

Environmental heterogeneity leads to higher plasticity in dry-edge populations of a semi-arid Chilean shrub: insights into climate change responses

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Summary

1. Interannual variability in climatic conditions should be taken into account in climate change studies in semi-arid ecosystems. It may determine differentiation in phenotypic plasticity among populations, with populations experiencing higher environmental heterogeneity showing higher levels of plasticity.

2. The ability of populations to evolve key functional traits and plasticity may determine the survival of plant populations under the drier and more variable climate expected for semi-arid ecosystems.

3. Working with populations of the semi-arid Chilean shrub *Senna candolleana* along its entire distribution range, we assessed inter- and intra-population variation in functional traits as well as in their plasticity in response to water availability. We measured morphological and physiological traits related to drought resistance in both field conditions and in a greenhouse experiment, where drought response was evaluated under two water availability treatments.

4. All populations responded plastically, but higher precipitation heterogeneity in dry-edge populations seemed to have selected for more plastic genotypes compared to populations growing at mesic sites and with more homogeneous environmental conditions.

5. Synthesis. Our results suggest adaptive plasticity since higher levels of phenotypic plasticity were positively associated with plant performance. However, we did not find evidence for genetic variation for plasticity within populations. To the extent that phenotypic plasticity may play a key role in future persistence, populations at mesic sites may be more vulnerable to climate change due to their lower plasticity and their current limitations to evolve novel norms of reaction. Conversely, although *Senna candolleana* populations at the dry edge are exposed to higher levels of stress, they may be less susceptible to climate change in view of their greater plasticity. We highlight the need to consider population differentiation in both mean traits and their plasticity to model realistic scenarios of species distribution under climate change.

Key-words: adaptive plasticity, drought, evolutionary potential, genetic variability, natural selection, population differentiation, precipitation heterogeneity, relative growth rate, *Senna candolleana*, water use efficiency

Introduction

Understanding the effects of climate change on natural populations has important consequences for long-term ecosystem functioning and biodiversity distribution (Chapin, Sala & Huber-Sannwald 2001; Cardinale *et al.* 2012). Research on cli-

mate change effects on plant populations typically considers the effects of shifts in mean climatic conditions (Bakkenes *et al.* 2002; Pearson & Dawson 2003; Badeck *et al.* 2004; Thuiller *et al.* 2005), but there is also evidence of increased climatic heterogeneity (Beniston *et al.* 2007; Jimenez *et al.* 2011; D'Odo-rico & Bhattachan 2012). Interannual variability in climatic conditions should be taken into account in climate change studies because it may significantly influence plant functional responses to environmental conditions (Easterling 2000; Knapp

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et al. 2002; Pennington & Collins 2007; Thomey *et al.* 2011). Phenotypic plasticity is an important mechanism by which individual organisms may cope with environmental heterogeneity in natural populations (Pigliucci 2001). This ability may be essential to avoid geographical shifts or local extinctions under climate change (Bradshaw & Holzapfel 2006; Hoffmann & Sgrö 2011). Plant populations along the distribution range are often exposed to different selection pressures, and hence, they may differ in their plasticity patterns (Sultan & Bazzaz 1993; Donohue *et al.* 2001; Heschel *et al.* 2004) as well as in their genetic variability (Young, Boyle & Brown 1996; Fischer, Van Kleunen & Schmid 2000; Matesanz, Horgan-Kobelski & Sultan 2014; Ramírez-Valiente, Valladares & Aranda 2014). Environmental heterogeneity may influence differentiation in phenotypic plasticity among populations (Galloway 1995; Ackerly *et al.* 2000; Weinig 2000; Matesanz, Gianoli & Valladares 2010). For instance, some studies suggest that higher environmental heterogeneity should select for higher plasticity (Alpert & Simms 2002; Gianoli 2004; Gianoli & González-Teuber 2005; Molina-Montenegro, Atala & Gianoli 2010; Baythavong 2011). In some cases, plasticity has been theorized to be higher at the edge (or peripheral) populations since they could be exposed to high environmental variability, and therefore, plastic genotypes could be advantageous (Volis *et al.* 1998; Valladares *et al.* 2014). However, available evidence does not always support this hypothesis (BlackSamuelsson & Andersson 1997; Volis *et al.* 1998; Berg, Becker & Matthies 2005; Mägi *et al.* 2011). Areas at the edge are characterized by small and isolated populations where genetic drift, founder events and population bottlenecks may lead to the loss of genetic variability (Ellstrand & Elam 1993; Arnaud-Haond *et al.* 2006) and consequently may reduce the magnitude of phenotypic plasticity compared to the centre of the species range (Berg, Becker & Matthies 2005; Becker *et al.* 2006). Moreover, marginal populations are often located at the extreme of the environmental stress gradient (Volis *et al.* 1998; Mägi *et al.* 2011), where the relative metabolic cost of plasticity may increase and exceed the benefits of maintaining plasticity (Parsons 1991; DeWitt, Sih & Wilson 1998; van Kleunen & Fischer 2005).

Plasticity, like any other trait, may evolve by natural selection (Via & Lande 1985; Ackerly *et al.* 2000; Richards *et al.* 2006). This requires the occurrence of intra-population phenotypic variation that must be related to fitness and genetically based (Pigliucci 2005; Richards *et al.* 2006). Genetic variation determines the potential for evolutionary response to selection in a specific trait (Scheiner 1993; Falconer & Mackay 1996). Several studies have addressed genetic variation for plant physiological traits related to drought tolerance and their plasticity (Sultan & Bazzaz 1993; Heschel *et al.* 2002; Geber & Griffen 2003; Ramírez-Valiente *et al.* 2010b; Godoy *et al.* 2011). However, this has rarely been evaluated in the context of plant evolutionary responses to climate change along distribution ranges.

Semi-arid ecosystems are characterized not only by permanent or seasonal water limitation but also by an ongoing increase in the interannual precipitation variability (D'Odorico & Bhattachan 2012), together with an intensification of

extreme events (Easterling 2000; Lloret *et al.* 2012). Functional traits related to drought tolerance, as well as their plasticity, may enhance plant fitness in water-limited environments (Dudley 1996; Heschel *et al.* 2002; Ludwig *et al.* 2004). Thus, their potential to evolve by natural selection may determine the survival of plant populations in semi-arid ecosystems under the expected drier and more variable climate (Beniston *et al.* 2007). In semi-arid central Chile, there is evidence of increased aridity, with expectations of both a 20–30% decrease in rainfall during the growth season and a moderate warming (Pezoa 2003; Fuenzalida *et al.* 2007). The aims of this study were as follows: (i) to determine population differentiation in functional traits and their adaptive plasticity in response to drought along the distribution range of the semi-arid Chilean shrub *Senna candolleana*; (ii) to evaluate whether higher environmental heterogeneity favours more plastic genotypes; and (iii) to assess intrapopulation variation of these traits and their plasticity as an estimate of their potential to evolve by natural selection. We selected four populations spanning the entire species' distribution and including habitats of contrasting aridity and precipitation heterogeneity. We measured plant performance and morphological and physiological traits related to drought tolerance in field conditions and in a greenhouse experiment, where plasticity to water availability was also assessed. We hypothesized that, since populations at the dry edge of the distribution have been exposed to long-term drought, they may have evolved efficient adaptations to low water availability. Moreover, we expected that higher environmental heterogeneity in the dry edge would have led to higher plasticity in functional traits of adaptive value compared to populations growing under more homogeneous environmental conditions.

Materials and methods

STUDY SPECIES AND POPULATIONS

Senna candolleana (Vogel) Irw. & Barn. (Fabaceae: Caesalpinioideae) is an evergreen shrub 1–2.5 m tall, with large, indehiscent pods (fruit size: 20 × 10 mm) and yellow flowers. Inflorescences are composed of racemes containing 8–10 flowers. Floral diameter is 2–3 cm with five subequal and imbricate petals, without nectar, apparently odourless, pollen being the main reward. Coloration of corolla and coloration of anthers are primary attractants (Hoffmann 1989). No information about the breeding system of *S. candolleana* was available in the literature, so we provided information of a close congener *Senna corymbosa*, as both species inhabit in similar latitudes and habitats, and preliminary, tentative field observations support this expectancy. The breeding system *S. corymbosa* supports that it is adapted for outcrossing, although it is self-compatible, that is induced geitonogamy and autogamy have been recorded (Laporta 2005). The main visitors and successful pollinators were large bees such as *Bombus atratus* Franklin. Most seeds fall by gravity near the mother plant; however, some entire seeds have been found in rodent dens/lairs, suggesting eventual medium dispersal events (pers. comm.). *Senna candolleana* is endemic to central Chile, and it occurs from sea level up to 700 m. a.s.l., usually within the coastal areas and interior valleys.

Study populations were located in four sites along a latitudinal and climatic gradient spanning the entire species distribution range

(Marticorena *et al.* 2001) (Fig. 1), with northern sites being drier than southern sites (Luebert & Plissock 2006). In these latitudes, El Niño and La Niña events impact precipitation patterns (Forte Lay & Aiello 2001) and soil water storage (Spescha & Forte Lay 2002; Forte Lay, Scarpati & Capriolo 2008) via for instance an increase in interannual precipitation heterogeneity. For the period 1920–2012, precipitation ranged from 3.8 to 895 mm (see Appendix S1 in the Supporting information for more detailed climatic information). Populations were located in two contrasting environments in terms of mean annual precipitation and interannual precipitation heterogeneity: mesic sites, with mean rainfall of 312–426 mm and moderate variability (41–50%), and xeric sites, with 98–126 mm of mean rainfall and high variability (68–77%) (Table 1). Temperature is probably buffered by the sea

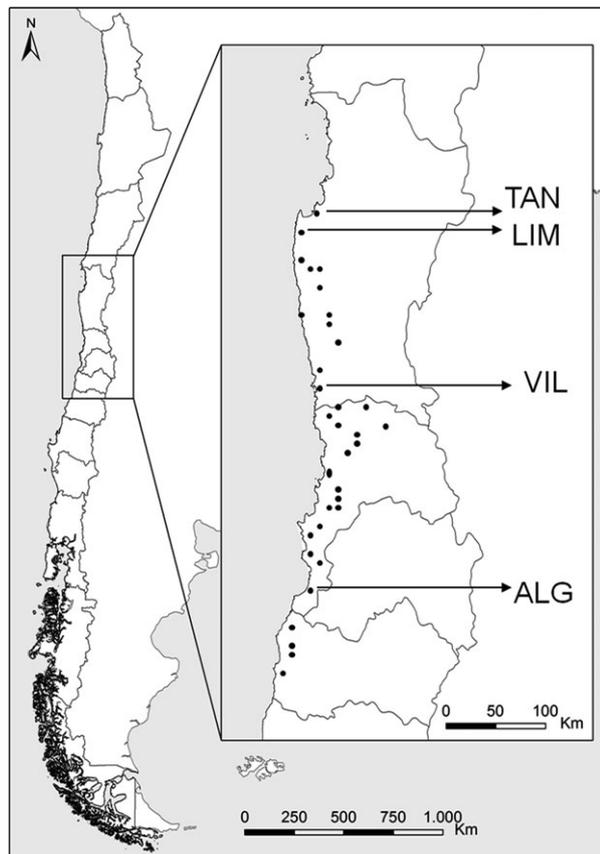


Fig. 1. *Senna candolleana* populations and study sites (codes in Table 1). Chilean Flora Data Base, University of Concepción.

Table 1. Location and climatic characterization of *Senna candolleana* populations. Population values of annual rainfall and annual temperature are means \pm SE. Values of interannual variation in annual rainfall are coefficients of variation ($CV = SD \text{ mean}^{-1}$) expressed as percentage. Data were obtained from the Chilean Meteorological Agency (www.meteochile.cl) for the period 1920–2012

Code	Population	Lat	Long	Alt (m a.s.l.)	Nearest meteorological station	Annual rainfall (mm)	Interannual variation (%)	Annual temperature (°C)
TAN	Tangué	−30.36	−71.56	75	Punta Tortuga , La Serena	98.75 (7.98)	77.93	14.18 (0.08)
LIM	Limarí	−30.66	−71.67	221	Ovalle , Fray Jorge, Los Peñones, P. Oscuro	126.04 (9.10)	68.11	15.03 (0.1)
VIL	Vilos	−32.15	−71.50	82	Los Vilos , Zapallar, Quintero	312.6 (16.57)	50.00	13.36 (0.14)
ALG	Algarrobo	−33.35	−71.65	27	Punta Angeles , Santo Domingo	426.02 (18.68)	41.59	14.15 (0.08)

Lat, latitude; Long, longitude.

Data were obtained from the nearest weather station (in bold), and missing data of the series 1920–2012 were completed with other nearby weather stations. See Appendix S2 for coordinates of weather stations.

influence and does not show considerable variation among populations (Table 1). We considered northern populations to be peripheral as they are located in the margin of the species distribution range and they are exposed to extreme climatic conditions of aridity and high interannual heterogeneity in comparison with the main species distribution. All populations were large and well-established (hundreds of adult individuals). Sites were of similar altitude and longitude (Table 1) and were not managed nor surrounded by highly disturbed areas. Location of studied populations and its nearest weather stations are shown in Appendix S2.

FIELD MEASUREMENTS

In January 2011, matching the reproductive peak of *S. candolleana*, 20–30 adult plants per population were sampled. The selected individuals grew at least 4 m apart from each other. For each individual, we measured maximum plant height, crown diameter, specific leaf area (SLA), leaf size, leaf thickness, stomatal conductance, photochemical efficiency, stem primary growth of the last year and fruit crop. All leaf traits were measured in healthy, fully expanded leaves. Morphological leaf traits were measured using five leaves per plant. SLA ($\text{cm}^2 \text{g}^{-1}$) was measured using the ratio of the one-side area of a fresh leaf divided by its oven-dry mass (Cornelissen *et al.* 2003). Stomatal conductance ($\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$) was measured with a leaf porometer (SC-1; Decagon Devices, Pullman, WA, USA) from 10 to 12 am in four leaves per plant. Maximum photochemical efficiency (F_v/F_m) was measured with a portable pulse-modulated fluorometer (FMS2, Hansatech, UK) from 09 to 12 am in three leaves dark-adapted for 30 min with leaf clips. Minimal (F_o) and maximal (F_m) fluorescence were measured, and these values were used to calculate maximum photochemical efficiency (F_v/F_m) as $F_v/F_m = (F_m - F_o)/F_m$, where F_v is the difference between F_m and F_o (Maxwell & Johnson 2000). Stem primary growth of the previous year was calculated as the average length of five non-lignified branches per plant. Finally, we collected pods from the same 20–30 field individuals. Seeds were cleaned, weighted (five seeds per individual) and stored in a cool and dry environment.

COMMON GARDEN AND PHENOTYPIC PLASTICITY ASSESSMENT

While evolutionary approaches to phenotypic plasticity should study it at the genotype level, ecological approaches to plasticity may sacrifice some precision in favour of more realistic evaluations of the role of plasticity in natural populations, studying it at the population or species level (Richards *et al.* 2006; Gianoli & Valladares 2012). Accordingly, we studied functional traits and their plasticity at both

the population and the genotype (maternal family) levels. In August 2011, 100 seeds from each field individual were stratified for 1 min in boiling water and then kept for 24 h in water at 10 °C, and finally sown in one individual tray per family (seedlings of the same mother and unknown father) with a 4:2:1:1 mixture of coco peat, sterilized peat moss, vermiculite (Projar, Spain) and expanded clay balls (Hnos. Aguado S.L., Spain). In December 2011, healthy seedlings were individually transplanted into 0.5-L pots filled with the same soil mixture. We selected 12 healthy seedlings from each of 10 randomly selected families per population. Six replicate seedlings per family were assigned to one of two contrasting water availability treatments (see below for treatment details). Each replicate was assigned to one of six different blocks (contiguous glasshouse benches) following a randomized complete block design to minimize microenvironmental biases (Zar 1999). The final sample was 480 plants (4 populations \times 10 families/population \times 2 treatments \times 6 replicates/treatment). To study drought response with established individuals with minimal confounding, maternal effects, all seedlings were grown in a glasshouse under standard conditions of high water and nutrient availability for 1 year before applying water treatments. In August 2012, we began the water availability treatment, and plants were grown for 3 months in each treatment. Maternal effects could influence patterns of population differentiation (Susko & Lovett-Doust 2000; Molinier *et al.* 2006). Typically, maternal environment is thought to have the greatest influence on traits expressed very early in life, such as germination timing and initial growth rate (Roach & Wulff 1987; Tielbörger & Petrü 2010). Therefore, our measurements were taken from 15-month-old plants (12 months of standard conditions + 3 months under treatments) trying to minimize them. Nevertheless, we have tested for an influence of seed mass [a common metric of maternal investment (Roach & Wulff 1987; Galloway 2001)] on our data, comparing factors effects in all functional traits with and without seed mass as a covariate.

We have simulated two contrasting regimes of water availability aimed at recreating the wide range of conditions experienced by plants over the last decades (see Appendix S1 for more detailed climatic information of study sites). In the moist treatment, plants were kept at field capacity (soil moisture measured 2 days after saturated irrigation), which for our specific soil mixture was equivalent to $68.67 \pm 0.62\%$ of soil water content (SWC). This treatment simulated years of high annual rainfall and periods where soil humidity is high for several days following heavy storms, two situations that are more frequent and important at the mesic locations. In the drought treatment, plants were kept at 22% of field capacity, equivalent to $14.95 \pm 1.36\%$ of SWC, which caused plants to wilt at midday daily. It has been shown that effects of water stress on seedling performance of semi-arid woody plants are noticeable for values of SWC around 6–15% (Valladares, Sanchez-Gomez & Zavala 2006). Moreover, studies performed in other semi-arid habitats (see Ogaya & Peñuelas 2004, 2007; Sardans *et al.* 2013) have reported a reduction of SWC at 15% when drought was experimentally induced simulating the projected climatic scenarios for future decades (IPCC 2012). Volumetric SWC was determined for a random subsample of 40 pots (10 pots \times 4 populations) once a week, and water was added to compensate for evapotranspiration losses. SWC was calculated as $(W - D) \times D - 1$, where W is the weight of the original sample and D is weight of the dried sample.

The experiment was designed to minimize confounding environmental effects and trying to mimic as possible natural conditions of study sites. All plants were provided with ample nutrients. Relative humidity fluctuated from 45% to 55% but was consistent through the greenhouse (as measured with Licor 1600). Temperature and photosynthetically active radiation (PAR) were recorded every 10 min dur-

ing the whole experiment with a HOBO H08-006-04 data logger (Onset, Pocasset, MA, USA). Mean temperature and maximum and minimum daily range for this period were 20.5, 22–26 and 9–12 °C, respectively. PAR was approximately $800\text{--}1000 \mu\text{mol m}^{-2} \text{s}^{-1}$.

DATA COLLECTION IN PHENOTYPIC PLASTICITY EXPERIMENT

Performance traits

We considered plant height, relative growth rate (RGR), photosynthetic rate and maximum photochemical efficiency (F_v/F_m) as estimates of plant performance. We measured plant height and RGR every 20 days during the 3 months of treatment, in a total of four censuses. Plant height was measured as the elongation above the cotyledons to the node of the most recent fully expanded leaf. RGR was calculated as $\text{RGR} = (\text{LnH}_4 - \text{LnH}_1)/3$, where H_4 is plant height at the end of treatment and H_1 at the beginning of treatment and 3 is the number of months in treatment. Photosynthetic rate and maximum photochemical efficiency were measured after 3 months in water treatment (see below for photosynthetic measurements details). Maximum photochemical efficiency was measured as described above for field measurements. RGR was considered a sound surrogate for fitness in many studies of woody plants (e.g. Ramírez-Valiente *et al.* 2010a, Salgado-Luarte & Gianoli 2010), and in our species, plant size is also a good surrogate for fitness since it was highly correlated with fruit crop ($r = 0.679$; $P < 0.001$, data from 92 individuals collected in the same study populations).

Water economy traits

These traits were measured after 3 months in treatment, from 10 am to 15 pm on seven comparable sunny days. Photosynthetic rate and stomatal conductance were measured on one fully expanded leaf of a primary branch per plant using a Licor 6400 (LI-COR, Lincoln, NE, USA) infrared gas analyzer. Leaves were exposed to an atmospheric CO_2 concentration of $400 \mu\text{mol CO}_2 \text{mol}^{-1}$ (using the built-in controller) and saturating light of $1100 \mu\text{mol m}^{-2} \text{s}^{-1}$. Stomatal ratio was set to 1 and gas flow to $500 \mu\text{mol s}^{-1}$. Measurements were performed at 25–26 °C (growth temperature) and relative humidity of 45–55%. When the leaf did not completely cover the chamber, leaf tracings were scanned on a LI-3000C portable area meter (LI-COR) to determine photosynthetic surface area for rate calculations. Intrinsic water use efficiency (iWUE) – a measure of carbon gain per unit water loss (Cohen 1970; Dudley 1996; Heschel *et al.* 2002, 2004) – was calculated as the ratio between photosynthetic rate and stomatal conductance.

Morphological leaf traits

After 3 months in treatment, one fully expanded leaf from a primary branch per plant was harvested and immediately weighed on a microbalance (Kern ALS 120-4N, Balingen, Germany) and scanned on a LI-3000C portable area meter (LI-COR). Then, we measured leaf thickness with a dial thickness gauge (Mitutoyo Co., Aurora, IL, USA) and finally oven-dried (at 65 °C for 48 h) and weighed the leaf to determine SLA (leaf area/leaf biomass) and leaf dry matter content (LDMC, oven-dry leaf mass/fresh leaf mass) (Cornelissen *et al.* 2003).

Allocation traits

At the end of the treatment, shoots and roots were harvested on a subsample of four blocks (four replicates of each family per population

and treatment, $N = 320$). Above-ground tissues were separated in two fractions: senescent leaves and fresh leaves. We determined fraction of senescent leaves as [senescent leaves biomass/(senescent leaves biomass + fresh leaves biomass)]. Root systems were manually washed, oven-dried (at 65 °C for 48 h) and weighed to determine root biomass. We calculated root:leaf biomass ratio as (root biomass/fresh leaves biomass).

STATISTICAL ANALYSIS

To assess differences among populations in field measurements, we performed one-way ANOVAS on all traits, with population as the main effect. To analyse inter- and intrapopulation differences in trait means and in their plasticity patterns in the greenhouse, mixed-model ANOVAS were used to test for the fixed effects of Population and Treatment, the random effect of Family (nested within Population) and Block, and the interactions Population × Treatment and Family (Population) × Treatment. To test the Population effect, we considered ‘families’ as replicates and no ‘individuals’, to avoid pseudoreplication. Differences in plasticity among populations were evaluated by comparing the slopes of the reaction norms via *t*-test, checking parameter estimates for each population and their confident limits at ±95% (Zar 1999). Individual analyses were performed within each Treatment to test the effects of Population and Block, followed by *post hoc* Tukey’s HSD, performed on Family means. To further identify those populations harbouring intrapopulation variation for functional traits, individual analyses were performed within each Population to test the effects of Family (random) and Family × Treatment. A sequential Bonferroni correction was used to account for multiple comparisons (Hill & Lewicki 2005). To assess the influence of maternal environment on interpopulation differences in all measured traits (i.e. maternal effects), we compared two-way ANOVAS (fixed factors were Population, Treatment and their interaction) with and without seed

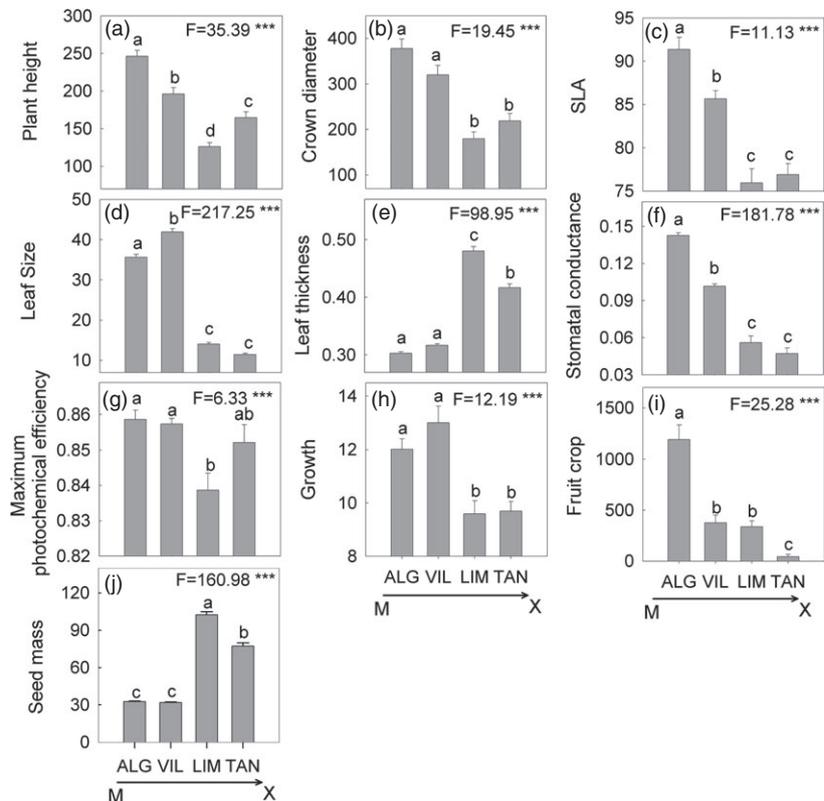
mass as a covariate for all traits (seed mass was included as a proxy of maternal effects). To test for an association between plasticity and plant performance, Pearson correlations were computed between phenotypic plasticity and the mean of RGR across treatments (as an estimate of performance). This was performed for traits where the interaction Population × Treatment was significant. Phenotypic plasticity was estimated as the percentage of change in the mean trait value from one environment to the other as $P = [(X_{Drought} - X_{Moist})/X_{Drought}] * 100$, where P is plasticity, $X_{Drought}$ is the mean trait value for each family under drought and X_{Moist} is the mean trait value for each family under moist conditions (Valladares, Sanchez-Gomez & Zavala 2006). In addition, a measurement of overall plasticity was estimated for each family as the arithmetic mean of the percentage of change observed for traits where the interaction Population × Treatment was significant (Molina-Montenegro & Naya 2012). Finally, we tested the relationship between phenotypic plasticity (% of change) for each family and the interannual precipitation variability of each population. Normality and homoscedasticity assumptions were tested prior to analyses by means of the Kolmogorov–Smirnov and the Levene test, respectively. Variables were log-transformed (seed number) or squared-root-transformed (plant height, F_v/F_m , leaf size and root:leaf biomass ratio) to meet the assumptions of ANOVA. All statistical analyses were performed using STATISTICA 8.0 (StatSoft Inc., Tulsa, OK, USA).

Results

PHENOTYPIC VARIATION AMONG POPULATIONS IN THE FIELD

We found significant phenotypic differences among populations in the field in all traits (Fig. 2). Plants in xeric populations had smaller size (height and crown diameters), lower SLA and leaf size, thicker leaves and lower stomatal conductance

Fig. 2. Population differences in functional traits related to drought resistance measured in the field along an aridity and precipitation heterogeneity gradient. (a) Plant height (cm), (b) crown diameter (cm), (c) SLA (cm² g⁻¹), (d) leaf size (cm²), (e) leaf thickness (mm), (f) stomatal conductance (mol H₂O m⁻² s⁻¹), (g) maximum photochemical efficiency (F_v/F_m), (h) Growth, stem primary growth of the last year (cm), (i) fruit crop (n fruits ind⁻¹) and (j) seed mass (mg). Population means ± SE are shown for 20–30 genotypes per population. Different letters indicate significant differences after Tukey’s HSD test. SLA, specific leaf area; M, mesic; X, xeric; study sites codes in Table 1. *F* ratios (*F*) and significance levels are shown (***) $P < 0.001$ (one-way ANOVAS).



(Fig. 2a–f). Maximum photochemical efficiency was significantly lower in one of the xeric populations (Fig. 2g). Populations in mesic sites had higher performance, estimated by stem primary growth, and fitness (fruit crop) than xeric ones (Fig. 2h,i). However, individuals from mesic populations produced lighter seeds than individuals from xeric sites (Fig. 2j).

POPULATION DIFFERENTIATION AND PHENOTYPIC PLASTICITY IN GREENHOUSE EXPERIMENT

Broad phenotypic differences were found among populations, among families within populations and across drought treat-

ments (Table 2; Figs 3 and 4). Overall, patterns of plasticity to water availability differed among populations but not among families within populations (Table 2).

Performance traits

Height and RGR were affected by drought treatment in all populations (Fig. 3a,b). Individuals from populations on xeric sites had higher plant height and RGR than those from populations on mesic sites in both treatments. Photosynthetic rate and maximum photochemical efficiency were also affected by drought in all populations (Fig. 3c,d).

Table 2. Mixed-model anovas of functional traits in *Senna candolleana* plants from four populations (10 maternal families per population) exposed to different water availability treatments in a greenhouse

	Population (d.f. = 3)	Family (population) (d.f. = 36)	Treatment (d.f. = 1)	Population × Treatment (d.f. = 3)	Family (population) × Treatment (d.f. = 36)	Block (d.f. = 5)	Error d.f.
Performance traits							
Height	58.024*	8.461*	98.293*	4.432*	0.826 NS	7.427*	395
RGR	3.999*	1.858 NS	94.180*	0.823 NS	1.116 NS	5.104*	395
Photosynthetic rate	18.516*	0.817 NS	78.368*	2.932*	0.767 NS	6.810*	385
Photochemical efficiency	2.527 NS	1.109 NS	36.262*	7.509*	0.752 NS	1.863 NS	395
Morphological traits							
Leaf size	53.314*	2.621*	121.569*	0.473 NS	0.847 NS	1.821 NS	378
SLA	0.918 NS	2.010 NS	2.151 NS	4.594 NS	1.005 NS	0.580 NS	378
Leaf thickness	1.309 NS	1.824 NS	75.495*	8.886*	1.172 NS	1.638 NS	378
LDMC	1.237 NS	1.252 NS	117.069*	1.564 NS	0.577 NS	0.497 NS	378
Water economy traits							
Stomatal conductance	18.971*	0.553 NS	64.952*	2.182*	0.939 NS	5.896*	385
iWUE	6.661*	1.014 NS	33.263*	7.120*	1.016 NS	1.536 NS	385
Allocation traits							
Root:leaf biomass ratio	13.051*	1.426 NS	69.075*	12.258*	0.666 NS	4.115 NS	234
Fraction of senescent leaves	22.492*	1.000 NS	346.495*	22.492*	1.250 NS	4.142 NS	234

RGR, relative growth rate; SLA, specific leaf area; LDMC, leaf dry matter content; iWUE, instantaneous water use efficiency. *F* ratios are shown.

*Significant using corrected *P*-value (sequential Holm–Bonferroni method); NS, not significant.

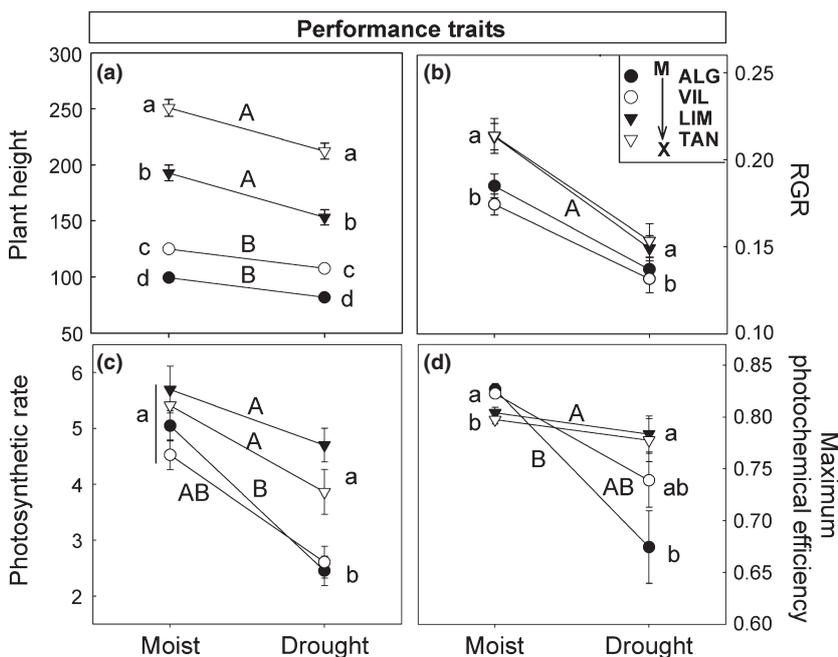


Fig. 3. Population differences in performance traits of *Senna candolleana* under two levels of water availability (moist and drought): (a) plant height (cm), (b) RGR (cm month^{-1}), (c) photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and (d) maximum photochemical efficiency (F_v/F_m). Population means \pm SE are shown for 10 families per population, with six replicates per family. Different capital letters indicate significant differences in the slope of the reaction norm among populations. Lowercase letters indicate significant differences in the average trait among populations within treatment after Tukey's HSD test. RGR, relative growth rate; M, mesic; X, xeric; study sites codes in Table 1.

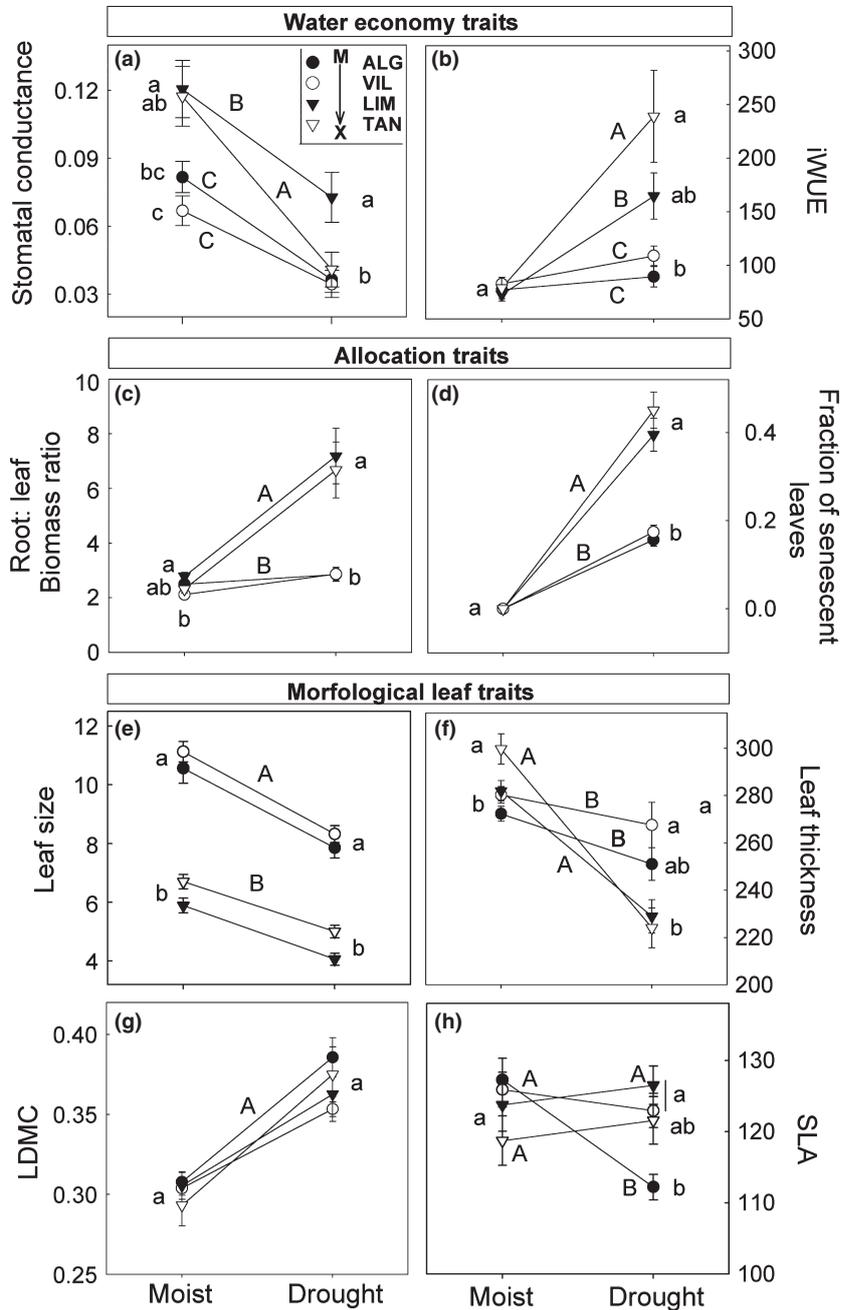


Fig. 4. Population differences in plastic responses in functional traits of *Senna candolleana* under two water availability levels (moist and drought): Water use efficiency traits: (a) stomatal conductance (mol H₂O m⁻² s⁻¹), (b) iWUE (μmol CO₂ mol⁻¹ H₂O). Allocation traits: (c) root: leaf biomass ratio, (d) fraction of leaves senescent. Morphological leaf traits: (e) leaf size (cm²), (f) leaf thickness (μm), (g) LDMC, (h) SLA (cm² mg⁻¹). Population means ± SE are shown for 10 families per population, with six replicates per family. Different capital letters indicate significant differences in the slope of the reaction norm among populations. Lowercase letters indicate significant differences in the average trait among populations after Tukey's HSD test within treatment. iWUE, water use efficiency; LDMC, leaf dry matter content; SLA, specific leaf area; M, mesic; X, xeric; study sites codes in Table 1.

Populations on xeric sites showed flatter norms of reaction and maintained higher values under drought than populations on mesic sites. We found significant intrapopulation variation for plant height (Table 2, Family (Population) term); plant height showed genetic variation in Algarrobo and Tangué, while RGR only in Limarí (Table 3). There was no intrapopulation variation for plasticity in any performance trait (Tables 2 and 3, Family (Population) × Treatment).

Water economy traits

Individuals from all populations decreased stomatal conductance in the drought treatment (Fig. 4a). However, popula-

tions on xeric sites showed slightly steeper slopes than populations on mesic sites. In the moist treatment, families from xeric populations had significantly higher stomatal conductance than families from mesic populations. However, plants from xeric populations decreased stomatal conductance to similar levels than plants from mesic ones under drought conditions, especially plants from the most xeric population. Plants from all populations increased iWUE in the drought treatment compared to the moist treatment (Fig. 4b). However, xeric populations showed greater plasticity than mesic ones. All populations had similar iWUE under moist treatment, but under drought, families from populations on xeric sites increased iWUE more than families from populations on mesic sites. No significant intrapopulation variation for

Table 3. Intrapopulation genetic variation in mean traits (family) and in their plasticity (family × treatment) within the four *Senna candolleana* populations of the study

	ALG		VIL		LIM		TAN	
	Family	Family × Treatment						
Performance traits								
Height	3.312*	1.978 NS	2.592 NS	1.203 NS	3.213 NS	1.041 NS	55.871*	0.236 NS
RGR	2.868 NS	0.452 NS	2.926 NS	0.591 NS	6.993*	0.293 NS	1.148 NS	2.319 NS
Photosynthetic rate	0.724 NS	1.181 NS	0.525 NS	0.972 NS	0.990 NS	0.749 NS	1.002 NS	0.534 NS
Photochemical efficiency	1.100 NS	0.642 NS	0.679 NS	1.140 NS	2.512 NS	0.556 NS	1.530 NS	0.514 NS
Morphological traits								
Leaf size	2.169 NS	1.001 NS	3.440 NS	0.670 NS	1.489 NS	1.235 NS	3.623*	0.955 NS
SLA	2.008 NS	0.933 NS	5.118*	0.831 NS	1.201 NS	1.475 NS	2.135 NS	0.727 NS
Leaf thickness	0.851 NS	1.534 NS	3.615 NS	0.870 NS	1.185 NS	1.978 NS	2.056 NS	0.713 NS
LDMC	0.675 NS	0.803 NS	2.450 NS	0.332 NS	1.064 NS	0.795 NS	1.719 NS	0.385 NS
Water economy traits								
Stomatal conductance	0.194 NS	3.299 NS	1.007 NS	0.665 NS	0.428 NS	0.808 NS	1.284 NS	0.557 NS
iWUE	0.431 NS	1.281 NS	1.806 NS	0.559 NS	0.856 NS	1.163 NS	1.094 NS	0.929 NS
Allocation traits								
Root:leaf biomass ratio	0.495 NS	1.170 NS	1.876 NS	0.786 NS	1.404 NS	0.413 NS	1.181 NS	1.135 NS
Fraction of senescent leaves	1.000 NS	0.518 NS	1.000 NS	1.036 NS	1.000 NS	1.017 NS	1.000 NS	1.699 NS

RGR, relative growth rate; SLA, specific leaf area; LDMC, leaf dry matter content; iWUE, instantaneous water use efficiency; study sites codes in Table 1.

F ratios are shown.

*Significant using corrected *P*-value (sequential Holm–Bonferroni method); NS, not significant.

water economy traits or for their plasticity was found (Tables 2 and 3).

Allocation traits

Only plants from xeric sites increased root:leaf biomass ratio in response to drought (Fig. 4c). Although xeric populations differed significantly from mesic populations in this trait under drought, all populations expressed similar phenotypes in the moist treatment. Families from all populations increased the fraction of senescent leaves under drought (Fig. 4d). However, populations from xeric sites showed steeper slopes than populations from mesic sites. No significant intrapopulation variation for allocation traits or for their plasticity was detected (Tables 2 and 3).

Morphological traits

Families from all populations decreased leaf size and leaf thickness and increased LDMC in the drought treatment compared to the moist treatment (Fig. 4e,f,g). Plants from xeric sites showed greater plasticity in leaf thickness and lower in leaf size than those from mesic sites (Fig. 4e,f). Plants from xeric populations had always smaller leaves than their mesic counterparts (Fig. 4e), while no such clear pattern was found for leaf thickness (Fig. 4f). All populations increased LDMC similarly, and there were no differences among populations in trait expression within treatment (Fig. 4g). SLA showed no consistent effect of drought (Fig. 4h). We found significant intrapopulation variation for leaf size but not for their plasticity at the species level

(Table 2). Leaf size showed significant intrapopulation variation in Tangue and SLA in Vilos.

Seed mass effects

No significant seed mass effects were detected in any functional trait. The significance of Population, Treatment and their interaction for most functional traits was very similar regardless of the inclusion of seed mass as a covariate. Only ≈8% of the models differed in term significance when seed mass was entered as a covariate (see Appendix S3).

Plasticity vs. performance

Significant positive correlations were found between trait plasticity (iWUE, leaf thickness, root:leaf biomass ratio and fraction of senescent leaves) and the mean of RGR across treatments (Fig. 5).

Plasticity vs. interannual precipitation variability

Plasticity in iWUE, leaf thickness, root:leaf biomass ratio, fraction of senescent leaves and overall plasticity was correlated with the interannual precipitation variability of each population (Fig. 6).

Discussion

Populations in peripheral, dry-edge populations showed higher plasticity than those from mesic, central ones. These results suggest that higher variability in the precipi-

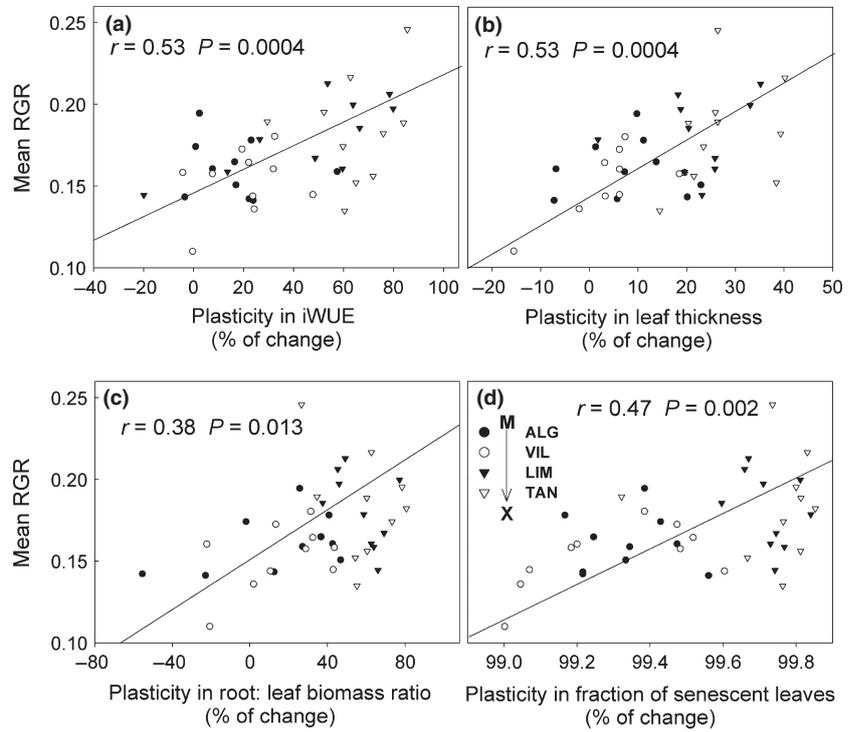


Fig. 5. Relationship between trait plasticity (calculated as percentage of change) and performance (measured as the mean of RGR across treatments) of each family for (a) iWUE, (b) leaf thickness, (c) root: leaf biomass ratio and (d) fraction of senescent leaves. RGR, relative growth rate; iWUE, water use efficiency; M, mesic; X, xeric; study sites codes in Table 1.

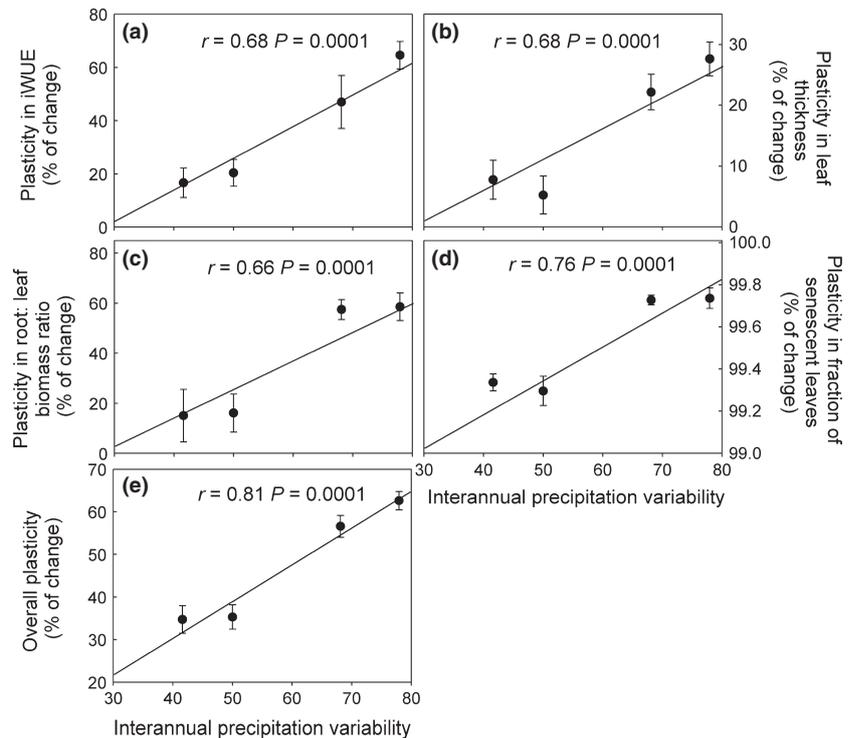


Fig. 6. Relationship between trait plasticity (measured as percentage of change) and interannual precipitation variability for (a) iWUE, (b) leaf thickness, (c) root: leaf biomass ratio, (d) fraction of senescent leaves and (e) overall plasticity (measured as the arithmetic mean of the percentage of change for the four traits). Population means \pm SE are shown for 10 families per population. iWUE, water use efficiency.

tation regime at the dry-edge populations could be selecting for more plastic genotypes. To the extent that phenotypic plasticity can play a key role in the performance of populations under climate change, higher plasticity in dry-edge populations can confer them with an advantage to cope with the predicted increase in aridity and climatic heterogeneity.

We found significant phenotypic variation among populations of *S. candolleana* along its distribution range. Individuals from populations on xeric sites showed ecophysiological traits aimed at reducing evaporative water losses and enhancing water use efficiency, such as smaller size, lower SLA, smaller and thicker leaves, and lower stomatal conductance. This interpopulation variability suggests that dry-edge popula-

tions are adapted to the local conditions, as has been broadly reported in water-limited environments (Givnish 1986; Dudley 1996; Ackerly 2004; Ludwig *et al.* 2004).

All populations responded plastically to drought; however, they differed in the magnitude of the phenotypic response. Overall, plants from populations on xeric sites and with higher interannual precipitation heterogeneity were more plastic (steeper reaction norms) than plants from populations on mesic sites and with lower variability. Our results seem to support the theoretical prediction that higher environmental heterogeneity should select for greater phenotypic plasticity (Alpert & Simms 2002; Bradshaw & Holzapfel 2006; Mate-sanz, Gianoli & Valladares 2010). Specifically, they agree with the outcome of an earlier study with a perennial herb in the same study area, which reported a positive association between phenotypic plasticity to drought in functional traits and interannual variability in rainfall (Gianoli & González-Teuber 2005). Moreover, our study represents the first evidence, to our knowledge, of phenotypic plasticity to be higher in peripheral populations in relation to the main distribution area of the species, a situation that has been theoretically considered and discussed (Valladares *et al.* 2014). Studies addressing this issue have reported either decreased plasticity at peripheral populations (Volis *et al.* 1998; Mägi *et al.* 2011) or similar phenotypic plasticity in marginal and central populations (Berg 1960; Stewart & Nilsen 1995; Black-Samuelsson & Andersson 1997). In *S. candolleana*, plants from dry-edge populations showed higher plasticity in water economy and allocation traits: they decreased stomatal conductance and increased iWUE, root: leaf biomass ratio and the fraction of senescent leaves stronger than populations from mesic sites. It is broadly known that one way that plants adjust to dry environments is by reducing water loss via decreased stomatal conductance and increased iWUE. Increasing root: leaf biomass ratio and the fraction of senescent leaves also represents a common plant strategy in water-limited environments as individuals modify resource allocation to enhance water uptake and reduce evaporative water losses (Lloret, Casanovas & Peñuelas 1999; Padilla & Pugnaire 2007). We found little among-population differences in morphological traits. Such a pattern of population differentiation in only some functional traits (or their plasticity) has been reported for other plant species facing water shortage (Heschel *et al.* 2002; Gianoli & González-Teuber 2005; Ramírez-Valiente *et al.* 2010a).

Population differences in performance traits suggest the adaptive nature of plasticity in *S. candolleana* populations, since phenotypic plasticity showed a positive association with plant performance. For example, families from xeric populations subjected to drought showed flatter reaction norms for photosynthetic rate and maximum photochemical efficiency than families from mesic populations, which performed poorly under drought conditions. Thus, the performance of plants from xeric populations remained relatively constant after being experimentally exposed to a stressful environment (drought), matching the concept of 'fitness homeostasis' (Hoffmann & Parsons 1991; Rejmánek 2000; Alpert & Sim-

ms 2002). This homeostatic pattern is likely to be explained by plasticity in underlying morphological and physiological traits such as leaf thickness, stomatal conductance, iWUE, and root: leaf biomass ratio (the change in root: leaf biomass ratio resulting mostly from increased leaf-shedding), which showed steeper reaction norms in response to drought in plants from xeric sites. Phenotypic plasticity in xeric populations is also likely to be associated with the observed faster growth and greater height in these plants. Growth-related traits are commonly used as proxies of fitness (see e.g. Gianoli & González-Teuber 2005; Ramírez-Valiente *et al.* 2010a). Phenotypic plasticity, like any other trait, could be subject to evolution by natural selection (Pigliucci 2005; Richards *et al.* 2006). However, no evidence for genetic variation for plasticity in any functional trait was found within populations of *S. candolleana*. Therefore, although currently adaptive, phenotypic plasticity in *S. candolleana* seems to present low evolutionary potential (since quantitative genetic variation is a key aspect of evolutionary potential), which may limit future responses to further selective pressures associated with climate change. These results concur with other studies that showed that heritabilities of plasticity are generally lower than those of mean trait values (Scheiner 1993).

Despite the lack of intrapopulation variation for plasticity in *S. candolleana*, we found intrapopulation variation for plant height, RGR and some morphological leaf traits. Therefore, these traits could be subject to evolution by natural selection and thus allow for further adaptations in *S. candolleana*. It is likely that morphological leaf traits would be the main drivers of future adaptive evolution in this shrub since (i) families from xeric populations had smaller leaf size than families from mesic ones, (ii) this variation was associated with higher performance, as individuals with smaller leaves had greater RGR, and (iii) we found genetic variation for these traits. Several studies have demonstrated that lower SLA and smaller and thicker leaves have fitness benefits in dry environments since they may decrease water loss and consequently they allow a more conservative water use (Givnish 1979; Dudley 1996; Ramírez-Valiente *et al.* 2010a). Although water economy and allocation traits showed significant differences among populations, there was no intrapopulation genetic variation for them, constraining their adaptive evolution in response to climate change; this agrees with several studies reporting that heritability and genetic variation were generally higher in morphological traits as compared to vegetative performance and physiological traits (Geber & Griffen 2003).

Significant differences in seed mass were found among populations, where individuals from xeric populations had heavier seeds than individuals from mesic ones. Seed mass and seed size are traits commonly used as a measure of maternal investment (Wulff, Caceres & Schmitt 1994; Lacey, Smith & Case 1997). However, the significance of the effects for most functional traits was very similar regardless of the inclusion of seed mass as a covariate. This suggests that maternal effects are likely to be minor in our study, although they cannot be discarded (Roach & Wulff 1987; Galloway 2001).

Under the expected increase in aridity and precipitation heterogeneity in semi-arid ecosystems (Beniston *et al.* 2007; D'Odorico & Bhattachan 2012), *S. candolleana* population success may be, at least in part, determined by the ability to express adaptive plasticity under drought stress. Our experiment suggests that, counterintuitively, populations on the mesic edge of *S. candolleana* distribution range may be more vulnerable to climate change. This is based both on their lower plasticity in functional traits related to drought tolerance and on their inability to evolve novel reaction norms. Conversely, higher levels of phenotypic plasticity in xeric populations may help to cope with the negative impact of increasing aridity and climatic heterogeneity. Although water availability is considered one of the most important factors in semi-arid ecosystems (Mooney 1989), other abiotic and biotic factors will likely affect population survival under climate change, such as pollination and efficient seed dispersal among populations, which could increase or decrease genetic variation within populations and in turn impact population vulnerability (Avisé 2004). Furthermore, reductions in rainfall may impact plants not only through direct effects on performance, as we have tested in our greenhouse experiment, but also through indirect processes such as disruptions of plant–plant or plant–animal interactions that could be occurring in natural conditions (Brooker 2006).

While several studies have shown that populations at the edge of species ranges have lower phenotypic plasticity, we demonstrate the opposite pattern and add experimental evidence to the importance of precipitation heterogeneity selecting for greater phenotypic plasticity. Our study also highlights the need to consider population differentiation in mean traits, as well as in their plasticity, to provide insights into species' range shifts under climate change, since despite of the important role of local adaptation and and/or plasticity in population persistence, species' distribution models in climate change scenarios rarely include intraspecific differences (Parmesan 2006; Lenoir & Svenning 2013; discussed in Valladares *et al.* 2014).

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Data accessibility

Data are available in Dryad <http://dx.doi.org/10.5061/dryad.bb7gp> (Lázaro-Nogal *et al.* 2015).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Annual rainfall and detailed climatic characterization of *Senna candolleana* studied populations.

Appendix S2. Location of *Senna candolleana* studied populations and the nearest weather stations.

Appendix S3. Analysis of variance of the effect of Population, Treatment and their interaction in functional traits of *Senna candolleana* with and without seed mass as a covariate.