Contemporary evolution of an invasive plant is associated with climate but not with herbivory

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Running headline: Contemporary evolution of an invasive plant

Summary

1. Divergence in plant traits and trait plasticity after invasion has been proposed as mechanisms favouring invasion success. Current hypotheses predict a rapid evolution in response to changes in the abiotic conditions in the area of introduction or to differences in the herbivore consumption pressure caused by a decrease in the enemies associated with the area of origin (e.g., evolution of increased competitive ability –EICA– hypothesis). The importance of these factors in determining plant geographical divergence has not been yet simultaneously evaluated.

2. *Senecio pterophorus* (Asteraceae) is a perennial shrub native to Eastern South Africa and a recent invader in Western South Africa (since ~100 years ago), Australia (>70-100 years) and Europe (>30 years). These areas differ in their summer drought stress (measured as the ratio between summer precipitation and potential evapotranspiration, *P*/PET) and their interactions with herbivores.

3. We performed a common garden experiment with *S. pterophorus* sampled throughout its entire known distributional area to determine: 1) whether native and non-native populations diverge in their traits, as well as the plasticity of these traits in response to water availability, and 2) whether climate and herbivory play a role in the genetic differentiation across regions.

4. Plants from the non-native regions were smaller and had a lower reproductive output than plants from the indigenous area. No geographical differences in phenotypic plasticity were found in response to water availability. Herbivory was not related to the plant geographical divergence. In contrast, our results are consistent with the role of climate as a driver for post-invasive evolution, as suggested by adaptation of plants to a drought cline in the native range, the analogous change in plant traits in independently invaded regions, and the convergence of vegetative traits between non-native plants and native plants under similar drought conditions.

5. Native and non-native populations of *S. pterophorus* differed in plant traits, but not in trait plasticity, in response to their local climatic conditions. Our results are contrary to the role of herbivory as a selective factor after invasion and highlight the importance of climate driving rapid evolution of exotic plants.

Key-words: adaptation, biological invasions, drought, ecological clines, evolution of increased competitive ability (EICA) hypothesis, herbivory, invasion ecology, phenotypic plasticity, plant traits, *Senecio pterophorus*

Introduction

The number of invasive plants has risen dramatically during the last decades, impacting the structure, function and dynamics of the receiver ecosystems (Mack *et al.* 2000). Successful plant invaders that become established and spread into a new habitat, however, represent only few of the overall introduced species (Williamson 1996; Kolar & Lodge 2001). Understanding why some species become invasive and others do not is essential to predicting the outcomes of future introductions (Sol *et al.* 2012) but it remains an open issue in the study of biological invasions.

During the last decade, the rapid evolution of exotic plants has been proposed as an important determinant for invasion success (Maron *et al.* 2004; Prentis *et al.* 2008; Buswell, Moles & Hartley 2011). Evolutionary change to novel environmental conditions is revealed by the divergence in genetically determined traits between the native and the invasive populations. It is commonly expected that plant genotypes with morphological and physiological traits related to higher fitness, such as an elevated growth, biomass, reproductive capacity and competitive ability, will increase their frequency in the newly established populations as a result of natural selection (Crawley 1987; Richards *et al.* 2006; Lachmuth, Durka & Schurr 2011). The characterization of the traits related to invasion, however, has proven difficult, in part because successful strategies may vary among ecosystem types and climatic conditions (Sakai *et al.* 2001; Pyšek & Richardson 2007). For example, small plants with narrow and thicker leaves perform better, and thus may be favourably selected, in warm, dry and nutrient-poor environments compared with large plants with a high foliar area (Westoby *et al.* 2002; Moles *et al.* 2009; Buswell, Moles & Hartley 2011). Plants may locally adapt to new climatic conditions after invasion, expressing those traits that confer them higher fitness in the invaded area (Colautti & Barrett 2013).

Contemporary evolution of exotic populations could also be driven by changes in the plant biotic environment. The tendency of exotic plants to perform better in the areas of introduction than in the native areas, expressed by an increased biomass, reproductive effort or competitive ability, has been attributed to the release from herbivorous natural enemies (Crawley, 1987; Blossey & Notzold 1995; Keane & Crawley 2002). The most cited hypothesis predicting post-invasive evolution, the Evolution of Increased Competitive Ability (EICA) hypothesis (Blossey & Notzold 1995) states that under a lower consumption pressure, genotypes allocating more resources to growth and reproduction and less to chemical defences would be favoured over the less-competitive and more heavily defended plants.

In addition to the adaptive changes of plant trait values driven by novel abiotic and biotic environmental conditions, invasive potential may also be determined by changes in the plastic response of those traits (Richards *et al.* 2006). A high phenotypic plasticity (i.e., the ability of an organism to express distinct phenotypes depending on the environmental conditions) expands the ecological niche and facilitates the colonization of novel habitats (Richards *et al.* 2006, Berg & Ellers 2010). Accordingly, invasive plants are expected to evolve an elevated plasticity in comparison with plants from the habitat of origin (Bossdorf *et al.* 2005). The hypothesis of the evolution of increased phenotypic plasticity after invasion, however, has been rarely tested (Bossdorf *et al.* 2005; Vanderhoeven *et al.* 2010; Godoy, Valladares & Castro-Díez 2011).

Here we study whether plant traits and trait plasticity rapidly evolve in response to new abiotic and biotic environmental conditions, using *Senecio pterophorus* DC (Asteraceae) as a model species. *S. pterophorus* is a perennial shrub native to Eastern South Africa that has expanded to Western South Africa (*ca.* 100 years ago) and has been introduced in Australia (> 70-100 years ago) and Europe (> 30 years ago) (Castells *et al.* 2013). These four regions differ in their climatic conditions and interactions with herbivores (Hijmans *et al.* 2005; Castells *et al.* 2013) (Table 1; see Fig. S1 in Supporting Information). The distribution of *S. pterophorus* in its native range occurs along an ecological cline of drought, but on average its native range is characterized by wetter and hotter summers compared with all non-native regions. A biogeographical study showed that non-native plants were released from herbivory after introduction, and this release was more intense in Europe, the region with a shorter time span since introduction (Castells *et al.* 2013). These differences make *S. pterophorus* a suitable model species to test the simultaneous role of key abiotic and biotic factors as determinants of plant geographical divergence.

We conducted a common garden experiment using 47 populations of *S. pterophorus* spanning its entire known distributional area across the native (Eastern South Africa), the expanded (Western South Africa) and two introduced ranges (Australia and Europe). We determined the genetic differences in individual-level traits, leaf-level traits and reproductive traits across regions and their response to water availability, and we asked two main questions: 1) Do plants from the expanded and introduced populations diverge in their traits and the phenotypic plasticity of those traits in comparison with the native populations? 2) Are climate and herbivory driving this genetic differentiation across regions?

The reported differences in the abiotic and biotic environment across regions allow us to make predictions about the factors determining plant adaptation. If climate is driving the biogeographical divergence in plant traits, we would expect a lower growth and leaf area in the introduced areas, where the plants are subject to drier conditions, compared with the native populations. Moreover, this pattern should be similar in all of the introduced areas because they share a similar climate. In contrast, if plant traits are explained by differences in herbivory among regions, the non-native plants should grow more and have a higher reproductive output compared with the native plants, especially in Europe, where herbivore release has been more intense (Castells *et al.* 2013). Finally, regardless of the factors driving post-invasive changes, plants from the non-native populations are expected to show a higher phenotypic plasticity in response to an environmental stress compared with the native populations.

Materials and methods

MODEL SPECIES

Senecio pterophorus (Asteraceae) is a perennial shrub of 0.4 to 2 m in height that colonizes open and disturbed environments, such as grasslands, forest margins and roads (Parsons & Cuthbertson 1992; Castells *et al.* 2013). *S. pterophorus* is native to the Natal province in Eastern Cape, South Africa and was introduced into the Western Cape Province circa 1918 (Hilliard 1977). The first citation in Australia is from 1908, but the species became invasive approximately 1930 along the southern coast (Parsons & Cuthbertson 1992). In continental Europe, *S. pterophorus* was first found near Barcelona, NE Spain, in 1982 and later in Liguria, NW Italy, in 1990 (see references in Castells *et al.* 2013). Since 1994, *S. pterophorus* has been considered a noxious weed subject to eradication by the Department of Environment and Primary Industries, Victoria (Australia), and it has been recently catalogued as an invasive species in Catalonia (NE Spain) (Andreu *et al.* 2012). A detailed species description, distribution and invasion history of *S. pterophorus* is provided in Castells *et al.* (2013).

FIELD SAMPLING

Senecio pterophorus was collected in 2009 and 2010 from 47 populations across the native range (Eastern Cape in South Africa), the expanded range (Western Cape in South Africa) and two invasive ranges (Australia and Europe) (Castells *et al.* 2013) (Table 1). Populations were at least 30 km apart in South Africa and Australia and 5 km apart in Europe, covering the entire species' known range, including the distribution limits (Castells *et al.* 2013). In each population we collected seeds from 6 to 15 individuals (referred to here as mother plants).

We calculated the ratio between summer precipitation and potential evapotranspiration (*P*/PET) (Thornthwaite 1948) for each population as a measure of drought stress. Mean temperature and precipitation during summer (June to August in the Northern Hemisphere and December to February in the Southern Hemisphere) were obtained from the WorldClim database (Hijmans *et al.* 2005). Summer *P*/PET was preferred over latitude or other climatic variables because it better relates to plant drought stress (e.g., Martínez-Vilalta *et al.* 2008). Populations in the native range had, on average, a higher summer *P*/PET (lower drought stress) compared with the non-native populations (Table 1). Additionally, native populations showed a latitudinal cline of summer *P*/PET, with an increasing summer *P*/PET (decreasing summer drought) towards the north (see Table S1).

The intensity of herbivore consumption on reproductive parts (heads and seeds) was characterized at the original sampling locations on the same individuals used in the common garden experiment. Native and expanded populations in South Africa had higher predation levels compared with the cross-continental introductions, with Europe showing nearly a complete release from herbivores (Castells *et al.* 2013) (Table 1).

COMMON GARDEN EXPERIMENT

Experimental design

In November 2010, seeds from six individuals from 47 populations (a total of 282 mother plants) were germinated in a mixture of *Sphagnum*, perlite and vermiculite (2:1:1) at the greenhouse facilities of the Faculty of Biology, University of Barcelona (Spain). Soil was watered regularly with a Hoagland nutrient solution. In February 2011, when the seedlings had four to five true leaves (100 days old approximately), two seedlings per mother plant were transplanted to the common garden. We ignored whether the seedlings from a mother plant were full-sibs or half-sibs, but for the sake of simplicity we refer to them as half-sibs throughout this study. The common garden was conducted at the experimental fields of the Autonomous University of Barcelona (41°29'53.3''N, 02°06'9.6''E) located in an old cultivated area surrounded by a *Pinus halepensis* forest. The soil is typic calcixerept (Soil Survey Staff 2010) and the mean annual temperature and precipitation are 14.9 ºC and 562.8 mm, respectively (Ninyerola *et al.* 2003). The weather in 2011, when the experiment was performed, was hotter and wetter than the average (15.6 ºC and 853.1 mm) (Meteorological Service of Catalonia 2015).

The field was divided into six plots of 58 m^2 separated by 1.5 m. Three plots were left without irrigation but receiving rainfall (Not Watered, NW) and three plots were assigned to a drip irrigation treatment (Watered, W) set at 4.5 L/day/plant. Treatments were randomly assigned to plots. Drought was selected as a stress treatment to characterize phenotypic plasticity, as growth and survival of *S. pterophorus* are strongly limited by water availability (Caño, Escarré & Sans 2007). Each treatment (NW and W) contained one half-sib per mother plant, with a total of 564 individuals (47 populations x six mother plants/population x two treatments) randomly distributed across plots within a treatment. Individuals within plots were separated by 75 cm.

Plants were watered during seven weeks after transplanting to minimize mortality and dead plants were replaced as necessary. The irrigation experiment started in April and ran until October. Plants from the water treatment received approximately 2212 L/m^2 throughout the experiment. Drip irrigation was applied continuously at a slow rate, and in consequence the soil was never saturated. Precipitation during the course of the experiment was 535 L/m^2; watered plants thus received 413% more water than non-watered plants.

Measurements

Plants were monitored for mortality and phenological stage (vegetative or reproductive) once a week throughout the experiment. The date of first flowering and the total length of the flowering period were recorded for each individual, as these characteristics have been related to invasiveness (Pyšek & Richardson 2007). We estimated relative growth rate (RGR) as the increase in plant height, measured at the beginning of the experiment (week 0) and at weeks 10, 16 and 23. RGR was calculated as the difference in log-transformed plant height between two consecutive periods divided by the corresponding time interval (first period: 0-10 weeks, second period: 10-16 weeks, and third period: 16-23 weeks). Plant reproductive effort was estimated by counting the number of flowering and fruiting heads in a plant subsample for each individual in June and August 2011. Because plant origin and response to irrigation could affect the blooming dynamics, we chose the highest number of heads counted at either census for each individual as an estimate of head production. To determine the average number of seeds per head, we counted the number of seeds (achenes) of three heads per individual plant. Total seed production was estimated by multiplying head production by the average of seeds per head. Shoot biomass was determined at the end of the experiment (September/October 2011) for all surviving individuals. Individuals were cut at ground level and leaves were separated from stems. Both fractions were oven-dried at 65ºC for two to three days and weighed.

Three leaves per plant were collected between September and October to estimate leaf-levels traits. Fresh leaves were scanned, and foliar area and shape was determined using ImageJ (Schneider, Rasband & Eliceiri 2012). Leaves were then oven-dried for 72 h at 65 °C and weighed. The SLA was calculated by dividing leaf area by dry weight. A high SLA is normally associated with higher productivity and invasiveness (Lake & Leishman 2004) and with shorter life-spans and vulnerability to herbivores and drought stress (Burke & Grime 1996; Maroco, Pereira & Chaves 2000). Leaf shape was estimated as $4 \cdot \pi \cdot$leaf area/leaf perimetre^2 (shape = 1 for a perfect circle). Leaves with more dissected margins are frequently associated with high evaporation and assimilation rates (Schuepp 1993). Total leaf area was calculated by multiplying leaf dry weight by SLA.

Leaf N concentration, C/N ratio and C isotopic composition were analysed in 116 individuals (see Table S1). Leaf N concentration was used as a surrogate for maximum photosynthetic capacity and, hence, potential growth (Reich, Ellsworth & Walters 1998) whereas $\delta^{13}C$ was used as a proxy of water-use efficiency (Farquhar, Ehleringer & Hubick 1989). All chemical analyses were carried out at the University of California Davis Stable Isotope Facility (USA) using an IRMS (PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer). The relationship between carbon stable isotopes was expressed in relation to a Pee-Dee Belemnite (PDB) standard. The accuracy of the measurements was 0.015‰.

To characterize the phenotypic plasticity in response to water availability, we calculated a plasticity index (PI) between half-sibs following Valladares *et al.* (2000):

$$PI = [Mean(W) – Mean(NW)] / Max[Mean(W), Mean(NW)]$$

where Mean(W) and Mean(NW) are trait values of half-sibs growing in the water and non-water treatments, respectively. PI ranges from 0 (no plasticity) to ±1 (maximum plasticity). A negative PI indicates a higher mean value under the NW treatment (control) compared with the W treatment.

STATISTICAL ANALYSES

A generalized binomial mixed model (logit transformation) was used to determine the effects of region, treatment and their interactions (fixed effects) on plant survival. Mother plants nested within populations and both crossed with plots were included as random effects. For the quantitative variables measured only once during the course of the common garden experiment (biomass, SLA, leaf shape, total leaf area, $\delta^{13}C$, N concentration, C/N, first flowering date, flowering period, seeds per head, number of heads and total number of seeds) a general linear mixed model was used, including region, water treatment and their interaction as fixed effects, and the same random structure as before. For RGR, a trait measured repeatedly throughout the experiment, the model also incorporated time as a fixed factor and individual as an additional random effect (nested within mother plant). The variables SLA, leaf shape, total leaf area, N concentration, C/N, number of heads and total number of seeds were normalized by a logarithmic transformation. Statistical analyses of plasticity were conducted using general linear mixed models with region as fixed effect and population and plot as random effects.

In a next step of our analysis we asked whether the effects of climate (*P*/PET) and herbivory (percentage of predated heads) could explain differences among regions in the studied plant traits. We did that by fitting additional linear mixed models including region, *P*/PET and predation as fixed effects. These models included the interaction between region and climate and between region and predation. As before, random effects (on the intercept of the model) included mother plant nested within population and both crossed with plot. Two separate models were fitted: one for control (NW) plants and the other for watered (W) plants. ANOVA Type I tables corresponding to these models are provided in the Supporting Information (see Tables S7, S8). In these sequential analyses *P*/PET was introduced before Region to test whether it explained the differences across regions obtained in the models presented in the previous paragraph. Note that *P*/PET and Predation were not correlated ($r^2 = 0.002$, $P > 0.05$). The variable *P*/PET was centred at the mean value for all populations before the analysis ($P/\mathrm{PET}_{centred} = 0.76$). The variables SLA, leaf shape, total leaf area, N concentration, C/N, number of heads, total number of seeds and predation were log transformed to meet normality. Significance for all statistical analyses was accepted at $P < 0.05$. All models were fitted using the R software v. 3.1.2 (R Development Core Team 2008) with packages nlme and lme4.

Results

Native and non-native populations of *S. pterophorus* differed in plant survival, aboveground biomass, total leaf area, leaf shape and reproductive effort (Fig. 1, see Table S2). Biomass was consistently lower in the non-native regions, and total leaf area was also lower in Australia and Europe compared with the native range (Fig. 1, see Table S2). Similarly, Australia and Europe had a lower reproductive output (seeds per head, total number of heads and total number of seeds) although these differences were not always significant (Fig. 1, see Table S2). Relative to native populations, survival was lower only for Australian plants. No genetic differences were observed between the native and non-native regions for SLA, $\delta^{13}C$, N concentration, C/N or phenology (Fig. 1, see Table S2).

Irrigation resulted in a higher biomass and total leaf area, and lower $\delta^{13}C$, C/N and number of seeds per head (Fig.1, see Table S2). Plant traits showed a plastic response to watering (i.e., plasticity index different from zero) except for leaf shape, C/N, first flowering date, flowering period and head and seed production (see Table S4). The response to watering was similar for all regions and most plant traits, except for phenology in the expanded region, biomass and N and C/N in Australia, and survival in Europe (Fig.1, see Table S2). However, the plasticity index was not different across regions for any plant trait (the only exception was flowering period in South Africa expanded range), indicating a similar phenotypic plasticity in the native and non-native populations (see Table S4). The effects of region and water treatment on the plant relative growth rate (RGR) were consistent with the pattern observed for biomass (see Fig. S2, Table S3).

The role of climate and herbivory on the differences across regions was evaluated simultaneously in a statistical model that incorporated summer P/PET and the intensity of predation measured at the population original areas. In the native region, P/PET was positively related with biomass, total leaf area and first flowering date and negatively related with leaf shape, N, flowering period and reproductive output variables (Figs 2 and 3; see Tables S5, S6). The general loss of a significant effect of Region when P/PET was included into the model (see Tables S7, S8) and the fact that the intercepts are similar across regions after accounting for $P/\mathrm{PET}_{\mathrm{centred}}$ (see Tables S5, S6) strongly suggests that the genetic differences in plant size and leaf area between the native and the non-native regions can be explained by differences in P/PET. Biomass and leaf area of the non-native populations tended to converge with the native populations with similar climatic conditions (Figs 2 and 3). In Australia, however, the effect of *P*/PET on some traits was different to the one observed for the native region, and was mostly driven by two populations with a much higher *P*/PET (Figs 2 and 3). Predation was generally unrelated to plant traits, particularly for control (NW) plants (see Tables S7, S8) and the corresponding model coefficients did not differ among regions (see Tables S5, S6). Finally, leaf shape and reproductive traits (flowering date, flowering period, number of seeds per head, number of heads and total number of seeds) were related to *P*/PET at the native range (Figs 2 and 3, see Tables S5, S6). However, climate did not completely explain the differences across regions; the overall effect of region for some of these traits was still significant after removing the variation due to *P*/PET in the ANOVA analyses, especially for water treatment (Tables S7, S8).

Discussion

DIFFERENCES BETWEEN NATIVE AND NON-NATIVE POPULATIONS

Our results are consistent with the presence of strong genetically based differences in trait values between the native and the non-native populations of *S. pterophorus*. Plants from the non-native areas were smaller and had lower leaf areas and lower reproductive capacities than plants from the native area. Because the introduction of *S. pterophorus* to novel areas is relatively recent (Western Cape ~ 100 years; Australia >70-100 years; Europe >30 years) (Castells *et al.* 2013), these results strongly suggest that plant traits can diverge rapidly after invasion. Moreover, the similar pattern found between Europe and the other two non-native areas suggests that changes may have occurred early on after the introduction. In contrast, *S. pterophorus* responded similarly to watering regardless of their geographic origin. The increased plasticity hypothesis predicts that plants from invasive populations should be more plastic than plants from the native populations (Richards *et al.* 2006). Contrary to this hypothesis, we found no differences in trait plasticity between native and non-native *S. pterophorus* populations. These results are consistent with previous studies finding that trait values were more important for determining plant invasibility than trait plasticity (Godoy *et al.* 2011; Matzek 2012).

CLIMATE DRIVES GEOGRAPHICAL DIVERGENCE IN PLANT TRAITS

S. pterophorus in the native region, in Eastern South Africa, is distributed along an ecological cline of drought, from the southernmost populations subject to a higher drought stress (lower summer *P*/PET) to the northernmost populations growing under wet and cool environments (higher summer *P*/PET). When plants from these populations grew under the same environmental conditions in the common garden, we observed a strong correlation between *P*/PET at the original sampling locations and most of the measured plant traits. Plants from drier areas were smaller and had lower leaf area, more dissected leaf margins, earlier blooming, longer reproductive period and higher seed production compared with plants from more humid areas. These genetically based trends along a climatic gradient suggest that plants are locally adapted to the conditions in the native area (Kawecki & Ebert 2004). Indeed, short stature, small size and low leaf area are believed to be advantageous under dry environmental conditions (Martínez-Vilalta *et al.* 2009; Hartmann 2011).

Because summer drought is more severe in the three non-native areas (Western South Africa, Australia and Europe) than in the native range, we hypothesize that climate may have also driven divergence of vegetative traits after invasion. Several pieces of evidence support this idea. First, the direct effect of region was no longer significant when $P/\mathrm{PET}_{\mathrm{centred}}$ was included into the statistical model (see Tables S7, S8).

Second, the estimated trait values at the mean *P*/PET were similar across regions (see Tables S5, S6) indicating that geographical differences in plant traits could be explained by differences in climate. Third, the direction of the changes across regions was, on average, similar for all of the introduced areas: introduced plants from Western South Africa, Australia and Europe had lower biomass and leaf area than the native populations. This pattern is only consistent with a response to similar climatic conditions, as these regions differed in their introduction time, distance from the source populations, and biotic environment. Finally, differences in individual-level and leaf-level plant traits after introduction were consistent with the climatic effects within the native region. Moreover, the value of individual-level and leaf-level traits in the non-native populations tended toward convergence with the native populations under similar climatic conditions, except for two Australian populations from New South Wales (A01 and A02; see Table S1) that experienced a much higher *P*/PET than the rest of populations from the same region.

The role of climate as a main driver for changes in reproductive traits between native and non-native populations was not as consistent as for vegetative traits. Because plants growing under drier conditions in the native region had a longer flowering period and a higher head and seed production, we would expect introduced plants to behave similarly in accordance with their climate. However, non-native plants tended to have a shorter reproductive season and lower seed production than native plants under similar *P*/PET conditions (Fig. 3). These results suggest that in addition to climate, other factors not included in this study were probably determining geographical differences in reproductive traits.

Genetically based differences between regions could also be caused by non-adaptative events such as demographic bottlenecks and genetic drift or the plant introduction routes (Keller & Taylor, 2008; Lachmuth *et al.*, 2011). We cannot reject that neutral events contributed to the geographical divergence, but the relationship between climate and plant traits within the native region and the convergence between introduced and native plants from similar climates are indicative of adaptive evolution. Moreover, the similar pattern observed in all non-native regions suggests that a directional change has occurred in three presumed independent events. Finally, the genetic similarity across the native and non-native areas obtained by neutral markers (AFLPs) (Vilatersana *et al.*, unpublished data) shows that the *S. pterophorus* in Western South Africa, Australia and Europe comes from multiple introductions spanning a range of climates in the native area. Thus, the convergence observed in the common garden between the introduced populations and the native populations with a matching climate cannot be explained solely by the invasion routes.

We acknowledge that our conclusions are limited by the fact that only one common garden located within the European invaded range was used. Reciprocal common garden experiments have been useful to reveal the interactions between genetically based plant trait expression and the environmental conditions from the species' distributional areas (Williams, Auge & Maron 2008; Colautti, Maron & Barrett 2009). However, we found no biogeographical divergence in trait plasticity in response to water availability (*P*/PET) and thus, *a priori*, we would not expect significant interactions between the relative change in plant traits across regions and the local environmental conditions in the native and introduced areas.

EVIDENCE AGAINST THE ROLE OF HERBIVORY

The release from natural enemies after invasion has been proposed as a driver for post-invasive evolution (Crawley, 1987; Blossey & Notzold 1995; Keane & Crawley 2002). One of the most invoked hypotheses explaining the success of invasive species, the

Evolution of Increased Competitive Ability (EICA) hypothesis (Blossey & Notzold 1995) states that under a lower consumption pressure by specialist herbivores, genotypes allocating more resources to growth and reproduction and less to chemical defences would be favoured in the introduced range. In our system, we did not find any relationship between herbivore release and genetically based plant traits across regions. Contrary to the predictions of the EICA hypothesis, plants from the introduced populations had lower growth and reproductive output compared with the native populations. Moreover, this pattern was similar for all non-native regions, even though the release in herbivory was more intense in Europe than in Australia (Castells *et al*. 2013).

Experimental support of the EICA remains controversial (Willis, Memmott & Forrester 2000; van Kleunen & Schmid 2003; Vilà, Gómez & Maron 2003; Jakobs, Weber & Edwards 2004). This lack of consistent results may occur, at least in part, because comparisons between native and introduced populations tend to use limited sample sizes and cover only part of the species' distributional areas. Under these conditions comparisons between native and introduced populations may not use the appropriate controls, particularly if the invasion routes are unknown (Bossdorf *et al.* 2005). Additionally, the release from herbivores in the invasive range, the first premise of the EICA hypothesis, is rarely evaluated quantitatively. We overcame most of these limitations by performing a common garden experiment with a large number of individuals and populations from nearly all of the species' known distributional range and incorporating data on herbivore consumption measured *in situ* on the same mother plants used in the experiment. However, our study was limited by the fact that herbivory was estimated only once on the reproductive parts, so we cannot discard that herbivory on shoots and roots along the entire plant life cycle could be related to plant divergence across regions.

In addition, changes in chemical defences after invasion are an important aspect of the EICA hypothesis that has not been covered in our study. We cannot reject that chemical defences of *S. pterophorus*, such as pyrrolizidine alkaloids (Castells *et al.* 2014), are evolving in response to herbivory independently from morphological traits.

To our knowledge, this is one of the first studies testing simultaneously, on the same plants, the role of climate and herbivory as the main drivers of post-invasive evolution. A recent meta-analysis on the North American invasive plant *Lythrum salicaria* (Lytharaceae) found that local plant adaptation was driven by both climatic and biotic effects (Colautti & Barrett 2013). The results obtained here for *S. pterophorus* are solely consistent with the role of climate as a driver for plant adaptation to novel environments. Our study adds to the recent reports of rapid evolution after invasion (Maron *et al.* 2004; Prentis *et al.* 2008; Buswell, Moles & Hartley 2011; Colautti & Barrett 2013) by showing that contemporary differentiation may occur in several independent events. The adaptation of *S. pterophorus* along a climatic gradient in the native range, together with multiple introductions in each non-native region, suggests that genotypes pre-adapted to drought (with a lower growth and leaf area) were favourably selected in the introduced areas, resulting in a rapid geographical divergence. Although reproductive traits also varied across a climatic cline in the native range, other factors in addition to drought contributed to their genetic divergence among regions. It remains unresolved whether genetic changes across regions increased plant fitness as a result of local adaptation, the so called "home site advantage" (Colautti & Barrett 2013), and whether the potential benefits at the individual level translate to higher invasion success at the population level. Understanding the mechanisms for rapid differentiation in response to novel climatic conditions improves our ability not only to explain the dynamics of biological invasions but also to predict the response of native populations under climate change (Hoffmann & Sgrò 2011).

Acknowledgements

We thank Miriam Cabezas, Maria Morante, Pere Losada, Guillem Esparza, Xavier Sans and Jose Manuel Blanco-Moreno for technical assistance. This research was conducted thanks to the financial support provided to E.C. by Ministerio de Ciencia e Innovación (Spain) (GCL2008-02421/BOS) and Ministerio de Economía y Competitividad (Spain) (GCL2011-29205). E.C. and J.M.V. belong to the research group "Response of ecosystems to climate change and environmental gradients" funded by Generalitat de Catalunya (Catalonia) (2014 SGR-453). We thank an anonymous reviewer for her/his exhaustive contributions which improved the quality of the final manuscript. The authors declare that they have no conflicts of interests.

Data Accessibility

Data deposited in the repository of Universitat Autònoma de Barcelona: https://ddd.uab.cat/record/131539

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Supporting Information

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

Table S1. Characteristics of the populations of *Senecio pterophorus* used in the common garden experiment

Table S2. Estimates and significance of the effects of region and treatment on individual-level traits, leaf-level traits and reproductive traits

Table S3. Estimates and significance of the effects of region and treatment on the relative growth rate

Table S4. Estimates and significance of the plasticity index in response to a water treatment

Table S5. Estimates and significance of the effects of region, drought index and herbivory on individual-level traits, leaf-level traits and reproductive traits of plants growing under a control (non-watered) treatment

Table S6. Estimates and significance of the effects of region, drought index and herbivory on individual-level traits, leaf-level traits and reproductive traits of plants growing under a water treatment

Table S7. ANOVA Type I table corresponding to the linear mixed model presented in Table S5

Table S8. ANOVA Type I table corresponding to the linear mixed model presented in Table S6

Fig. S1. Monthly temperature and precipitation at the *S. pterophorus* sampling locations averaged by region

Fig. S2. Plant height of *S. pterophorus* from the native region compared to three introduced regions in a common garden under a water treatment

Table 1. Characteristics of the *Senecio pterophorus* populations used in the common garden experiment, averaged by region in the native, expanded and introduced ranges (Mean ± SE). Different letters indicate significant differences between regions by a Tukey post-hoc contrast in a t-test

Region	Plant Status	Populations and individuals	Elevation* (m)	Mean Annual Temperature* (ºC)	Mean Annual Precipitation* (mm)	Summer *P*/PET†	Predation* (% damaged heads)
South Africa	Native	18 pop, 107 ind	792.7 ± 96.3[a]	16.6 ± 0.2[a]	746.2 ± 31.8	1.27 ± 0.11[a]	25.2 ± 1.6[a]
	Expanded	5 pop, 29 ind	133.0 ± 56.1[b]	16.1 ± 0.4[ab]	856.4 ± 86.5	0.36 ± 0.04[b]	33.4 ± 2.8[a]
Australia	Introduced	12 pop, 70 ind	140.7 ± 46.9[b]	15.1 ± 0.4[b]	754.4 ± 63.0	0.51 ± 0.09[b]	15.4 ± 1.5[b]
Europe	Introduced	12 pop, 72 ind	244.5 ± 46.9[b]	15.3 ± 0.2[b]	667.0 ± 19.9	0.37 ± 0.02[b]	0.2 ± 0.1[c]

* Data obtained from Castells *et al.* 2013

†Ratio of precipitation to potential evapotranspiration during summer (December-February in the southern hemisphere and June-August in the northern hemisphere).

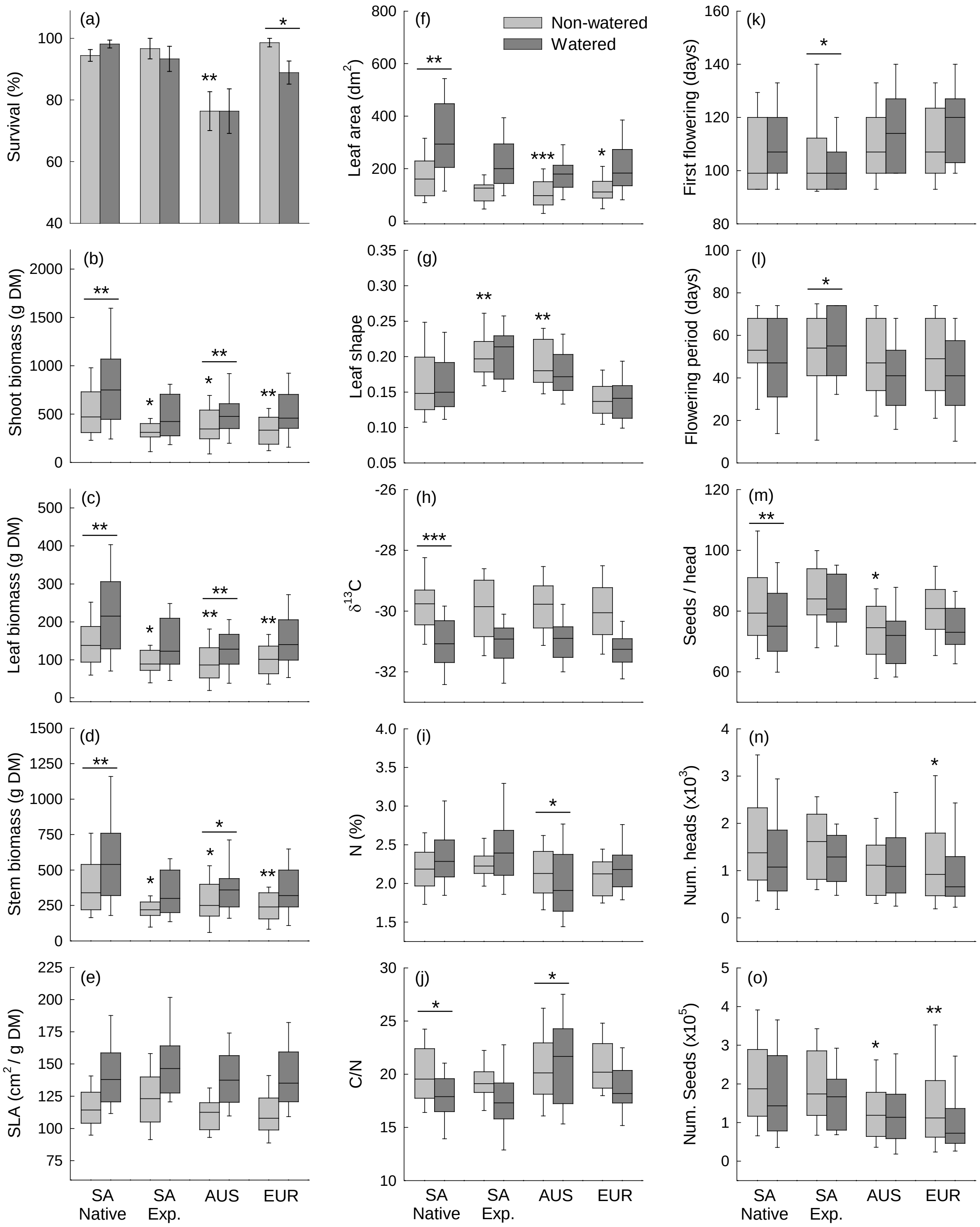

Fig. 1 Individual-level traits (a-d), leaf-level traits (e-j) and reproductive traits (k-o) of *S. pterophorus* from the South Africa native range (SA Native), South Africa expanded range (SA

Exp.) and two introduced regions in Australia (AUS) and Europe (EUR), growing in a common garden experiment without irrigation (non-watered; light grey) and with irrigation (watered; dark grey). Means ± SE are shown for percentage survival. The boxplots for the other variables show the 25th and 75th percentiles (box limits), the median (inner line), and the 10th and 90th (below and above whiskers, respectively). Statistical significance corresponding to the linear mixed models of Table S2 is shown as *$P < 0.05$, **$P< 0.01$ and ***$P < 0.001$. The reference level (intercept) is South Africa native range and non-watered treatment. Asterisks on top of a non-watered treatment in the non-native regions (South Africa expanded, Australia and Europe) indicate significant differences in this control treatment between each region and South Africa native range. Significant differences between regions for the water treatment are not shown for simplicity. Asterisks on top of a horizontal bar in South Africa native range indicate a significant effect of water treatment in this region. Finally, asterisks on top of a horizontal bar in the non-native regions indicate differences in the treatment effect (Region:Treatment) between each of these regions and South Africa native range.

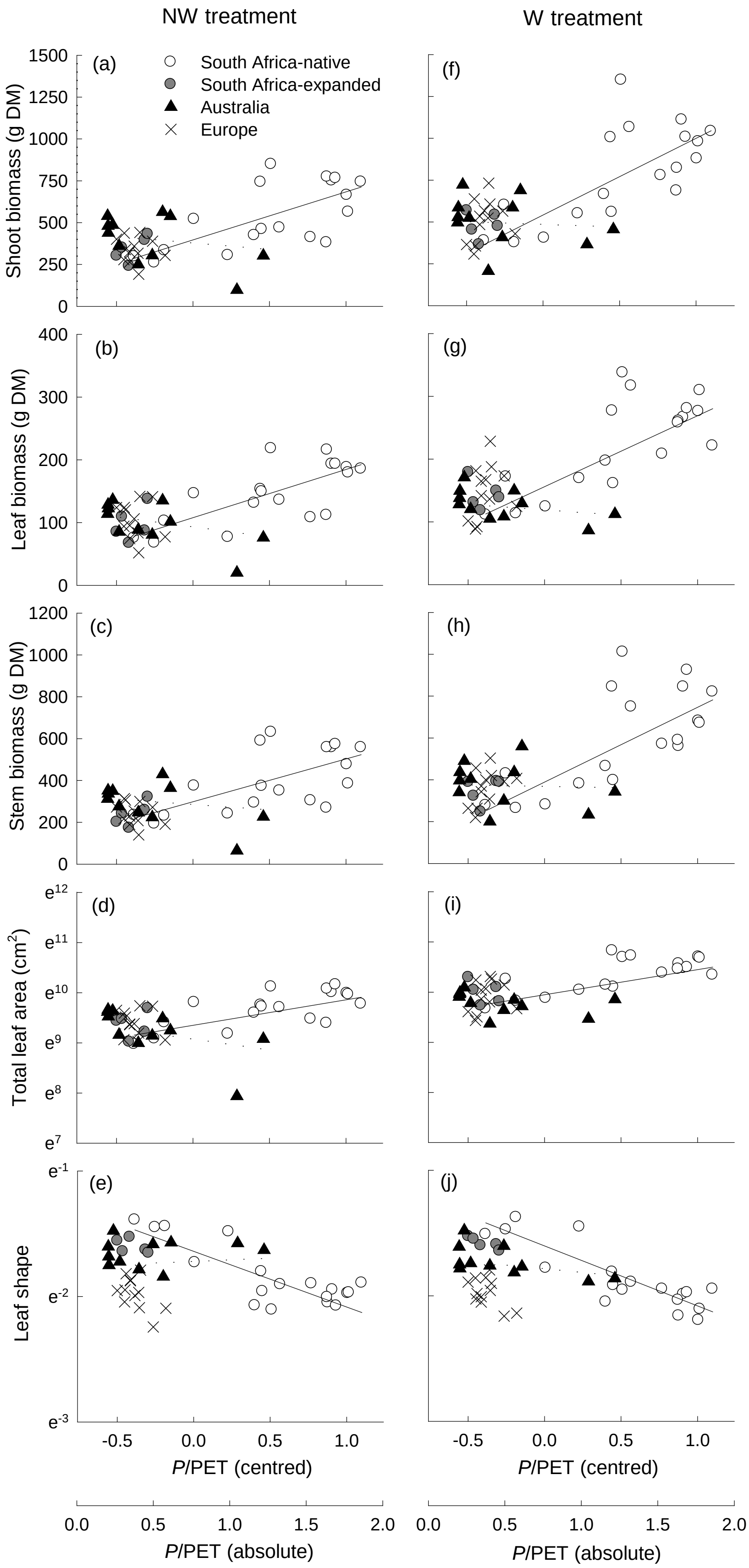

Fig. 2 Relation between drought index and individual-level and leaf-level traits of *S. pterophorus* from the native region in South Africa, the expanded region in South Africa and the introduced

regions in Australia and Europe. Drought index was expressed as the ratio of summer precipitation to potential evapotranspiration -*P*/PET- centred at the average for all populations. Plants were grown in a common garden experiment without irrigation (NW treatment) (a-e) and with irrigation (W treatment) (f-j). Each dot represents a population average. Depicted lines represent the relationships as obtained from the coefficients of the corresponding linear mixed models (see Tables S5, S6). For clarity, relationships are only shown for the native region (solid line) and for any region when the slope of the relationship was significantly different from South Africa-native (in our case only Australia, dashed line). An additional x-axis with the absolute value of *P*/PET is shown as a reference.

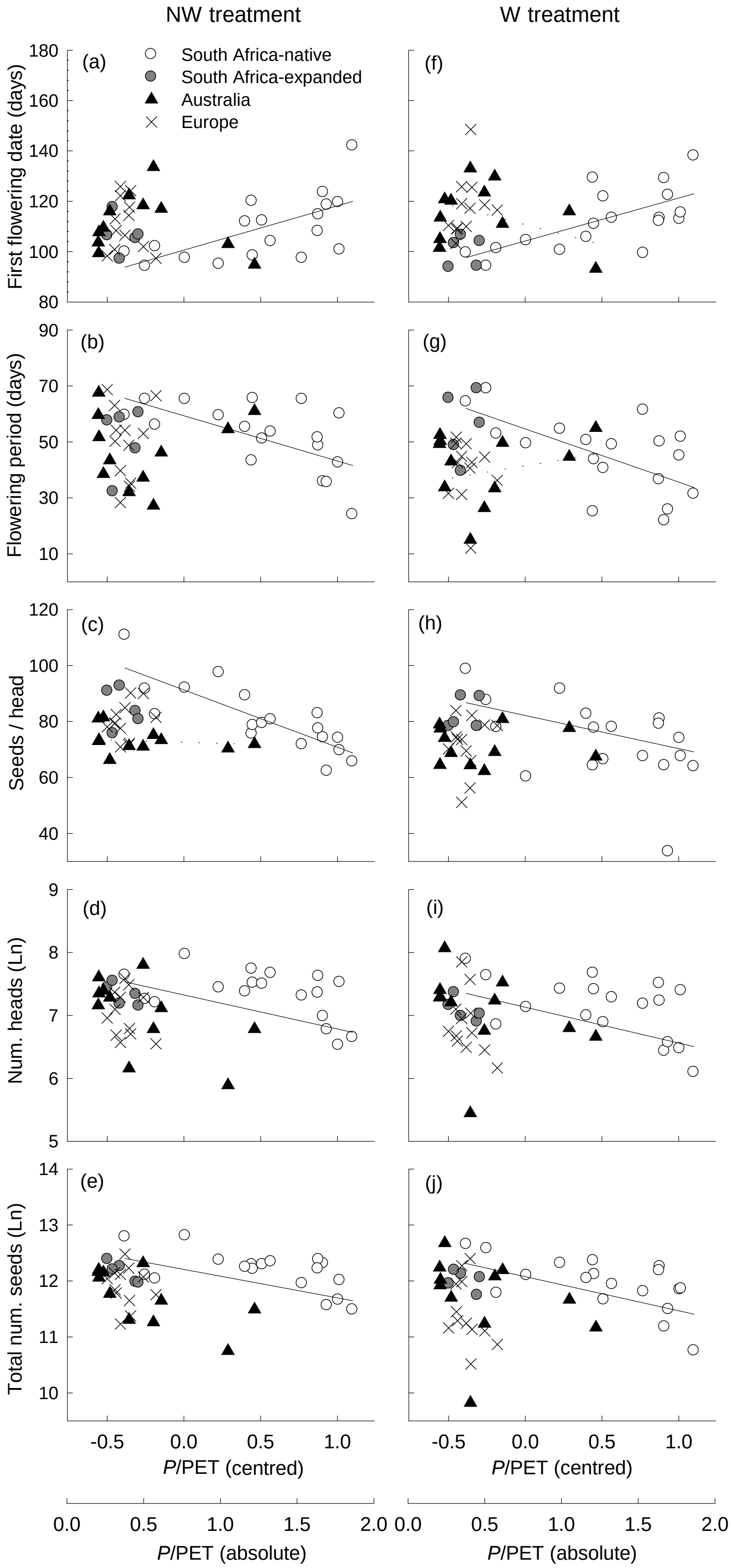

Fig. 3 Relation between drought index and reproductive traits of *S. pterophorus* from the native region in South Africa, the expanded region in South Africa and the introduced regions in Australia

and Europe. Drought index was expressed as the ratio of summer precipitation to potential evapotranspiration -*P*/PET- centred at the average for all populations. Plants were grown in a common garden experiment without irrigation (NW treatment) (a-e) and with irrigation (W treatment) (f-j). Each dot represents a population average. Depicted lines represent the relationships as obtained from the coefficients of the corresponding linear mixed models (see Tables S5, S6). For clarity, relationships are only shown for the native region (solid line) and for any region when the slope of the relationship was significantly different from South Africa-native (in our case only Australia for some traits, dashed line). An additional x-axis with the absolute value of *P*/PET is shown as a reference.