in the recent colonization of
10 Valdivian forest understory by the perennial alien herb *Prunella vulgaris* from nearby
11 populations in open sites. Using reciprocal transplants, we found local adaptation
12 between populations. Field data showed that the shade environment selected for taller
13 plants and greater specific leaf areas. We found population differentiation and within-
14 population genetic variation in both mean values and reaction norms to light variation of
15 several ecophysiological traits in common gardens from seeds collected in sun and
16 shade populations. The colonization of the forest resulted in a reduction of plastic
17 responses to light variation, which is consistent with the occurrence of genetic
18 assimilation and suggests that *P. vulgaris* individuals adapted to the shade have reduced
19 probabilities to return to open sites. All results taken together confirm the potential for
20 rapid evolution of shade tolerance in *P. vulgaris* suggesting that this alien species may
21 pose a threat to the native understory flora of Valdivian forest.

22

23 **Keywords:** adaptive divergence; evolutionary response; genetic assimilation; invasive
24 plants; temperate rainforest.

25

1 **Introduction**

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

17 One possible mechanism involved in plant invasion success is phenotypic
18 plasticity (Hulme 2008; Pyšek, Richardson 2007; Richards et al. 2006). Ecological
19 niche breadth of an alien plant species -and therefore invasion success- may be
20 enhanced by the ability to express advantageous phenotypes under a great variety of
21 environmental conditions (Pigliucci 2001). Adaptive phenotypic plasticity may first
22 grant access to the novel environment and further allow the occurrence of rapid adaptive
23 evolution by exposing plants to new selective pressures, thus facilitating colonization of
24 habitats in the introduced range (Bossdorf et al. 2005; Sakai et al. 2001; Sexton et al.
25 2002). Because not all genotypes of the invasive plant species would be successful

1 colonizers, adaptation to the novel environment may involve a decrease in the invasive
2 population size and at the same time an increase in genetic differences with the source
3 population (Dlugosch, Parker 2008; Lavergne, Molofsky 2007). This process may be
4 fostered by genetic drift and inbreeding in the newly adapted population (Allendorf,
5 Lundquist 2003; Kawecki, Ebert 2004; Sexton et al. 2002). Associated to this genetic
6 differentiation, evolution of phenotypic plasticity may also occur provided that there is
7 genetic variation for plasticity of traits under selection. Several studies suggest that the
8 evolution of phenotypic plasticity may be beneficial for alien species (Agrawal 2001;
9 Bossdorf et al. 2008; Etterson 2004). However, in a low resources scenario (e.g. low
10 light) this evolution tends to lower values of phenotypic plasticity (Valladares,
11 Niinemets 2008; Valladares et al. 2000), presumably due to associated fitness costs (see
12 Crispo 2007; Pigliucci et al. 2006), thus reducing the capacity of the evolved population
13 to colonize again its original environment.

14 The present study was carried out in Puyehue National Park, which is a
15 protected area within the Valdivian forest. Although we found several alien species
16 occurring in disturbed areas and forest gaps, e.g., *Ranunculus repens* (Ranunculaceae),
17 *Anthemis cotula*, *Taraxacum officinale* (Asteraceae), and *Lotus corniculatus* (Fabaceae),
18 we decided to investigate *Prunella vulgaris* (Lamiaceae) because it is the only exotic
19 species invading the mature forest understory (Saldaña et al. personal observations).
20 *P.vulgaris* is a short lived, perennial herb native from Europe that is widely distributed
21 in South America and along Chile (Matthei 1995). It was first introduced in Chile 130
22 years ago for gardening purposes (Philippi 1881) and now it can be found from 31° 30'
23 S to 42° 53' S, and from sea level to 1500 m (CONC Herbarium, 2005). Previous studies
24 have documented substantial genotypic and phenotypic variation in growth,
25 morphology, physiology and life history among populations from different habitats and

1 geographic regions (Winn, Evans 1991; Winn, Werner 1987). Specifically, *P. vulgaris*
2 showed significant phenotypic plasticity to contrasting light environments (Winn, Evans
3 1991). This species is self-compatible, being capable of producing numerous seeds in
4 the absence of pollinators (Winn, Werner 1987). Thus, a hypothetical abiotic filter for
5 some pollinators exerted by the shade, as has been observed for another Lamiaceae
6 species (Herrera 1995), should not hamper the naturalization of *P. vulgaris* in the forest
7 understory.

8 We investigated the role of phenotypic plasticity, genetic differentiation and
9 local adaptation in the invasion of the forest understory (shade) by *P. vulgaris* from
10 nearby populations in open sites (sun). We evaluated in the field whether there is
11 phenotypic differentiation between sun and shade populations and examined the
12 adaptive value of plant traits in each environment using phenotypic selection analysis
13 (Lande, Arnold 1983). We further tested for the occurrence of local adaptation using
14 reciprocal transplants (Kawecki, Ebert 2004). Working with seeds of *P. vulgaris*
15 collected in sun and shade populations, we evaluated in greenhouse common gardens
16 whether there is genetic differentiation between populations in both mean phenotypic
17 values and phenotypic plasticity to shading for several plant characters functional
18 involved in light harvesting and exploitation. This was done using several genetic
19 families per light environment in order to further determine whether there is genetic
20 variation for plant traits and their phenotypic plasticity. If there is genetic variation for
21 the characters shown to be under selection in the field, we then would prove that
22 evolutionary change in these traits could occur in the invasive populations of *P. vulgaris*
23 (see Geber, Griffen 2003).

24

25 **Material and Methods**

1 *Study site*

2 The lowland forest of Puyehue National Park (40° 39' S, 72° 11' W; 350-400 m a.s.l),
3 located in the western foothills of the Andes in south-central Chile, was the site selected
4 for the present study. The climate is maritime temperate, with an average annual
5 precipitation of 2800 mm and a mean temperature of 9.8 °C (Dorsch 2003). This old-
6 growth temperate rainforest is dominated by broad-leaved evergreen trees including
7 *Laureliopsis philippiana* (Atherospermataceae), *Aextoxicon punctatum* (Aextoxicaceae),
8 *Nothofagus dombeyi* (Fagaceae) and *Eucryphia cordifolia* (Cunoniaceae) (Saldaña,
9 Lusk 2003), and woody vines are fairly abundant (Gianoli et al. 2010).

10

11 *Field experiments*

12 To characterize the patterns of abundance of *P. vulgaris* across light environments in the
13 forest, six random transects of 50 meters (three in shade and three in sun conditions)
14 were established to evaluate cover and density of the study species in October 2008.
15 Every 5 m a quadrat of 1 m² was set up to count *P. vulgaris* individuals and estimate %
16 cover area of: bare soil, litter, herbs and *P. vulgaris*. Additionally, in each point, light
17 environmental conditions (direct and diffuse radiation) were characterized with
18 hemispherical photographs taken with a Nikon Coolpix 900 digital camera and
19 processed with HemiView 2.1 (Delta-T Devices, Cambridge, U.K.).

20 To test for the occurrence of local adaptation to light conditions in populations
21 of *P. vulgaris*, a reciprocal transplant experiment was carried out in the forest. In
22 November 2008, 50 seedlings were transplanted to a common garden in a sun site
23 (100% canopy openness; maximum radiation ca. 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and 50 seedlings
24 were transplanted to a shade common garden (8% canopy openness; maximum radiation
25 ca. 160 $\mu\text{mol m}^{-2} \text{s}^{-1}$). In both cases, half of the plants (25 seedlings) were from a sun

1 population (100% canopy openness) and the other half (25 seedlings) from a shade
2 population (10% canopy openness). We measured several plant traits at the start of the
3 experiment: plant height (cm), plant size (projected cover area from a vertical view,
4 cm²), number of leaves per individual, and SLA (specific leaf area) in one fully
5 expanded leaf per individual (cm² g⁻¹). These measurements provided data to determine
6 initial phenotypic differentiation (subset of plants transplanted into their original
7 environment) and also allowed the evaluation of phenotypic selection (Lande, Arnold
8 1983) by conducting multiple regression analysis considering fitness traits (survival,
9 number of flower heads) at the end of the experiment –six months later– as dependent
10 variables and plant traits as independent variables. We also measured at the end of the
11 experiment the same plant traits –with the exception of SLA– to perform another
12 selection analysis with number of flower heads as fitness component and change in each
13 of these traits as predictor variable.

14

15 *Greenhouse experiments*

16 Seeds of *P. vulgaris* were collected in Puyehue National Park during late austral
17 summer 2007 (February–March). We sampled 10 widely spaced plants at each light
18 environment, forest understory (4-8 % canopy openness) and large gaps (> 35 % canopy
19 openness), and collected 20–30 seeds per plant, hereafter termed “family”. In September
20 2007, seeds were placed on moistened paper in the dark at room temperature to allow
21 germination. Seeds failed to germinate in three understory families and three gap
22 families. Ten days after emergence, 14 individuals of each family (seven families per
23 original light environment) were transplanted to a greenhouse at Universidad de
24 Concepción (36° 49' S, 73° 03' W), where a factorial experiment was carried out
25 crossing origin (understory / gap) with light treatment (shade / sun). Sun treatment was

1 55% of full sunlight (daily range: 600–1200 PAR $\mu\text{mol m}^{-2} \text{s}^{-1}$), which are the light
2 conditions within the greenhouse. Shade treatment was 6% full sunlight (35–200 PAR
3 $\mu\text{mol m}^{-2} \text{s}^{-1}$), which was obtained by placing plants under a double-layer dark mesh
4 hung 1.5 m above the greenhouse benches. Measurements of photosynthetic active
5 radiation (PAR) were done with a LI-250 Light Meter (LI-COR). Seven individuals
6 from each family and origin were assigned to each light treatment (total initial N = 7
7 replicates * 7 families * 2 origins * 2 treatments = 196 seedlings). Plants were watered
8 every three days in all treatments. After five months, for each individual plant,
9 maximum photosynthetic rate (A_{max}), stomatal conductance (g_s) and instantaneous
10 water use efficiency ($\text{WUE} = A_{\text{max}}/g_s$) were measured at a saturating not inhibitory
11 light (PAR 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) using a CIRAS II portable infrared gas analyzer and leaf
12 chamber (PP Systems, Hitchin, England). Additionally, in five individuals of five sun
13 and shade families, total chlorophyll content (Chl) and chlorophyll a: chlorophyll b ratio
14 (Chl a:b) was measured with a hand-held chlorophyll meter (SPAD-502, Minolta
15 Camera Co., Osaka, Japan). Five separate measurements on one completely full-
16 expanded leaf of each individual were performed. The SPAD-502 calculates an index in
17 ‘SPAD units’ based on absorbance at 650 and 940 nm. We used the arithmetic mean of
18 these measurements for all subsequent analyses. The non-invasive optical method
19 provided reliable estimates of relative leaf Chl (Richardson et al. 2002). Afterwards, in
20 two mature leaves per plant, leaf size (area) was estimated by means of digital
21 photography and later analysis with Sigma-Scan Pro 5 software (SPSS Inc, Chicago, IL,
22 USA). Leaves were then oven-dried at 70°C for 48 h and weighed. Specific leaf area
23 (SLA) was determined for each sample.

24

25 *Statistical analyses*

1 A one-way ANOVA was used to compare the mean phenotype of study populations in
2 the field before the reciprocal transplant. To test for local adaptation in the field, a two-
3 way ANOVA was used to detect significant Origin x Light interactions in fitness
4 estimators (survival and reproduction). To identify plant traits that could explain local
5 adaptation, selection gradients (Lande, Arnold 1983) were calculated in each light
6 environment. Directional selection gradients (β) were obtained from multiple regression
7 analysis using a general lineal model (GLM). These metrics assessed the relationship
8 between relative fitness and the standardized measured traits, taking into account the
9 effect of correlated characters. In the case of plant survival, being a dichotomous
10 response variable and because it may violate parametric assumptions of normality
11 (Janzen, Stern 1998), multivariate nonparametric logistic regressions were used instead
12 of linear regressions. To test for genetic differentiation between sun and shade
13 populations and for genetic variation (differences between maternal families) in both
14 mean values and reaction norms of ecophysiological traits measured in the greenhouse,
15 a GLM was applied. In greenhouse analyses, Origin and Light were selected as fixed
16 factors whereas Family nested in Origin was selected as random.

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

22

23

24 **Results**

25 *Patterns of abundance*

1 Although *P. vulgaris* has the capacity to invade the forest understory, its abundance was
2 significant higher in forest gaps (Cover (%): Shade = 6.5 ± 2.7 , Sun = 18.7 ± 4.8 ; $F_{1,60}$
3 = 5.6, $P < 0.05$; Mean density per quadrat (# individuals m^{-2}): Shade = 5.3 ± 2.2 , Sun =
4 12.3 ± 1.25 , $F_{1,60} = 6.4$, $P < 0.05$). The abundance of *P. vulgaris* was positively
5 correlated with % bare soil and direct radiation, and negatively correlated with % soil
6 surface covered by litter (Table 1).

7

8 *Field experiments*

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

22 In the sun, a significant selection gradient indicated that plants displaying leaves
23 with lower SLA were favored in terms of reproductive fitness, and increased plant
24 survival was associated with reduced size and lower SLA (Table 3). In contrast, plants

1 with higher SLA and greater height produced more flowers in the shade, and survival
2 was enhanced when plants were taller (Table 3).

3 4 *Greenhouse experiments*

5 *P. vulgaris* phenotype was affected by origin (forest understory or canopy gap), light
6 treatment in the greenhouse (sun or shade), the maternal family and the interaction
7 among them for all five traits taken together (Table 4). Specifically, all measured traits
8 showed significant differences according to origin, which indicates the occurrence of
9 population differentiation, and were significantly affected by the experimental
10 treatment, which indicates plasticity to light availability (Table 4). Furthermore, there
11 was a significant Origin x Light effect for three traits (WUE, Chl and Chl a:b ratio),
12 which indicates differential plasticity to shading in plants from the forest understory and
13 from canopy gaps. The mean value of SLA, WUE, Chl and Chl a:b showed differences
14 among families and there was genetic variation within populations for plasticity to
15 shading in Amax, WUE, Chl and Chl a:b (Table 4). Overall, plants subjected to shading
16 exhibited higher SLA and Chl, and lower Amax, WUE, and Chl a:b (Fig. 2). *P. vulgaris*
17 individuals from shade origin showed higher SLA and lower Amax, WUE, Chl and Chl
18 a:b ratio than plants from sun origin (Fig. 2). Plants from the shade showed lower
19 plasticity in WUE, Chl and Chl a:b ratio than plants from the sun (Fig. 2).

20 21 22 **Discussion**

23 Invasive plant species spread over novel environments mainly by two mechanisms that
24 are not mutually exclusive: local adaptation and “general purpose genotype” typically
25 relying on high phenotypic plasticity (Ross et al. 2009; Bossdorf et al. 2008; Parker et

1 al. 2003). Our results indicate that both mechanisms are involved in the successful
2 invasion of the Valdivian forest understory by *P. vulgaris*. On one hand, reciprocal
3 transplant experiments demonstrated that phenotypic differentiation between sun and
4 shade populations has an adaptive value in each light environment. On the other hand,
5 phenotypic plasticity to shading in sun populations of *P. vulgaris* most likely allowed
6 the initial establishment of *P. vulgaris* individuals in the shaded understory, which was
7 probably followed by –still detectable– selective processes that shaped the observed
8 shade phenotypes (see Ghalambor et al. 2007; Sexton et al. 2002). Furthermore, we
9 detected genetic variation for the same ecophysiological traits that are target of
10 selection, hence demonstrating the potential for evolutionary responses to occur in the
11 respective selective scenarios. Examples of adaptive responses to environmental change
12 during the spread of biological invasions are numerous (Blair, Wolfe 2004; Bossdorf et
13 al. 2005; Sakai et al. 2001; Sexton et al. 2002; Strauss et al. 2006). However, most of
14 them have focused on exotic plant species invading areas with some degree of
15 disturbance. Our results show that adaptive evolution of invasive plants can also occur
16 in relatively pristine, well-conserved forests and adds to the debate of whether mature
17 forests are particularly resistant to plant invasion (Martin et al. 2009).

18 We found population differentiation in plasticity to shading, with reduced
19 plasticity exhibited by individuals from the forest understory. Considering that open,
20 sunny sites is the habitat from which *P. vulgaris* individuals invaded the understory, the
21 reduction in plastic responses of shade individuals suggests that adaptation to deep
22 shade conditions entails a relative canalization of these phenotypes by a genetic
23 assimilation process (see Pigliucci et al. 2006; Crispo 2007). A reduction in the plastic
24 responses of ecophysiological traits that may confer performance advantages in the sun
25 but not in the shade, such as WUE, is somewhat expected. The genetic assimilation

1 hypothesis posits that the novel phenotype resulting from plasticity becomes genetically
2 fixed following directional selection on the optimum phenotype in the novel habitat, and
3 the magnitude of phenotypic plasticity might be overall reduced because of fitness costs
4 of plasticity (Crispo 2007). There are reports of excessive plasticity being maladaptive
5 for plants, especially under relatively homogeneous environments (Valladares et al.
6 2007; van Kleunen, Fischer 2005). For instance, woody plant species typical of forest
7 understory tend to show low values of phenotypic plasticity compared to their
8 counterparts in the nearby open (Valladares et al. 2000).

9 There are early reports of population differentiation in ecophysiological
10 responses to light availability in *P. vulgaris*, but comparing very distant populations
11 (Winn, Evans 1991). In the present study, small-scale population differentiation in both
12 mean value and plasticity of traits that are relevant to performance under low light may
13 be fostered by the small population size in the forest understory, where only a subset of
14 the pool of sun genotypes is represented, the likely decrease in outcrossing rate due to
15 decreased pollination in the cool forest shade, and the ensuing population genetics
16 phenomena (Allendorf, Lundquist 2003; Dlugosch, Parker 2008; Kawecki, Ebert 2004).
17 Moreover, reproduction type was rather contrasting between sun and shade populations.
18 While sun individuals mainly exhibited sexual reproduction by profuse flowering, shade
19 individuals barely produced inflorescences but showed greater vegetative growth. It is
20 important to recall that *P. vulgaris* is able to display clonal behavior (Macek, Lepš
21 2003).

22 *P. vulgaris* individuals showed functional phenotypic adjustments in response to
23 the low light environment. Both populations initially differed in plant height and SLA.
24 Accordingly, variation in these morphological traits was associated with local
25 adaptation in each population. After the reciprocal transplant, plants in the shade

1 showed increased height and SLA, and reduced number of leaves. Thus, the invasion of
2 the understory by this perennial herb has imposed variations in plant architecture in
3 order to optimize light harvesting, which is a typical feature of the shade-avoidance
4 syndrome (Smith, Whitelam 1997). Results also suggest that *P.vulgaris* suffers a
5 limitation of carbon acquisition in the shade because only those individuals with higher
6 SLA and height –and hence with greater capabilities of carbon acquisition– were able to
7 produce expensive plant tissues such as inflorescences. It is important to note that those
8 traits favoring fitness in the shade had the contrary effect in the sun. For instance, higher
9 SLA promotes flowering and clonal reproduction (size) in the shade but both traits
10 (SLA and size) are negatively related to the survival in the sun. Likewise, adaptation to
11 the shade implies being taller and produce fewer leaves, while in open sites being taller
12 reduces the number of inflorescences formed and leaf production is associated with
13 competitive ability. These patterns would hold back the return to sun conditions of the
14 individuals adapted to shade.

15 Although *P. vulgaris* is one of the few exotic plant species able to invade the
16 deep shade understory of the undisturbed Valdivian temperate rain forest, its level of
17 invasion is still low. Furthermore, the species abundance is positively related to the %
18 of bare soil and negatively related to the % of soil surface covered by litter, which
19 suggests some dependence on fine-scale disturbance. However, some preventive actions
20 are already necessary at this first invasion stage in order to reduce the costs and
21 augment the success of controlling this species latter on (Hulme 2006). We have to keep
22 in mind that this species has already invaded an enormous variety of environments
23 along 4000 km in Chile within the last 100 years (Matthei 1995). Although the lag-time
24 to invade forests is generally long and invasions are not as dramatic as in other
25 ecosystems (Martin, Marks 2006), evolutionary changes may occur rapidly (Maron et

1 al. 2004) and forest understories are vulnerable to exotic species that rapidly adapt to
2 low light. This study has documented the potential for rapid evolution of shade
3 tolerance in *P. vulgaris* and hence this alien species may pose a threat to native
4 understory flora of Valdivian forest.

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Tables & Figures

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 the Puyehue rainforest: bare soil cover (%), litter cover (%), herbaceous cover (%), and direct and diffuse radiation (mol m⁻² yr⁻¹) estimated from the analysis of hemispherical pictures. The model fit is shown (R²) together with partial regression coefficients.

	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

*** 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. 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 (d.f. for (O) and (L)=97; d.f. for OxL=96) , with the exception of Survival, where values of χ^2 are shown.

	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 x L	13.8***	9.1**	0.7	2.5	6.6**	5.5*	45.2***	15.3**

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

Table 3. Linear standardized selection gradients (β) of reproduction (# flowers) and coefficients of multiple logistic regressions (B) of survival of *Prunella vulgaris* against traits in the sun and shade environments of a southern temperate rainforest. R^2 indicates the the model.

	Sun		Shade	
	Reproduction	Survival	Reproduction	Survival
	β	B	β	B
R^2	0.24*	0.75***	0.21*	0.4
Plant height	-0.24	0.11	0.37*	1.3
# leaves	0.10	2.27	-0.23	0.0
Plant size	0.25	-4.27*	0.23	0.0
SLA	-0.43*	-7.70***	0.47*	-0.0

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

Table 4. GLM of variation in *P. vulgaris* traits in greenhouse common gardens. 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 (d. f.) are shown for Traits and each individual trait respectively.

	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 x L	50.9 (5)***	0.2 (1)	1.1 (1)	0.1 (1)***	212.2 (1)***	19.7 (1)***
(F in O) x L	5.1 (40)**	1.6 (12)	3.2 (12)**	3.4 (12)***	11.3 (8)***	4.9 (8)***

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

Figures caption

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. Trait values, their change during the experiment, and fitness estimates are shown. Statistical analyses are given in Table 2.

Fig. 2 Phenotypic plasticity of Specific Leaf Area (SLA), Maximum Photosynthetic Rate and instantaneous Water Use Efficiency ($iWUE$) to shading in *Prunella vulgaris* seedlings 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 are identified by different symbols (filled symbols= families from shade plants; empty symbols= families from sun plants). Differences between sun and shade treatment within families of the same origin were calculated with Fisher LSD post hoc analysis and represented with *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. Further statistical analyses are provided in Table 4.

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.

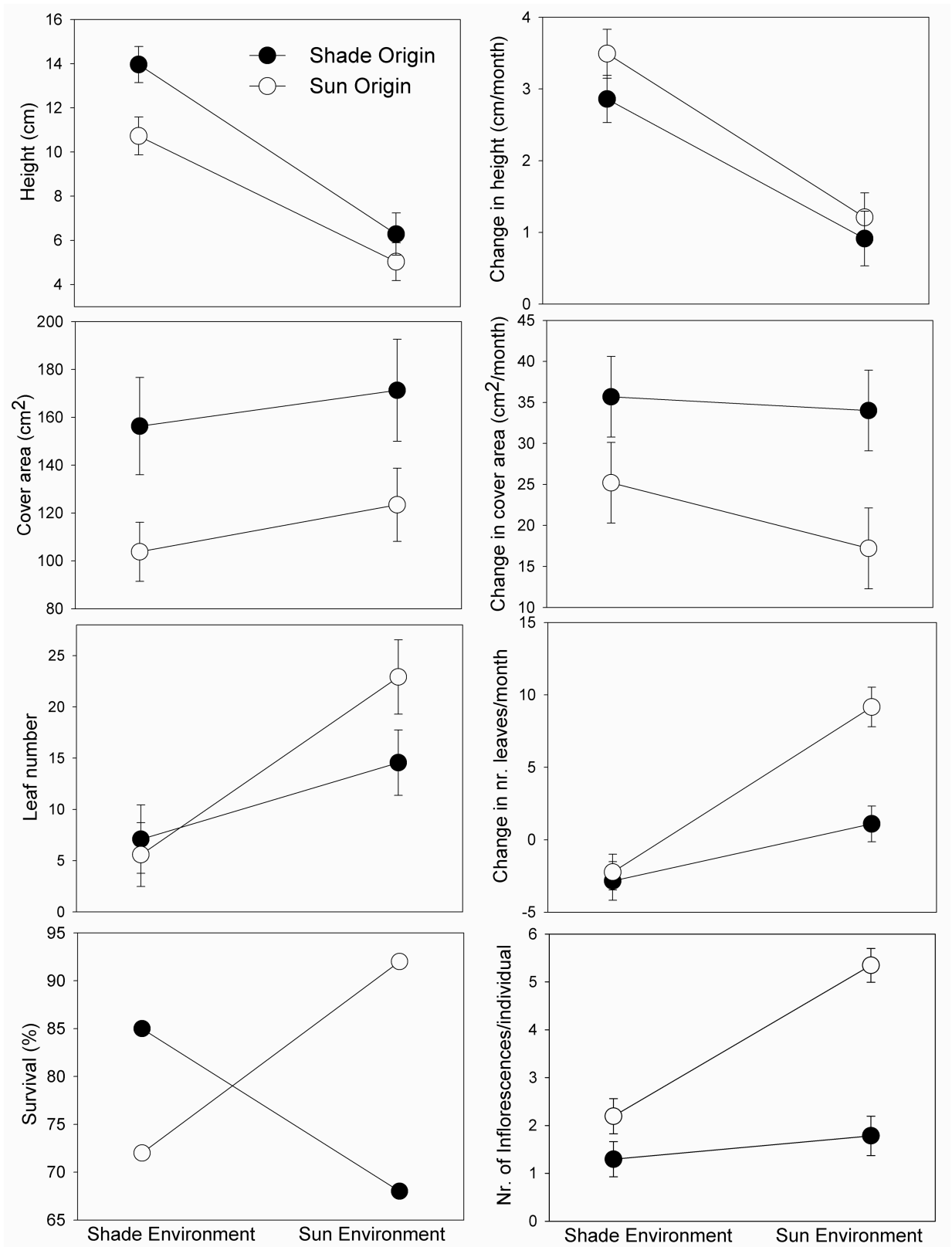


Figure 1

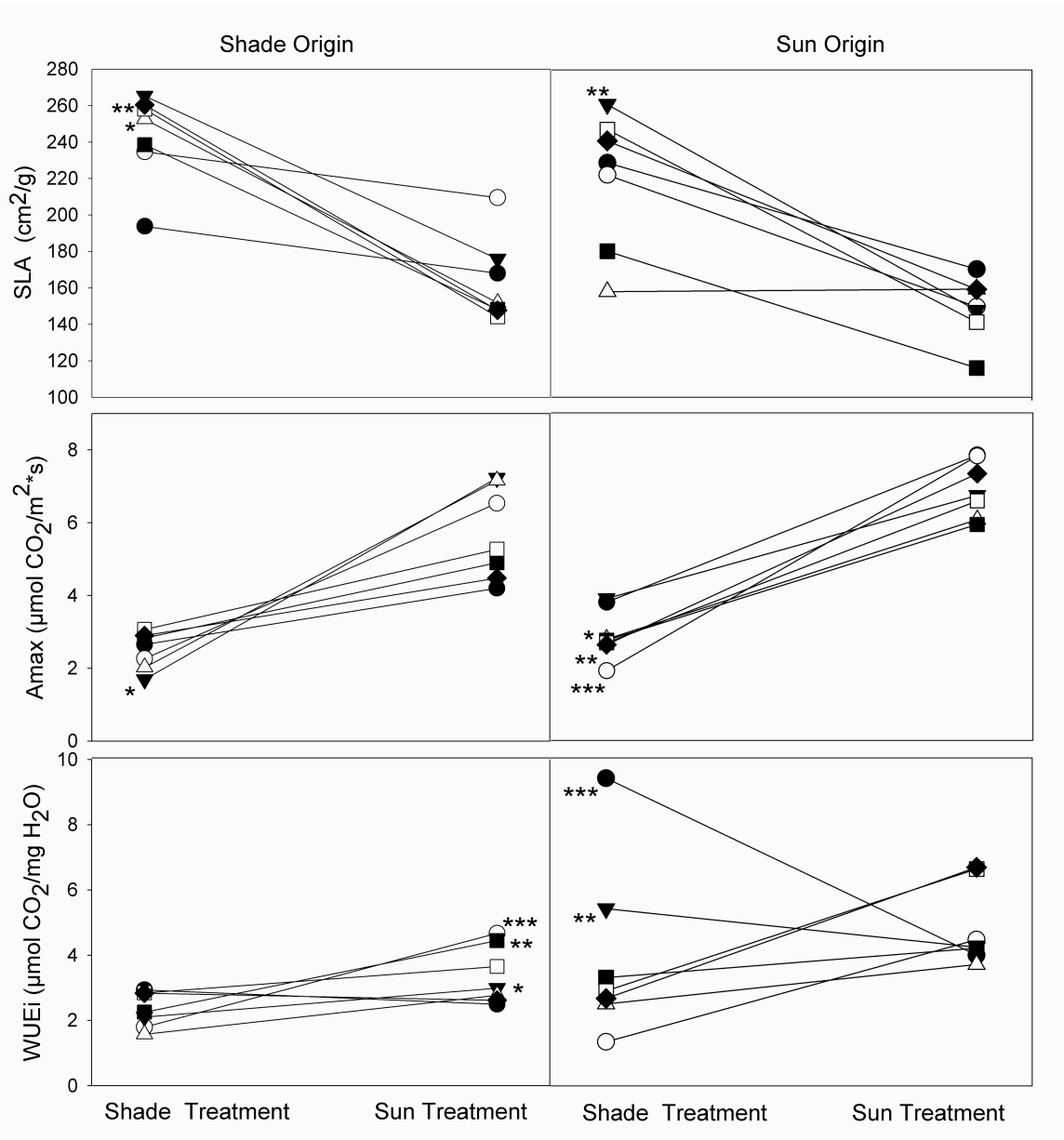


Figure 2

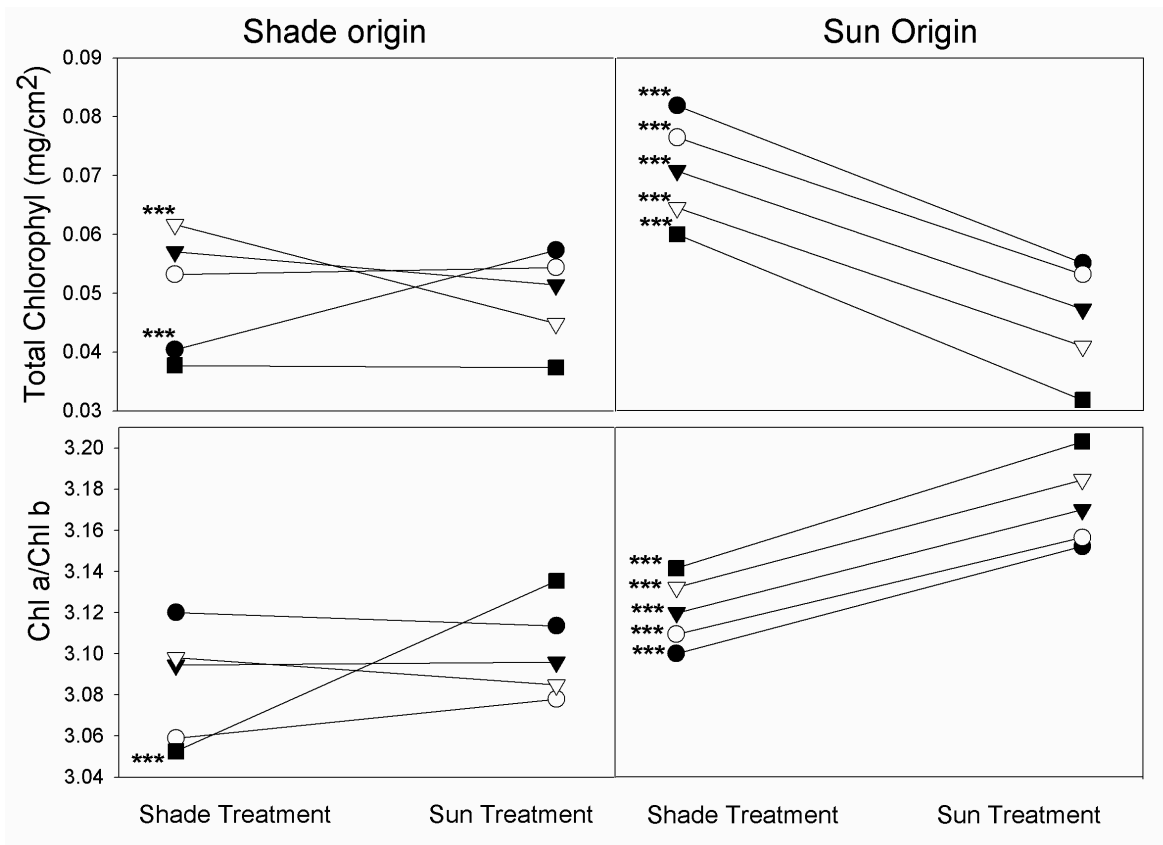


Figure 3