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**Abstract**

Rapid adaptive evolution has been advocated as a mechanism that promotes invasion. Demonstrating adaptive evolution in invasive species requires rigorous analysis of phenotypic shifts driven by selection. Here, we document selection-driven evolution of *Phyla canescens*, an Argentine weed, in two invaded regions (Australia and France). Invasive populations possessed similar or higher diversity than native populations, and displayed mixed lineages from different sources, suggesting that genetic bottlenecks in both countries might have been alleviated by multiple introductions. Compared to native populations, Australian populations displayed more investment in sexual reproduction, whereas French populations possessed enhanced vegetative reproduction and growth. We partitioned evolutionary forces (selection *vs*. stochastic events) using two independent methods. Results of both analyses suggest that the pattern of molecular and phenotypic variability among regions was consistent with selection-driven evolution, rather than stochastic events. Our findings indicate that selection has shaped the evolution of *P. canescens* in two different invaded regions.

## Introduction

Rapid adaptive evolution has been advocated as an important mechanism contributing to successful invasions ((Maron *et al.* 2004; Lavergne & Molofsky 2007; Cano *et al.* 2008). When species are introduced into a new region, they may face novel selective regimes, under which genetically based shifts of phenotypic traits may affect the fitness of individuals, the viability of populations, and the process of range expansion of the invading species (Suarez & Tsutsui 2008). Therefore, adaptive responses to these new selective pressures could play a major role in promoting invasion (Sakai *et al.* 2001; Cox 2004; Sax *et al.* 2007). Convincing evidence of rapid adaptive evolution has been reported in some invasive animals (e.g. cane toad, Phillips *et al.* 2006), but such compelling evidence has not been documented in 2 invasive plants (Barrett *et al.* 2008). In an increasing number of studies, phenotypic shifts in invasive plants have been detected, e.g. larger size (Buckley *et al.* 2003; Leger & Rice 2003), faster growth rate (Siemann & Rogers 2001; Blair & Wolfe 2004; Guesewell *et al.* 2006), reduced defences (Rogers & Siemann 2004; Meyer *et al.* 2005), enhanced reproductive rates (Wolfe *et al.* 2004; Brown & Eckert 2005), and increased plasticity (Lavergne & Molofsky 2007; Cano *et al.* 2008). However, the role of selection was not explicitly tested in most works (Keller & Taylor 2008, but see Maron *et al.* 2004; Maron *et al.* 2007; Colautti *et al.* 2009; Chun *et al.* 2009).

High genetic diversity may help maximize the evolutionary potential of alien species (Sakai *et al.* 2001). Although founder effects are frequently associated with introduced populations, resulting in reduced genetic diversity, this effect can be negated by various mechanisms (Bossdorf *et al.* 2005; Novak & Mack 2005; Roman & Darling 2007). For example, multiple introductions alleviate genetic bottlenecks (Chen *et al.* 2006; Kolbe *et al.* 2007), enable new genetic combinations (Voisin *et al.* 2005; Kolbe *et al.* 2007; Lavergne & Molofsky 2007), and may increase the

evolutionary potential of introduced populations. Therefore, understanding the introduction history and its genetic consequences on introduced populations may contribute to predicting the adaptive potential and identifying the force(s) prompting evolution of an invader. In general, we may predict that selection-driven evolution is more likely to occur in genetically diverse invaders that have been introduced a numberof times, whereas stochastic evolution might play a more important role in invaders experiencing severe genetic bottlenecks (but see Dlugosch & Parker 2008b).

Phenotypic shifts during invasion may be caused not only by selection, but also by stochastic processes, such as founder effects and/ or other chance demographic events (Eckert *et al.* 2003; Kliber & Eckert 2005; Dlugosch & Parker 2008a; Facon *et al.* 2008). Thus, stochastic evolution should be tested as a null expectation when interpreting phenotypic evolution in an invasive species (Keller & Taylor 2008). However, in most cases where phenotypic evolution in invasive plants has been documented, researchers either did not simultaneously examine the prior evolutionary history and genetic diversity of the invaders or the effect of selection against an appropriate null model that accounts for stochastic evolution (but see Chun *et al.* 2009). Although several studies on adaptive clinal variation addressed the role of selection *vs.* stochastic events (Maron *et al.* 2004; Maron *et al.* 2007; Colautti *et al.* 2009), their approach only applied to the adaptive evolution that related to a geographic gradient. Generalized conceptual designs to partition selection from chance events were not systematically presented until recently (Keller & Taylor 2008), mainly including two different, but not mutually exclusive methods: (1) comparing phenotypic variations between invasive and native genotypes descending from the same ancestor (e.g. Dlugosch & Parker 2008b), and (2) comparing the estimation of genetic differentiation (among ranges) at neutral loci, e.g. $F_{st}$, relative to that of phenotypic differentiation ($Q_{st}$) (Spitze 1993; Steinger *et al.* 2002; Chun *et al.* 2009). To date, few experimental data have been published to test these frameworks.

Here, we explicitly assess the role of selection in the phenotypic evolution of the invasive plant *Phyla canescens* (Kunth) Greene (Verbenaceae, common name: lippia), across its native (Argentina) and invaded regions (Australia and France). First, molecular markers were used to analyse the genetic diversity and structure in native and invasive populations and the genetic relationship among individuals. Then, we assessed phenotypic differentiation across the native and invaded regions using a common garden (greenhouse) experiment. After that, we used two methods (ancestordescendent comparisons and $Q_{st}$ *vs* $F_{st}$ test) to test the hypothesis that selection, rather than stochastic processes, was the primary force that prompted a genetically based phenotypic shift. Finally, *post-hoc* explanations of post-immigration evolution were explored by examining the relationships between phenotypic traits and environmental factors. Our findings indicate that selection has shaped the evolution of *P. canescens* in two different invaded regions.

## Materials and Methods

### Species and material
*Phyla canescens* is a perennial herb that forms a mat-like groundcover on floodplains and in pastures. With favourable moisture conditions, *P. canescens* flowers from spring to autumn and prodigiously produced seeds (McCosker 1994). Plants are not automatically able to self-pollinate and self-pollination produces less seeds than outcrossing (Gross *et al*. unpublished data). *P. canescens* also propagates vegetatively from stem nodes (Lucy *et al*. 1995).

The plant was commercially introduced into Australia and France and has become invasive (*SI Materials*). Recent taxonomic and genetic studies on *P. canescens* indicated that the source of invasive populations in this study was limited to central Argentina (*SI Materials*). Plant specimens were sampled at five localities to represent the geographic distribution of *P. canescens* within three regions: central Argentina (native), southeastern Australia and southern France (invasive), during January to March 2007 (*SI Methods, Table 1*). A total of 146 (6-10 per site) individuals were collected and maintained under greenhouse conditions. Rarefaction analysis suggested 6-10 individuals per population and about 20 individuals per region provided good representation of genetic diversity at population and region level (*SI, Figure 1*).

### Genetic analysis
We conducted PCR amplifications of 12 inter-simple sequence repeat (ISSR) primers (*SI Methods*). ISSR bands were scored as binary data (present or absent) that were analysed as dominant diploid data. Genetic diversity was measured for each population through band richness (Br), percentage of polymorphic loci (%P) (AFLPDIV, Petit,

INRA-Bordeaux) and Nei's gene diversity ($H_j$) (AFLP-SURV, Vekemans 2002, distributed by the author). The hierarchical partitioning of genetic variation within and among populations and regions was assessed using analysis of molecular variance (AMOVA, Armstrong & De Lange 2005) based on the pairwise δ squared Euclidean distance among molecular loci (GenAlEx version 6.2, Peakall and Smouse, Australian National University).

Genetic relationships among native and invasive individuals were determined by constructing a UPGMA endrogram using unweighted pairwise genetic distance matrices (SPSS 17.0, SPSS Inc., Chicago, USA). Such a multilocus analysis is the preferred approach in case admixture might occur in invasive populations (Keller & Taylor 2008).

*Greenhouse experiment*

All 146 *P. canescens* individuals were grown initially in a controlled-environment greenhouse (CSIRO European Laboratory, near Montpellier, France) for at least two months. We replicated the experiment temporally by growing and measuring plants in early May and again in late June, 2007. Two healthy 5-node cuttings (counting the tip as one node, with all branches or flowers removed), of approximately equal size, were selected from each individual. One cutting was used to determine initial length and dry weight, which was used to calculate relative growth rate and as covariance in the analysis (*SI Methods*). The other cutting was planted in a 1.3L pot with the fifth node buried in the potting mix (0.5:1:1 peat: washed sand: nursery soil and 4g/L of pelleted fertilizer Osmofit®, InfinIT, Cincinnati, USA). Plants were watered as required and their positions in the greenhouse were randomized every ten days. Plants were grown at an average temperature of 19–24ºC (night-day, range from 13–30ºC), RH 65% (30%–90%) and 15-hours light, with little environmental difference during the two experiment periods (e.g. < 1ºC for average 1 temperature and 1% for average RH). Eight phenotypic traits of three categories were determined for each individual (*SI Methods*) after seven (replicate 1) to eight (replicate 2) weeks, including: Category I: vegetative growth and allocation (total vegetative biomass, ratio of below-ground to above-ground vegetative biomass (R:S)); Category II: morphology (leaf size, leaf morphology, internode length, stem diameter); Category III: sexual reproductive investment (inflorescence number, ratio of reproductive to vegetative biomass (S:V)). Principal component analysis (PCA) was conducted on these phenotypic parameters based on the trait mean values for each population to examine the phenotypic differentiation of *P. canescens* populations. The relative growth rate during the whole growth period (RGR) was calculated for each population (*SI Methods*). Analysis of traits and initial cutting size suggested that maternal effects were eliminated in our experiment (*SI Methods*).

*Selection vs. stochastic processes*

Two approaches were used, termed "ancestor-descendent comparisons" and "$Q_{st}$ *vs.* $F_{st}$ test" (Keller & Taylor 2008), to partition the evolutionary force of phenotypic differentiation (selection *vs.* stochastic processes).

First, based on the structure of the UPGMA dendrogram, individuals were assigned to demes (demes represent a subset of individuals within or among populations that clustered together in the dendrogram because they share similar multilocus genotypes). After fitting the replicate term as a fixed block effect (*SI Methods*), region and deme were used to execute a factorial ANOVA of the combined data, with the effect of individuals (clonal replicates) nested within the region by deme interaction. The F-ratios of region, deme and their interaction were calculated against the mean square of individuals. In this design, deme effect controlled for the phenotypic divergence caused by shifts in deme frequencies during invasion. Region effect took the phenotypic divergence that persisted after controlling for divergence among demes; thus it explained evolution under region-specific selection. Finally, the region by deme interaction indicated deme-specific evolution. Invasive individuals whose source could not be identified were not included in this analysis.

Second, comparison was made between the distribution of genetic variance at neutral loci ($F_{st}$) and phenotypic traits ($Q_{st}$) among regions and within each region. $F_{st}$ was estimated with a Bayesian approach for dominant markers (Hickory 1.0, Holsinger *et al.* 2002). For each phenotypic trait, a hierarchical linear mixed effect model was fitted by restricted maximum-likelihood approach using R software (O'Hara & Merila 2005) to estimate the components of variance of the nested effects (region, population and individual). Trait means were compared among regions and populations by nested ANOVA. The replicate error (residual variance, $V_r$) used for the calculation of components of variance was reduced by a replicate term (as fixed effect, *SI Methods*). After that, three components of variance were estimated: among-region 1 ($V_{reg}$), among-population (within region) ($V_{pop}$), among-individual (within population) 2 ($V_{ind}$). The proportion of total variance residing among regions ($Q_{ct}$) was calculated by $Q_{ct}=V_{reg}/(V_{reg}+V_{pop}+2V_{ind})$.

For each region, $Q_{st}$ was calculated among populations by $Q_{st}=V_{pop}/(V_{pop}+2V_{ind})$ (Spitze 1993). The confidence intervals of $Q_{st}$ and $F_{st}$ were determined by drawing 1000 bootstraps over individuals or loci, and $Q_{st}$ and $F_{st}$ were compared by $t$ test, with $|Q_{st}–F_{st}|>2sqrt(SE_Q^2+SE_F^2)$ suggesting significant difference at $P=0.05$ level. If the differentiation between regions or populations could be attributed to stochastic processes, divergence of quantitative traits would approximate that of neutral loci, and $Q_{st}$ would be equal to $F_{st}$ (Spitze 1993; Steinger $et$ $al.$ 2002).

Combining hierarchical ANOVA and $Q_{st}/F_{st}$, results of the $Q_{st}$ vs. $F_{st}$ test can be interpreted as follows: (1) significant region or population effect and $Q_{st}>F_{st}$ suggests that selection drives phenotypic divergence, (2) significant region or population effect but $Q_{st}=F_{st}$ indicates phenotypic divergence driven by stochastic processes, (3) nonsignificant region or population effects and $Q_{st}<F_{st}$ means that stabilizing selection maintains similar traits, and (4) non-significant region or population effects and $Q_{st}=F_{st}$ suggests no statistically detectable phenotypic evolution (Keller & Taylor 2008). This method is appropriate for various demographic scenarios, including bottlenecks or admixture.

### Relationships between traits and environmental factors
We analysed relationships between phenotypic traits and five environmental factors (drought and flood frequency, average precipitation and temperature, and human footprint (HFP), a parameter indicating human disturbance level (*SI Methods*), over all populations. To control the influence of evolutionary history and chance genetic effects, a multiple regression analysis was used (Keller & Taylor 2008). Each trait was analysed against two independent variables, including one environmental factor and one principal coordinate based on ISSR loci (*SI Figure 3*); the latter controlled the influence of evolutionary history and chance genetic effects on trait variation. Population mean values of principal coordinates and phenotypic traits were used in this analysis.

### Results

### Genetic diversity and structure
A total of 37 bands, ranging in size from 200 to 2000bp, were reliably scored from the 12 ISSR primers we used. French populations displayed significantly higher genetic diversity than Argentine and Australian populations (Figure 1a). For example, in the French populations, 84% of bands were polymorphic, compared with only 64% in the Argentine and Australian populations. Rarefaction analysis suggested that total band richness was the same among these three regions (*SI Figure 1*), thus a higher 9 population level diversity in France compared with the other regions was attributable 0 to higher gene flow among closely located French populations, rather than sampling bias.

AMOVA results revealed that most genetic variation (73%) was distributed 3 within populations, while inter-regional differences only contributed 6% of the variation (Figure 1a). In invasive populations 10% (France) and 25% (Australia) of the total genetic diversity was distributed among populations, whereas the native populations possessed higher levels of genetic structure (35%, Figure 1a).

The UPGMA dendrogram showed that *P. canescens* individuals from native populations clustered into two groups (Deme-1, UBB + HUR; and Deme-2, RIO + SMM + TAN; Figure 2). All Australian individuals and about half of French individuals were distributed across these two demes. The remaining French individuals formed a distinctive cluster (the "French-Deme" in Figure 2), but its source was not identified. The clustering pattern of invasive individuals appears unrelated to their geographic distribution. Overall, individuals from nine out of 10 invasive populations occurred in both Deme-1 and Deme-2.

### Phenotypic differentiation
Principal Component Analysis (PCA) of traits revealed among-region phenotypic differentiation of *P. canescens* populations. The first two principal components (PC) explained 81% of phenotypic variation among populations, and populations from different regions were well separated (Figure 1b). PC1 is associated with plant size (i.e., biomass, leaf size and morphology, stem diameter), and investment in sexual reproduction; while PC2 is mainly related to the investment in sexual reproduction (inflorescence number and S:V) as well as internode length, and R:S (Figure 1c). In Argentina, the RIO population differs from the other four populations (Figure 1b). When compared with native Argentine populations, Australian populations showed enhanced investment in sexual reproduction and less allocation to below-ground biomass. French populations had greater biomass, larger leaves, and thicker stems (*SI Figure 2*). Within invaded regions, Australian populations displayed significant divergence, while French

populations were phenotypically uniform (Figure 1b). The RGR of these populations ranged between 0.75 to 0.92 g g$^{-1}$biomass day$^{-1}$. However, differences among regions were not significant ($P$=0.42, ANOVA), although there was a marginal population effect ($P$=0.02, ANOVA). Thus, the greater biomass of French individuals was caused by 4 larger initial fragment biomass ($P$<0.001, ANOVA) which was associated with wider leaves and thicker stems, rather than differences in RGR.

### Selection vs. stochastic events

In the "ancestor-descendent comparison" analysis, traits were compared among individuals from Deme-1(Argentina: n=20, Australia: n=20, France: n=10) and Deme-2 (Argentina: n=26, Australia: n=30, France: n=17); individuals in FrenchDeme (n=23) were not used in this analysis because their sources were not identified. Six out of eight henotypic traits showed significant region effect (Figure 3), indicating that inter-regional phenotypic divergence of *P. canescens* was mainly driven by selection ($\lambda$=0.53, $P$<0.0001, MANOVA). Leaf characters (Figure 3c, d) and parameters for sexual reproduction (Figure 3g, h) displayed some region×deme interaction, with Deme-2 showing a weaker shift between Argentina and Australia than Deme-1. Two traits that appeared to vary, R:S and internode length, did not possess a statistically significant region effect (Figure 3b, e), probably because of the large variance associated with these traits. The absent or weakly significant deme effect (Figure 3) suggest there was little difference in traits between demes. Amongregion phenotypic divergence was not attributed to the change of deme frequencies across ranges ($\lambda$=0.92, $P$=0.14, MANOVA), indicating that regional differentiation was not influenced by stochastic events.

In the hierarchical ANOVA and $Q_{st}$ vs $F_{st}$ test, six traits showed significant differences across all three regions and had a $Q_{ct}$ significantly higher than $F_{st}$, indicating selection-driven divergence. Conversely, R:S and internode length displayed marginally or non-significant region effects and had similar $Q_{ct}$ and $F_{st}$ values (Table 1). Within the native region, three traits (R:S, internode length, and S:V) were not similar among populations and had a $Q_{st}$ significantly lower than $F_{st}$, suggesting stabilizing selection. In contrast, four traits (vegetative biomass, leaf size, leaf morphology, and inflorescence number) differed among populations, but $Q_{st}$ vs. $F_{st}$ was not significant indicating divergence driven by stochastic processes (Table 1).For Australian populations, most traits possessed among-population differences and $Q_{st}$ was comparable to $F_{st}$ (Table 1), thus phenotypic divergence seemed to be influenced by stochastic processes. French populations were phenotypically uniform in general and evolution was not detected for most traits (Table 1).

The evolutionary force indicated by the two approaches matched very well. Both methods diagnosed that the phenotypic divergence pattern among regions was consistent with selection-driven evolution, and both indicated that disruptive selection among regions occurred for six traits (biomass, leaf size and morphology, stem diameter, inflorescence number and S:V). The absence of selection-driven differences in the native range, as illustrated by the $Q_{st}$ vs. $F_{st}$ test, also matched the non-3 significant deme effect observed in the ancestor-descendent comparison. In both analyses, the significant region effect, for each trait, mainly occurred between the native and one of the two invaded regions (Fisher's LSD, Figure 3; *SI Figure 2*), also supporting the conclusion that phenotypic differentiation in *P. canescens* is driven by different selection regimes across its native and invaded regions.

### Trait – environmental factor relationships

Because phenotypic divergence of *P. canescens* mainly occurred among regions, we addressed the correlations between traits and environmental factors across all populations. After holding the effect of evolutionary history constant, traits related to plant size (biomass, leaf size, leaf morphology and stem diameter) were positively correlated to flood frequency (Figure 4c-e) and human footprint (HFP, Figure 4i-l), but negatively correlated to drought frequency (Figure 4a-b). In contrast, sexual reproductive traits (inflorescence number, S:V) were positively associated with average temperature (Figure 4g-h), and S:V is also negatively correlated to HFP (Figure 4m). Finally, R:S displayed a negative correlation with precipitation (Figure 4f). Correlations with HFP and average temperature were partially attributable to differences in environmental factors among regions.

## Discussion

### Multiple introductions and their genetic consequence

Our study provides clear evidence of the release of evolutionary potential following the negation of genetic bottleneck in *P. canescens*. An invader's ability to 5 respond to new selective pressure is contingent on sufficient genetic diversity within invasive populations (Sakai *et al.* 2001). Multiple introductions are suggested as a pathway

to negate genetic bottlenecks and have been widely observed in invasive species (Bossdorf *et al.* 2005; Novak & Mack 2005). The mixing of lineages from different sources within invasive populations of *P. canescens* (e.g. individuals from 0 RAY clustered with individuals from HUR, SMM, and RIO, Figure 2) and the unsuccessful identification of the sources of "French-Deme" suggests that multiple introductions might have occurred. This provides the most parsimonious explanation for higher genetic diversity and the decreased genetic structure within and among invasive populations (Figure 1), and may facilitate evolutionary responses to new selection regimes. Genetic analysis of additional populations using co-dominant markers would be required to confirm this finding.

Multiple introductions also facilitate the creation of novel genotypes by genomic recombination of isolated native gene pools (Ellstrand & Schierenbeck 2000; Lockwood *et al.* 2005; Roman & Darling 2007). This effect could lead to phenotypic differentiation, arising from novel interactions among alleles, and might confound our ability to determine whether such differentiation is the result of selection (Keller & Taylor 2008), but this was not the case in our study. If phenotypic shifts in invasive populations are mainly attributed to new genotypes generated by recombination, higher within-population phenotypic variation and genetic diversity would be expected in invaded regions (Ellstrand & Schierenbeck 2000). However, in our study of *P. canescens*, phenotypic variances among invaded and native regions were similar (*SI Figure 2*), and Australian populations displayed similar genetic diversity compared to Argentine populations (Figure 1). The higher average genetic diversity in French populations most likely stems from genetically diverse source populations and high gene flow among populations. In addition, the $Q_{st}$ *vs*. $F_{st}$ test which was not affected by genetic admixture gave the same results as the ancestor-descendent comparison. Thus, all of our results suggest that genetic recombination would not confound our conclusions, i.e. even if genetic recombination occurred within invasive 4 populations of *P. canescens*, these novel genotypes also appear to respond to selection. 5 Because this study used a dominantly-expressed molecular marker (ISSRs), assessing whether genomic recombination occurred could not be specifically tested. Assessment of the role of recombination in the phenotypic evolution of *P. canescens* could provide interesting future research.

### *Selection-driven phenotypic evolution among regions*
Significant inter-regional phenotypic divergence in the reproductive systems of *P. canescens* was observed between native and invasive populations. Such divergent phenotypic shifts could be simply explained by demographic admixture, i.e. the number and frequency of different genotypes/haplotypes (Kolbe *et al.* 2007), or latitudinal clines (Colautti *et al.* 2009) across introduced populations. Thus, it is essential to exclude stochastic forces as the cause of divergence before discussing these phenotypic changes in the context of adaptive evolution. When partitioning evolutionary forces, our results using two independent methods indicated that the molecular and phenotypic variability of *P. canescens* are consistent with the pattern expected with evolution driven by selection, rather than stochastic events. The ancestor-dependent comparison suggested that most traits were significantly influenced by region effect, after controlling for evolutionary history (Figure 3).  Similarly, the $Q_{st}$ *vs.* $F_{st}$ test showed that *P. canescens* populations had significantly higher phenotypic differentiation than neutral marker differentiation among regions (Table 1). These consistent results provide compelling evidence that the inter-regional phenotypic divergence of *P. canescens* was driven by region-specific selective pressure during invasion. While some aspects of our experimental design (e.g. number of clonal replicates and the number of populations within regions) could be strengthened, we feel that these issues do not affect the overall conclusions of our study (*SI Methods*).

We attempted to account for two concerns with the interpretation of our data. First, in the ancestor-descendent comparison, if invasive individuals constituted a biased sample of their source deme, ancestor-descendent comparisons may not be a valid neutral expectation for phenotypic divergence (Keller & Taylor 2008). We checked ISSR diversity for each invasive deme relative to its native counterpart and the indices of invasive demes were similar or slightly higher than that of the native deme (e.g. Br, Deme-1/Deme-2, Argentina: 1.61/1.59, Australia: 1.67/1.63, France: 1.77/1.74, rarefaction of 10 individuals), suggesting that invasive individuals were representatively sampled. Second, in the $Q_{st}$ *vs.* $F_{st}$ test, we might have underestimated disruptive selection among regions. Sampling in the native range did not identify the source of the "French Deme". This will lead to an overestimate of the genetic differentiation among regions. However, this was not responsible for the interregional phenotypic divergences observed in the greenhouse experiment (Figure 1, *SI Figure 2*) because no significant phenotypic differences were found among the "French-Deme" and the French individuals in Deme-1 and Deme-2 (Fisher's LSD, Figure 3). Hence, estimates of phenotypic differentiation among regions are not likely to be affected. Thus, our estimation of $Q_{ct}/F_{st}$ is conservative and we still observed significantly higher $Q_{ct}$ than $F_{st}$.

Phenotypic changes across the native and invaded regions have previously been demonstrated (Maron *et al.* 2004; Lavergne & Molofsky 2007; Dlugosch & Parker 2008b). In some cases, analysis of clines (e.g. along latitude) and transplant experiments showed that the phenotypic evolution could be locally adaptive (Maron *et al.* 2004; Maron *et al.* 2007; van Kleunen & Fischer 2008). In *P. canescens*, because inflorescence number is positively correlated with fecundity under field conditions (Macdonald 2008; Gross *et al*. unpublished data), more investment in flowers in Australian populations indicates evolution towards increased sexual reproduction. In contrast, French populations displayed enhanced capacity for vegetative reproduction (Figure 3), as reflected by larger fragment size (leaf size and morphology, stem diameter). Similar results were found in *Paris quadrifolia* by Jacquemyn *et al.* (2008).

These phenotypic shifts are consistent with predictions that invasive populations may allocate more resources to sexual reproduction or grow more vigorously in their new ranges (e.g. EICA hypothesis, Blossey & Notzold 1995).

Significant correlations between reproductive traits and environmental factors of sampling sites suggest that the genetically-based phenotypic shifts of *P. canescens* are adaptive. The positive correlations of plant-size-related traits to flood frequency and HFP suggest that enhanced vegetative reproduction and growth may benefit from disturbance that stimulates fragmentation, because larger fragments may have higher survival rates during dispersal and establishment (Jacquemyn *et al.* 2008). In apparent agreement, a negative S:V-HFP correlation probably indicates decreased allocation to flowering. Sexual reproduction was positively associated with temperature, which agrees with previous observations that introduced plants in lower latitudes flowered earlier and displayed higher fecundity (O'Neil 1999; Kollmann & Banuelos 2004). This relationship suggests that increased fecundity in *P. canescens* might be an adaption to the novel climate conditions in Australia, and these new traits might facilitate its expansion into lower latitudes. Although R:S did not differentiate among regions, the negative correlation with precipitation matches with the theory that plants allocate more resources to root biomass in dry environments (Bloom *et al.* 1985). However, given that correlations between traits and some environmental factors (temperature and HFP) were partially attributable to the environmental differences among regions, care should be taken when interpreting these correlations. In future studies, reciprocal transplant experiments and demographic models could be combined to address the fitness consequences, e.g. survival, growth, and fecundity, of these genetically-based phenotypic shifts under local and regional conditions.

### Evolutionary forces within the native and invaded region

Our study showed that the pattern of phenotypic differentiation among populations of *P. canescens* within regions was mainly driven by stabilizing selection and chance events. Although Argentine populations were sampled from sites over a 9 distance of 850 km and displayed significant genetic structure, the insignificant deme effect and the similar or lower values of $Q_{st}$ *vs.* $F_{st}$ for all traits did not provide support for disruptive selection. Three traits were similar due to stabilizing selection, and the differentiation of another four traits appeared to be influenced by chance events. These observations indicate that local adaptation was limited among native populations. Differentiation for six phenotypic traits among Australian populations also seemed driven by stochastic processes. Similar pattern has been recently 6 observed in purple loosestrife, within whose native (Europe) and introduced (North America) range stabilizing selection was found in some traits, but no evidence of disruptive selection was revealed (Chun *et al* 2009).

In our study, the lack of detectable local adaption may be associated with the dispersal and recruitment characteristics of *P. canescens*: a floodplain species. Recruitment of *P. canescens* (either by seeds and/ or vegetative fragments) occurs almost exclusively on floodplains below the flood line (Macdonald 2008); thus the habitats that populations initially colonize are relatively homogenous, and this may lead to stabilizing selection. Dispersal of *P. canescens* is dependent on floods; and in its in invaded range, dispersal is also facilitated by the ornamental plant industry. These processes can cause founder effects (Van Looy *et al.* 2009), but are less likely to lead to selection-driven local adaptations. However, after introduction to other regions, invasive populations experienced environmental conditions that differed from those of the native range. Thus, region-specific selection would be expected to be stronger than disruptive selection within regions, and prompt inter-regional differentiation. Our results suggest that evolutionary forces shaping the genetic structure of native populations could be broken during invasion; even when populations do not exhibit adaptive phenotypic differentiation in their native range, they may still possess the potential for adaptive evolution when invading new regions. However, given the conservative estimate of $Q_{st}$ in our study (see above and *SI Methods*), our results may not be sensitive enough to detect within-region local adaptation in some traits. Additional studies based on more populations from each region are required to verify the pattern of within-region phenotypic differentiation 9 reported here.

*Artificial versus natural selection in the invasion process*

The method we applied to partition selection and stochastic processes could not reveal the stage of the invasion during which selection occurred (Keller & Taylor 2008). There are two reasons to consider that phenotypic divergence in *P. canescens* is mainly driven by natural selection. First, although *P. canescens* was widely introduced as an ornamental plant, herbarium records suggest that the introduction of *P. canescens* was *ad hoc*, and not systematic. Also, we did not find records of breeding programs, as often occurs for many deliberately introduced grasses or legumes (Cook & Dias 2006). Second, artificial selection could not explain significant correlations between phenotypic traits and local environmental factors, which were consistent with the expectation of inter-regional phenotypic differentiation. Phenotypic shifts due to natural selection may have enhanced the invasiveness of *P. canescens* in both France and Australia, and can affect our estimate of its invasive potential and impact.

## Acknowledgements

# References

Armstrong T.T.J. & De Lange P.J. (2005). Conservation genetics of *Hebe speciosa* (Plantaginaceae) an endangered New Zealand shrub. *Bot. J. Linnean Soc.*, 149, 229-239.

Barrett S.C.H., Colautti R.I. & Eckert C.G. (2008). Plant reproductive systems and evolution during biological invasion. *Mol. Ecol.*, 17, 373-383.

Blair A.C. & Wolfe L.M. (2004). The evolution of an invasive plant: An experimental study with *Silene latifolia*. *Ecology*, 85, 3035-3042.

Bloom A.J., Chapin F.S. & Mooney H.A. (1985). Resource limitation in plants – an economic analogy. *Annu. Rev. Ecol. Syst.*, 16, 363-392.

Bossdorf O., Auge H., Lafuma L., Rogers W.E., Siemann E. & Prati D. (2005). Phenotypic and genetic differentiation between native and introduced plant populations. *Oecologia*, 144, 1-11.

Brown J.S. & Eckert C.G. (2005). Evolutionary increase in sexual and clonal reproductive capacity during biological invasion in an aquatic plant *Butomus umbellatus* (Butomaceae). *Am. J. Bot.*, 92, 495-502.

Buckley Y.M., Downey P., Fowler S.V., Hill R., Memmot J., Norambuena H., Pitcairn M., Shaw R., Sheppard A.W., Winks C., Wittenberg R. & Rees M. (2003). Are invasives bigger? A global study of seed size variation in two invasive shrubs. *Ecology*, 84, 1434-1440.

Cano L., Escarre J., Fleck I., Blanco-Moreno J.M. & Sans F.X. (2008). Increased fitness and plasticity of an invasive species in its introduced range: a study using *Senecio pterophorus*. *J. Ecol.*, 96, 468-476.

Chen Y.H., Opp S.B., Berlocher S.H. & Roderick G.K. (2006). Are bottlenecks associated with colonization? Genetic diversity and diapause variation of native and introduced *Rhagoletis completa* populations. *Oecologia*, 149, 656- 667.

Chun Y.J., Nason J.D. & Moloney K.A. (2009). Comparison of quantitative and molecular genetic variation of native vs. invasive populations of purple loosestrife (*Lythrum salicaria* L., Lythraceae). *Mol. Ecol.*, 18, 3020-3035.

Colautti R.I., Maron J.L. & Barrett S.C.H. (2009). Common garden comparisons of native and introduced plant populations: latitudinal clines can obscure evolutionary inferences. *Evol. Appl.*, 2, 187-199.

Cox G.W. (2004). *Alien species and evolution: The evolutionary ecology of exotic plants, animals, microbes, and interacting native species*. Island Press, Washington DC.

Dlugosch K.M. & Parker I.M. (2008a). Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Mol. Ecol.*, 17, 431-449.

Dlugosch K.M. & Parker I.M. (2008b). Invading populations of an ornamental shrub show rapid life history evolution despite genetic bottlenecks. *Ecol. Lett.*, 11, 701-709.

Eckert C.G., Lui K., Bronson K., Corradini P. & Bruneau A. (2003). Population genetic consequences of extreme variation in sexual and clonal reproduction in an aquatic plant. *Mol. Ecol.*, 12, 331-344.

Ellstrand N.C. & Schierenbeck K.A. (2000). Hybridization as a stimulus for the evolution of invasiveness in plants? *Proc. Natl Acad. Sci. U. S. A.*, 97, 7043- 7050.

Facon B., Pointier J.P., Jarne P., Sarda V. & David P. (2008). High genetic variance in life-history strategies within invasive populations by way of multiple introductions. *Curr. Biol.*, 18, 363-367.

Guesewell S., Jakobs G. & Weber E. (2006). Native and introduced populations of *Solidago gigantea* differ in shoot production but not in leaf traits or litter decomposition. *Funct. Ecol.*, 20, 575-584.

Holsinger K.E., Lewis P.O. & Dey D.K. (2002). A Bayesian approach to inferring population structure from dominant markers. *Mol. Ecol.*, 11, 1157-1164.

Jacquemyn H., Brys R. & Hutchings M.J. (2008). Biological flora of the British Isles: *Paris quadrifolia* L. *J. Ecol.*, 96, 833-844.

Keller S.R. & Taylor D.R. (2008). History, chance and adaptation during biological invasion: separating stochastic phenotypic evolution from response to selection. *Ecol. Lett.*, 11, 852-866.

Kliber A. & Eckert C.G. (2005). Interaction between founder effect and selection during biological invasion in an aquatic plant. *Evolution*, 59, 1900-1913.

Kolbe J.J., Glor R.E., Schettino L.R., Lara A.C., Larson A. & Losos J.B. (2007). Multiple sources, admixture, and genetic variation in introduced *Anolis* lizard populations. *Conserv. Biol.*, 21, 1612-1625.

Kollmann J. & Banuelos M.J. (2004). Latitudinal trends in growth and phenology of the invasive alien plant *Impatiens glandulifera* (Balsaminaceae). *Divers. Distrib.*, 10, 377-385.

Lavergne S. & Molofsky J. (2007). Increased genetic variation and evolutionary potential drive the success of an invasive grass. *Proc. Natl Acad. Sci. U. S. A.*, 104, 3883-3888.

Leger E.A. & Rice K.J. (2003). Invasive California poppies (*Eschscholzia californica* Cham.) grow larger than native individuals under reduced competition. *Ecol. Lett.*, 6, 257-264.

Lockwood J.L., Cassey P. & Blackburn T. (2005). The role of propagule pressure in explaining species invasions. *Trends Ecol. Evol.*, 20, 223-228.

Lucy M., Powell E., McCosker R., Inglis G. & Richardson R. (1995). Lippia (*Phyla canescens*): a review of its economic and environmental impact on floodplain ecosystems in the Murray-Darling Basin. In. Queensland Department of Primary Industries Pittsworth Qld, p. 40.

Macdonald M.J. (2008). Ecology of *Phyla canescens* (Verbenaceae) in Australia. In: *Botany*. University of New England Armidale, p. 195. 0

Maron J.L., Elmendorf S.C. & Vila M. (2007). Contrasting plant physiological adaptation to climate in the native and introduced range of *Hypericum perforatum*. *Evolution*, 61, 1912-1924.

Maron J.L., Vila M., Bommarco R., Elmendorf S. & Beardsley P. (2004). Rapid evolution of an invasive plant. *Ecol. Monogr.*, 74, 261-280.

McCosker R.O. (1994). Lippia (*Phyla nodiflora*) an invasive plant of floodplain ecosystems in the Murray-Darling Basin. In: *A report on the distribution and ecology of lippia in the lower Gwydir Valley and the Murray-Darling Basin prepared for the Gingham Watercourse Landcare Group*. Department of Ecosystem Management, University of New England Armidale, p. 17.

Meyer G., Clare R. & Weber E. (2005). An experimental test of the evolution of increased competitive ability hypothesis in goldenrod, *Solidago gigantea*. *Oecologia*, 144, 299-307.

Novak S.J. & Mack R.N. (2005). Genetic bottlenecks in alien plant species: influence of mating systems and introduction dynamics. In: *Species invasions: Insights into ecology, evolution and biogeography* (eds. Sax DF, Stachowicz JJ & Gaines SD). Sinauer Associates, Inc. Sunderland, MA, pp. 201-228.

O'Hara R.B. & Merila J. (2005). Bias and precision in $Q$ST estimates: Problems and 8 some solutions. *Genetics*, 171, 1331-1339.

O'Neil P. (1999). Selection on flowering time: An adaptive fitness surface for nonexistent character combinations. *Ecology*, 80, 806-820.

Phillips B.L., Brown G.P., Webb J.K. & Shine R. (2006). Invasion and the evolution of speed in toads. *Nature*, 439, 803-803.

Rogers W.E. & Siemann E. (2004). Invasive ecotypes tolerate herbivory more effectively than native ecotypes of the Chinese tallow tree *Sapium sebiferum*. *J. Appl. Ecol.*, 41, 561-570.

Roman J. & Darling J.A. (2007). Paradox lost: genetic diversity and the success of aquatic invasions. *Trends Ecol. Evol.*, 22, 454-464.

Sakai A.K., Allendorf F.W., Holt J.S., Lodge D.M., Molofsky J., With K.A., Baughman S., Cabin R.J., Cohen J.E., Ellstrand N.C., McCauley D.E., O'Neil P., Parker I.M., Thompson J.N. & Weller S.G. (2001). The population biology of invasive species. *Annu. Rev. Ecol. Syst.*, 32, 305-332.

Sax D.F., Stachowicz J.J., Brown J.H., Bruno J.F., Dawson M.N., Gaines S.D., Grosberg R.K., Hasting S.A., Holt R.D., Mayfield M.M., O'Connor M.I. & Rice W.R. (2007). Ecological and evolutionary insights from species invasions. *Trends Ecol. Evol.*, 22, 465-471.

Siemann E. & Rogers W.E. (2001). Genetic differences in growth of an invasive tree species. *Ecol. Lett.*, 4, 514-518.

Spitze K. (1993). Population-structure in *Daphnia obtusa* - quantitative genetic and allozymic variation. *Genetics*, 135, 367-374.

Steinger T., Haldimann P., Leiss K.A. & Muller-Scharer H. (2002). Does natural selection promote population divergence? A comparative analysis of population structure using amplified fragment length polymorphism markers and quantitative traits. *Mol. Ecol.*, 11, 2583-2590.

Suarez A.V. & Tsutsui N.D. (2008). The evolutionary consequences of biological invasions. *Mol. Ecol.*, 17, 351-360.

van Kleunen M. & Fischer M. (2008). Adaptive rather than non-adaptive evolution of *Mimulus guttatus* in its invasive range. *Basic Appl. Ecol.*, 9, 213-223.

Van Looy K., Jacquemyn H., Breyne P. & Honnay O. (2009). Effects of flood events on the genetic structure of riparian populations of the grassland plant *Origanum vulgare*. *Biol. Conserv.*, 142, 870-878.

Voisin M., Engel C.R. & Viard F. (2005). Differential shuffling of native genetic diversity across introduced regions in a brown alga: Aquaculture vs. maritime traffic effects. *Proc. Natl Acad. Sci. U. S. A.*, 102, 5432-5437.

Wolfe L.M., Elzinga J.A. & Biere A. (2004). Increased susceptibility to enemies following introduction in the invasive plant *Silene latifolia*. *Ecol. Lett.*, 7, 813-820.

**Table 1.** Comparison of $Q_{st}$ (or $Q_{ct}$) and $F_{st}$ among and within the native (Argentina) and invaded (Australia and France) regions. Values are means (95% confidential interval), and $Q_{st}$ (or $Q_{ct}$) values in bold font indicate significant higher (+) or lower (-) than $F_{st}$ at $P = 0.05$ level. Effects of region and population are shown for each trait (hierarchical ANOVA [*]$P<0.05$, [**]$P<0.01$, [***]$P<0.001$) and the comparison (Fisher's LSD) among regions are shown. Interpretations for the forces promoting the evolution of traits are given.

| | $F_{st}$ | $Q_{ct} / Q_{st}$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Biomass | R : S | Leaf size | Leaf morphology | Internode length | Stem diameter | Inflorescence No. | S : V |
| **Among regions** | 0.082 (0.052-0.121) | **0.535** (+) (0.429-0.626) | 0.049 (0-0.123) | **0.266** (+) (0.156-0.387) | **0.463** (+) (0.340-0.601) | 0.027 (0-0.105) | **0.254** (+) (0.139-0.357) | **0.228** (+) (0.135-0.335) | **0.261** (+) (0.173-0.355) |
| ANOVA | | Au=Ar<F*** | Au>Ar=F* | Au=Ar<F** | Au=Ar<F*** | ns | Au=Ar<F*** | Au>Ar=F** | Au>Ar=F** |
| Evolutionary force | | Selective divergence | Stochastic divergence | Selective divergence | Selective divergence | Evolution not detected | Selective divergence | Selective divergence | Selective divergence |
| **Within regions** | | | | | | | | | |
| Argentina | 0.314 (0.232-0.403) | 0.276 (0.054-0.566) | **0.036** (-) (0-0.209) | 0.181 (0-0.49) | 0.244 (0-0.55) | **0.036** (-) (0-0.236) | 0.099 (0-0.382) | 0.216 (0-0.533) | **0.049** (-) (0-0.306) |
| ANOVA | | *** | ns | *** | *** | ns | ns | *** | ns |
| Evolutionary force | | Stochastic divergence | Stabilizing selection | Stochastic divergence | Stochastic divergence | Stabilizing selection | Evolution not detected | Stochastic divergence | Stabilizing selection |
| Australia | 0.234 (0.165-0.311) | 0.353 (0.162-0.624) | 0.343 (0-1.0) | 0.354 (0.09-0.735) | 0.287 (0.060-0.604) | 0.391 (0.173-0.683) | **0.034** (-) (0-0.231) | 0.190 (0.012-0.427) | 0.371 (0.149-0.770) |
| ANOVA | | *** | ns | *** | *** | *** | ns | *** | ** |
| Evolutionary force | | Stochastic divergence | Evolution not detected | Stochastic divergence | Stochastic divergence | Stochastic divergence | Stabilizing selection | Stochastic divergence | Stochastic divergence |
| France | 0.091 (0.051-0.139) | 0.033 (0-0.201) | 0.025 (0-0.219) | 0.083 (0-0.845) | 0.039 (0-0.224) | 0.024 (0-0.160) | 0.104 (0-0.280) | 0.007 (0-0.072) | **0.004** (-) (0-0.062) |
| ANOVA | | ns | ns | ns | ns | ns | ** | ns | ns |
| Evolutionary force | | Evolution not detected | Evolution not detected | Evolution not detected | Evolution not detected | Evolution not detected | Stochastic divergence | Evolution not detected | Stabilizing selection |

**Figure Legends**

**Figure 1.** Site location, genetic diversity and structure (a), phenotypic divergence (b, c) of *P. canescens* populations from the native (Argentina) and invaded (Australia and France) regions. The distribution of *P. canescens* in Australia and the source range of invasive populations (Argentina) are shown in grey shading. Values of diversity indices[*] shown are mean (SE) and values marked with different letters are significantly different at *P*=0.05 level (Fisher's LSD) among regions. Pie charts show the genetic structure among regions, among populations within regions, and within populations (AMOVA). Phenotypic divergence among populations, as illustrated by Principal Component Analysis (PCA), is shown in b and c. Principal components 3 diagrams with populations (b) and loading values of traits (c) are projected on the factor-plane of the first and the second principal component.

[*] Br, brand richness; $H_j$, Nei's gene diversity; %P, percentage of polymorphic bands

**Figure 2.** ISSR based UPGMA dendrogram of all *P. canescens* individuals, showing division of demes. The font shows the region of origin (central Argentina: bold, southeastern Australia: highlighted, southern France: grey). The distance is calculated based on squared Euclidean distance method and the average linkage is used between groups. Deme represents a subset of individuals within or among populations that clustered together in the dendrogram because they share similar multilocus genotypes; bootstrap support greater than 50% are shown. Ourgroup are individuals from two northern Argentina populations that are genetically distinct from the fifteen populations used in this study.

**Figure 3.** Trait shifts of *P. canescens* demes across the native (Argentina) and invaded (Australia and France) regions. Solid circles, open circles and solid triangles respectively represent Deme-1, Deme-2 and French-Deme. Values are mean ± SE. Effects of region, deme, and region by deme interactions, on traits are shown (ANOVA, [*]$P<0.05$, [**]$P<0.01$, [***]$P<0.001$).

**Figure 4.** Relationships between phenotypic traits and environmental factors (only significant correlations are shown). Residuals of a simple linear regression on traits against principle coordinates of ISSR loci, which represents trait values after adjustment of the genetic effect, are plotted against environmental factors. Values for partial correlation coefficients for environmental factors in multiple regression (r) and the significance level ([*]$P<0.05$, [**]$P<0.01$, [***]$P<0.001$) are shown.
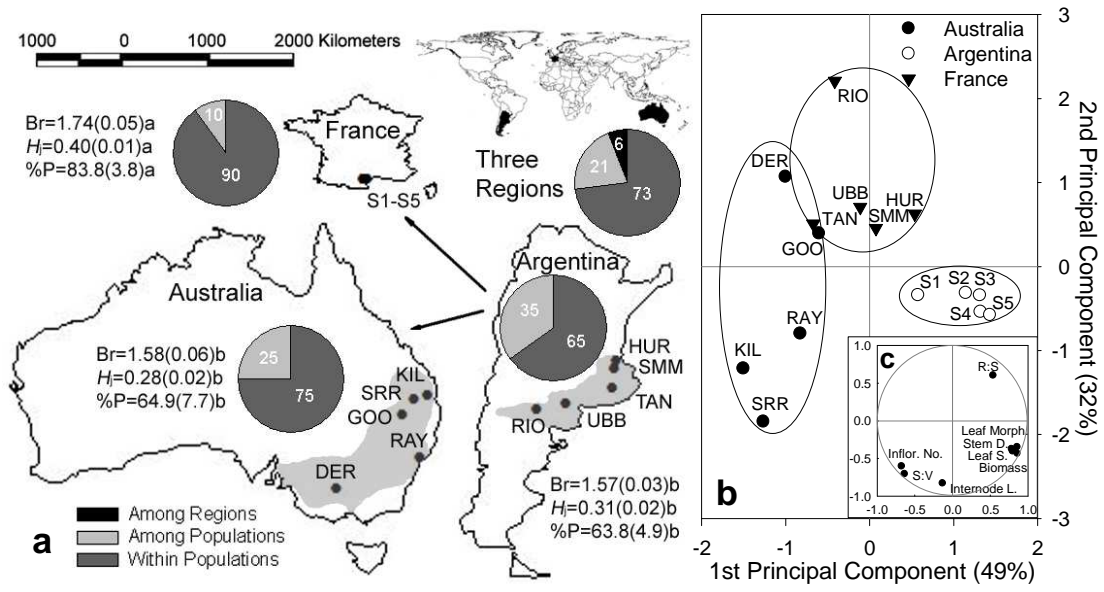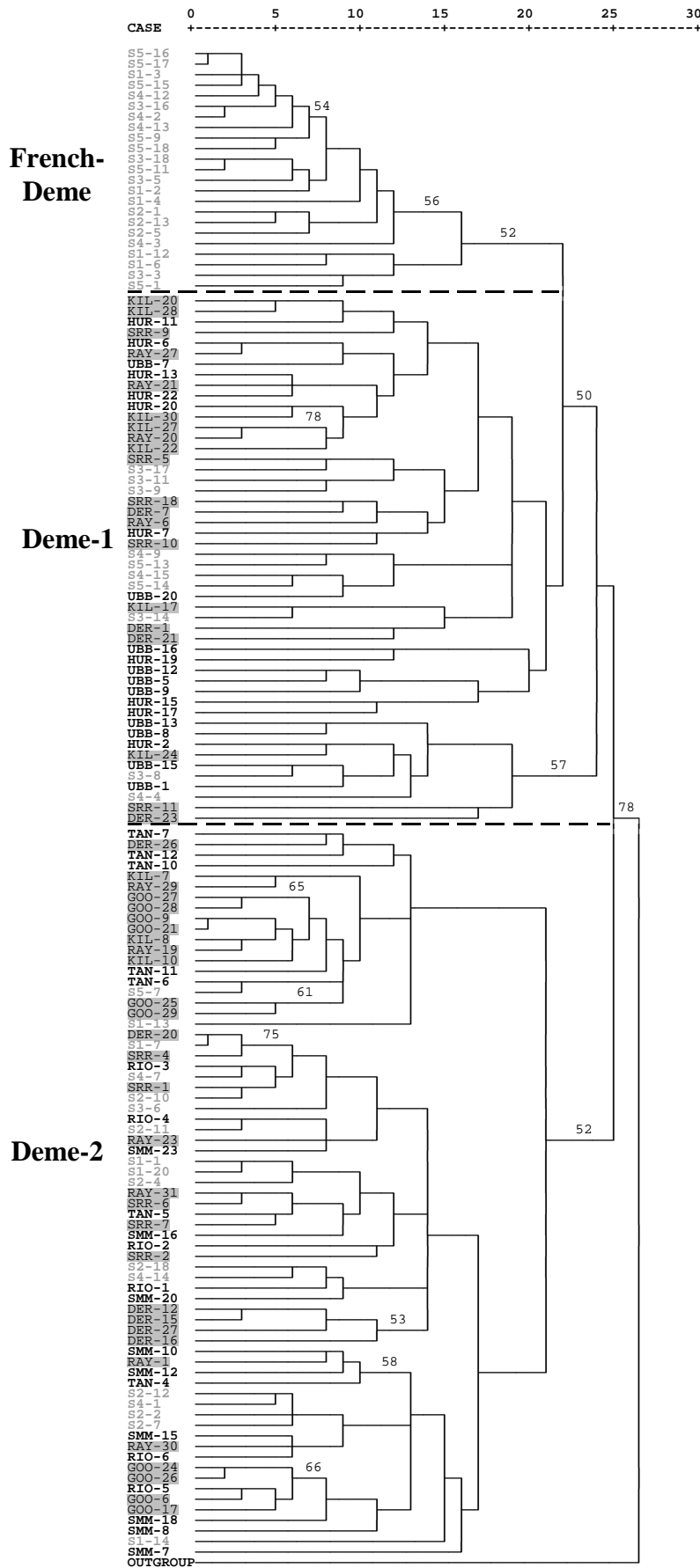
Figure 1.

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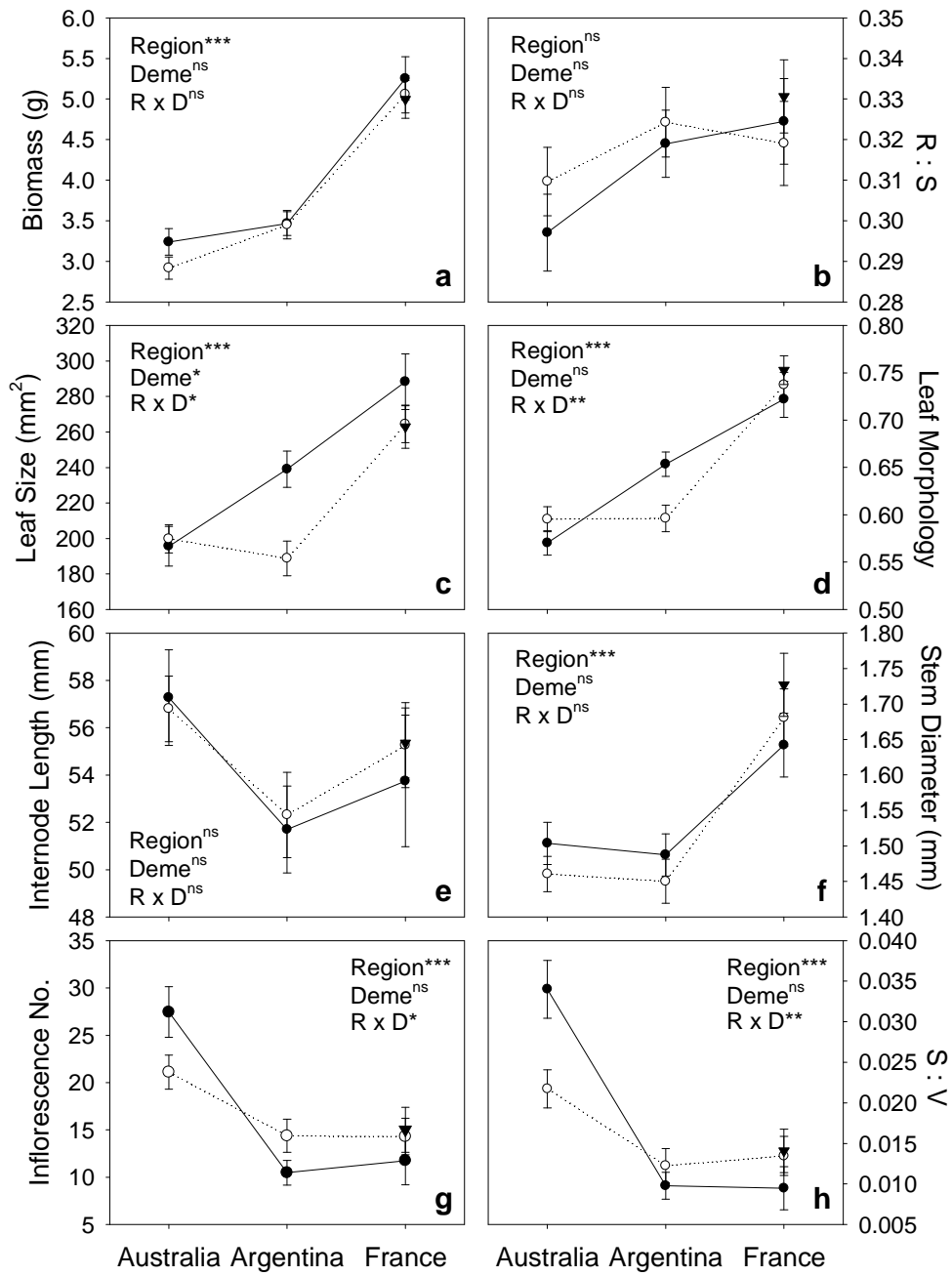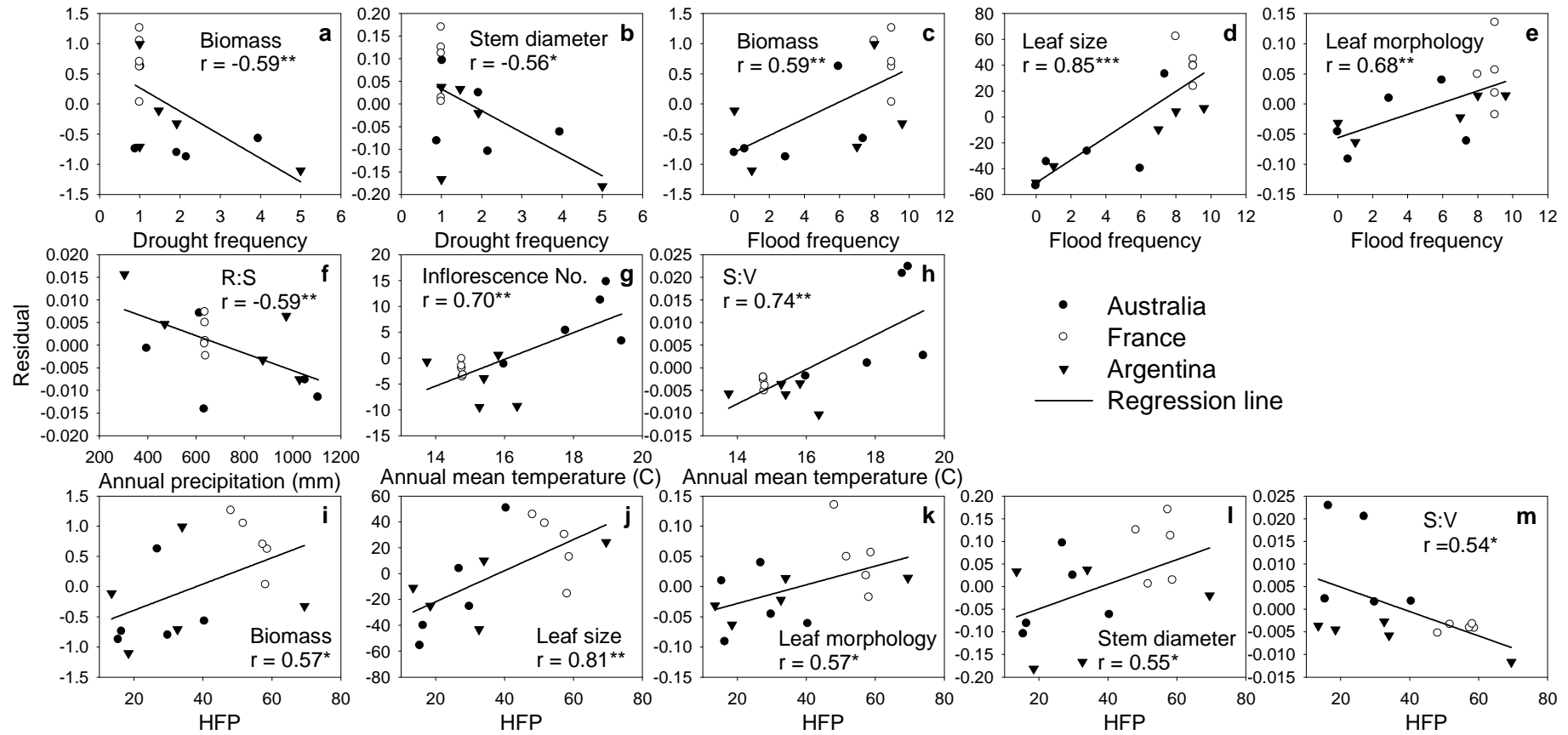
Figure 4.

## Supplemental Information

## Materials

### Invasion history of Phyla canescens in Australia and France

*P. canescens* was introduced into Australia by the 1930s as an ornamental and lawn plant (Lucy *et al.* 1995). Currently, *P. canescens* has invaded grazing lands throughout the Murray-Darling Basin, affecting 5.3 million hectares of land with a 38 million Australian dollars loss in cattle production alone (Earl 2003). Other negative environmental effects include drawing moisture from deep in the soil profile (Lucy *et al.* 1995; Xu unpublished data), and increasing the amount of erosion and soil slumping along river banks (Lucy *et al.* 1995; Earl 2003). Consequently, the total annual environmental cost of *P. canescens* has been estimated at 1.8 billion Australian dollars (Earl 2003).

In France, *P. canescens* was first reported to be cultivated in Paris in 1826, and was recorded in southern France (Hyères, Var Department) in 1870. Now, *P. canescens* mainly occurs in the Mediterranean region, where it has been listed as an invasive plant species of concern (Agence Méditerranéenne de l'Environnement *et al.* 2003). It has become naturalized widely along the lower Aude River floodplain in Hérault and Aude provinces, especially near the towns of Capestang, Nissan, Fleury, and Vendres. In this region, 66% of 1000 ha of local pasture were infested with ground-cover up to 100% (Conservatoire Régional des Espaces Naturels Languedoc-Roussillon 2006). It has also invaded drains, banks of waterways, headlands and roadsides, and threatens some protected plants, e.g., *Bellevalia romana*, *Plantago cornuti*, (Olivier *et al.* 1995) in critical conservation habitats such as the Zones Naturelles d'Intérêt Ecologique Faunistique et Floristique - ZNIEFF.

## Methods

### Sampling strategy

The literature indicates that the geographic distribution of *Phyla canescens* is centred in central to northern Argentina and extends into neighbouring countries (southern Bolivia, southern Paraguay, Uruguay, and the Rio Grande do Sul Province of Brazil); some populations were also recorded in Ecuador, Peru, and Chile. During field surveys based on herbarium records, we widely sampled populations of *P. canescens* through Argentina, Bolivia, Peru and Chile. Subsequent taxonomic identification (Sosa, Julien, and Traversa, personal observation) and molecular analysis (Fatemi and Gross, unpublished data) suggested that the source of the invasive populations analysed in our study was limited to central Argentina; including Buenos Aires Province (where it is widespread), northern Rio Negro Province, and eastern La Pampa Province. In Rio Negro and La Pampa, the plant is found within the few floodplains that occur in these two relatively dry provinces. Given that the match of the source and descendent is essential for partitioning selection vs. stochastic processes, only the most likely native source populations were used in our study. These samples were collected along a northeast-southwest geographic transect over 850 km in central Argentina, providing good geographical coverage of the species' native range. We tested how representative our estimates of genetic diversity were using the rarefaction approach (AFLPDIV, Coart *et al.* 2005). The results showed that 6-10 individual per population would provide good estimate of population-level

genetic diversity, and about 20 individuals would be sufficient to estimate the majority of the regional-level genetic diversity (Figure 1).

Because *P. canescens* is not autogamous and showed limited selfing-compatibility relative to outcrossing, we anticipated that the species may mainly generate seeds through outcrossing and that the genetic diversity of the species would mainly be distributed within populations (i.e., genetic structuring of populations would be relatively weak). Thus, it is reasonable to use relatively few populations, but more individuals within each population in this study. In the field, individuals were collected at least 5 meters apart. Genetic analyses confirmed that this distance was sufficient to minimize duplicate sampling of clones as most samples possessed different genotypes (Fatemi and Gross, unpublished data).

To match the spatial scale of populations in Australia and Argentina, we surveyed localities in France where *P. canescens* had been previously recorded based on herbaria records.  Our search extended from the Spanish border to Toulon, east of Marseille (and included Corsica). Despite this effort, invasive populations were only found on the lower Aude River floodplain. Other previously reported localities appear to have been disturbed through urban development or consisted of cultivated ornamental plants that have not become naturalised. Therefore, all French specimens were collected in the lower Aude river valley from five adjacent populations, which were located 5 to 12 km apart.

### ISSR procedure
Total genomic DNA of each individual was extracted from silica-gel-dried leaves using DNeasy Plant Minikit (QIAGE Inc., Hilden, Germany) or the CTAB method. One hundred inter-simple sequence repeat (ISSR) primers with 15-23 base pairs in length (UBC primer set no.9, Biotechnology Laboratory, University of British Columbia, Vancouver, Canada) were screened using a subset of ten samples to select primers producing highly resolved and consistently expressed bands. In our analysis, 12 primers were used for PCR amplification. Each 25 µL amplification reaction contained 16 mM $(NH_4)_2SO_4$, 67 mM Tris-HCl (pH 8.8), 0.01% Tween-20, 4 mM $MgCl_2$, 0.4 mM of each dNTP, 0.5 unit *Taq* Polymerase, and 20 ng genomic DNA. Amplifications were performed in a PC-960C thermal cycler (Corbett Research, Sydney, Australia) under the following conditions: template denaturation at 94 °C for 5 min; followed by 30 cycles of denaturation at 94 °C for 30 s, primer annealing at 49 °C for 30 s (adjusted for each primer), and primer extension at 72 °C for 1 min; followed by a final extension step at 72 °C for 10 min. PCR products were separated on 2% agarose gel buffered with TBE containing SYBR Safe DNA gel stain (Invitrogen, Osaka, Japan) and visualised for photography under a UV illuminator.

### Measurement of phenotypic traits
During harvest, plants were divided into below ground, above ground, and reproductive tissues; then dried at 60°C for at least 48 hours until all tissues maintained constant weight, at which time plant parts were weighed separately. Total biomass, ratio of below ground to above ground vegetative biomass (R:S), and ratio of reproductive to vegetative biomass (S:V) were calculated. The number of inflorescences was counted. The length and width of one leaf on the third node of the longest stem was measured. Leaf size and leaf morphology were calculated as width × length and width to length ratio, respectively. The internode length and stem diameter

of the internode below the third node of the longest stem were measured. Relative growth rates (RGR = [ln(harvested biomass) - ln(starting biomass)]/growth period) were estimated for each population by calculating the slope of a linear regression fitting ln(biomass) to growth time for all individuals of that population. This parameter was used to explicitly assess the influence of initial cutting weight on the biomass.

### *Statistical analysis*

We hypothesized that the initial cutting size would affect the expression of plant traits; hence we intended to use it in a covariate analysis. The effect of cutting stem length was not significant in ANCOVA. Initial cutting weight showed a significant region effect, which we attributed to among-region genetic differences, especially due to leaf size and stem diameter (see below). In this case, we can't distinguish whether the effect of the covariate was attributed to initial cutting weight or genetic differences among regions. Thus ANCOVA, which mathematically requires a covariate to be independent of the factors being analyzed, was not used in our study.

Because our experiment included two sequential replicates, trait variations caused by differences in the growth conditions of these two replicates needed to be adjusted before any other factors were assessed. The effects of replicate and its interactions were therefore fitted in factorial and hierarchical ANOVA models used in this study before region, deme, and population effects were determined. The replicate effect accounted for differences in growth conditions of the two replicates. Although significant replicate effects were observed in some traits (total biomass, R:S, inflorescence number, and S:V), these effects appeared to be simply related to differences in the time-course of the two greenhouse experiments (seven weeks for the first experiment and eight weeks for the second experiment). Effects of interactions with the replicate term (e.g. deme × replicate) were mostly non-significant. In the few cases in which significant interactions were observed, significance levels of all main factors remained consistent, regardless of whether interactions were included in the ANOVA design, or not. These assessments suggested that it was reasonable to treat the replicate term as a fixed block effect without considering interactions and after this adjustment, the two replicates of the same individual could then be treated as sub-clonal replicates.

### *Maternal effect on initial cutting weight*

Because the inter-regional difference of some traits (e.g. biomass) appear attributable to differences in initial cutting weight, it was necessary to examine whether the inter-regional difference of the initial cutting weight was genetically based or caused by maternal effects. We did the following to eliminate maternal (environmental) effects, and to test for any possible residual maternal effects that might be present in our experiment:

(1) All plants were initially grown in a common greenhouse environment for at least 2 months before experimentation and only greenhouse-grown plant tissues were used for cuttings.

(2) Maternal effects in our study were likely to reflect differences in growth conditions among regions and it was reasonable to expect that they would decline with time after plants were grown in a common environment. The two replicates in our experiment used materials generated from two successive, vegetatively propagated, generations. Thus, if inter-regional differences for initial cutting weight

was attributable to maternal effects, initial cutting weight would display a region by replicate interaction. We conducted a region by replicate factorial ANOVA on the initial cutting weight, but the interaction was not significant (P = 0.21).

(3) Later, we examined the size of cuttings, collected using similar methods, from these populations after one year of growth in the same common greenhouse and observed the same regional effect (ANOVA, P=0.01, Fisher's LSD, France: 0.050[a], Australia: 0.046[ab], Argentina:0.041[b]).

Taken together, these results support our conclusion that the influence of maternal effects was limited (if not entirely eliminated) in our experiment and the observed regional effect on initial cutting weight was mainly genetically based.

### Technical issues on the $Q_{st}$ vs $F_{st}$ test

$Q_{st}$ was formally used to partition additive genetic variance which requires a complex design to isolate. However, recent reviews suggested that including some non-additive genetic effects (e.g. dominance variance) may not significantly affect the $Q_{st}$ *vs*. $F_{st}$ test (Goudet & Buchi 2006; Leinonen *et al.* 2008). Furthermore, given that we had only two clonal replicates, the within- and among-individual variation were likely to be overestimated, so our estimation of $Q_{ct}$ (or $Q_{st}$) tended to be conservative. Because the null hypothesis of no phenotypic differentiation is associated with $Q_{ct}=F_{st}$ among regions, the detection of any differences among regions probably represents an underestimate of differentiation; this problem was not likely to affect the conclusions of our study. Finally, the precision of $Q_{st}$ estimates using current statistical approaches has been a concern. Although previous simulation studies showed that the precision of $Q_{st}$ could be low when making comparisons among a small number (< 20) of populations (O'Hara & Merila 2005), a more recent review of empirical data from 2– 31 populations suggested that the effect of population number on the precision of $Q_{st}$ was very low (Leinonen *et al.* 2008). In our study, we were mainly concerned with inter-regional comparisons, and the region number was more important in influencing the precision of $Q_{ct}$. The influence of region number on the hierarchical $Q_{st}$ *vs*. $F_{st}$ test is not clear and has never been tested. However, our study included 3 regions, more than most other native vs. invasive comparative studies. These problems can only be improved with more population and region sampling, new approaches, and statistical innovations (Leinonen *et al.* 2008).

### Source of environmental factors data

Geographic distribution data of drought frequency, flood frequency, annual average temperature and precipitation, and HFP were obtained from the Center for International Earth Science Information Network (CIESIN) at Columbia University (Wildlife Conservation and Center for International Earth Science Information Network 2005; Dilley *et al.* 2005) and Worldclim (Hijmans *et al.* 2005). Drought and flood frequency represent the occurrence rate of extreme climate events; average temperature and precipitation reflects the general climate conditions, and HFP indicates the level of anthropogenic influence on natural systems. Mean values of these environmental parameters for all grids within the area of a circle centring the sample site, with a radius of 5 km for French sites and 25 km for Argentine and Australian sites, were calculated. The relationship between phenotypic traits and environmental factors at the sample site were explored by multiple regression analysis. Other factors were also assessed but not presented because they either do not directly reflect environmental factors, e.g., latitude, do not have sufficient variance for

regression analysis (altitude), or are highly inter-correlated (GDP and population density).

Table 1. Collection sites of native (Argentina) and invasive (Australia and France) *Phyla canescens* populations

| Country | Code | Site | Longitude | Latitude | Habitat | Individuals |
|---|---|---|---|---|---|---|
| Argentina | UBB | Universidad Nacional del Sur, Bahia Blanca, southern Buenos Aires Province. | 63º 16' W | 38º 42' S | Pampas | 10 |
| | HUR | Hurlingham SABCL, northern suburbs of Buenos Aires City | 58º 37' W | 34º 35' S | Disturbed urban area | 10 |
| | RIO | Chimpay, Rio Negro Province | 66º 08' W | 39º 10' S | Transitional area between southern Chaco, Pampas and Patagonia regions | 6 |
| | SMM | San Miguel del Monte, Laguna de Monte, Buenos Aires Province | 58º 47' W | 35º 27' S | Wet Pampas | 10 |
| | TAN | Near Tandil, central Buenos Aires Province. | 59º 03' W | 37º 11' S | Wet Pampas | 10 |
| Australia | KIL | Wivenhoe Dam region near Kilcoy, State of Queensland | 152º 34' E | 26º 58' S | Reservoir area, pasture | 10 |
| | SRR | St Ruth's Reserve, Dalby, State of Queensland | 151º 15' E | 27º 20' S | Dark clay soil, mixed pasture and woodland | 10 |
| | GOO | "Limebon" Boggabilla NSW, Goondiwindi, State of New South Wales | 150º 04' E | 28º 47' S | Dark soil plains, pasture | 10 |
| | RAY | Heatherbrae, Raymond Terrace, State of New South Wales | 151º 43' E | 32º 46' S | Dark sedimentary floodplain soil, annually inundated | 10 |
| | DER | Derang Lakes Ramsar Wetlands, State of Victoria (Ramsar wetlands) | 143º 53' E | 35º 41' S | Grazed pasture by creek bank | 10 |
| France | S1 | Nissan-lez-Ensérune (Domaine de la Plaine), Hérault Department | 3° 08' E | 43° 16' N | Pasture with cultivation history | 10 |
| | S2 | Les Cabanes de Fleury (Le Bouquet), Aude Department | 3° 10' E | 43° 14' N | Pasture with cultivation history | 10 |
| | S3 | Nissan-lez-Ensérune, Hérault Department | 3° 08' E | 43° 15' N | Pasture with cultivation history | 10 |
| | S4 | Capestang (Le Viala) , Hérault department | 3° 03' E | 43° 17' N | Pasture with cultivation history | 10 |
| | S5 | Nissan-lez-Ensérune (Périès) ), Hérault Department | 3° 04' E | 43° 16' N | Pasture with cultivation history | 10 |

Figure 1. Band richness at the region (a) and population (b) level as a function of individuals sampled. The error bars show the standard error among populations in each region.
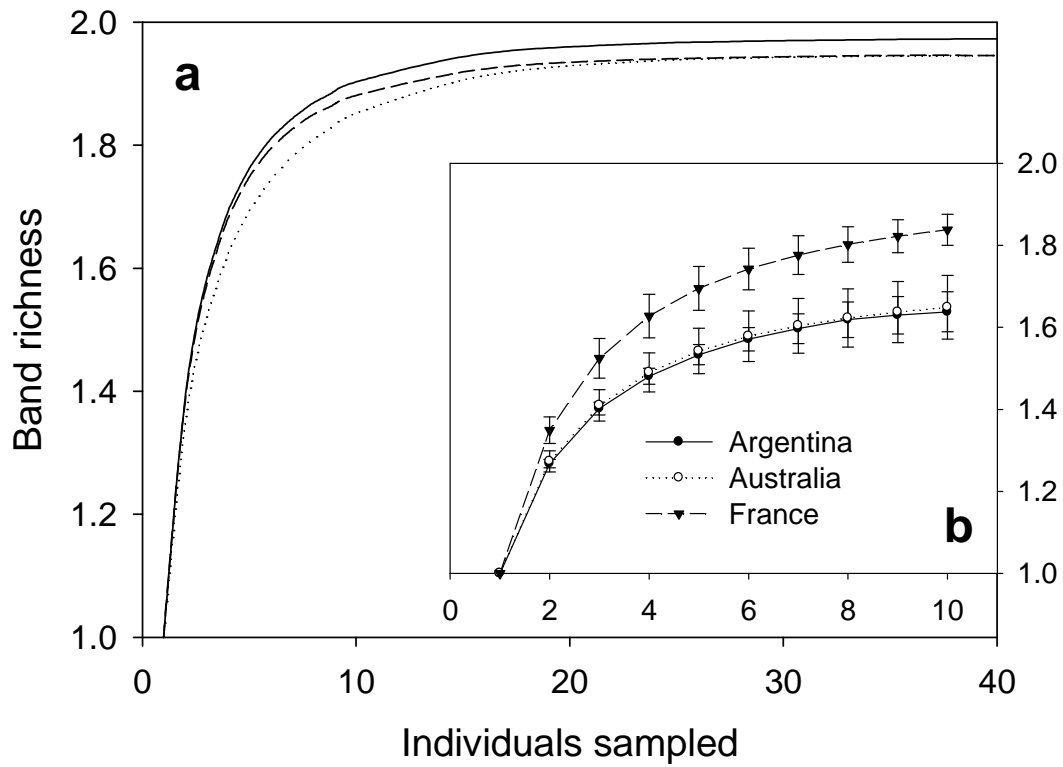
Figure 2. Phenotypic traits of *P. canescens* populations in the native (Argentina) and invaded regions (Australia and France). Values shown are mean ± SE. Means are compared between regions with LSD and regions followed by the same letter are not significantly different at *P* =0.05 level. R:S = root to shoot ratio, S:V = ratio of reproductive to vegetative biomass.
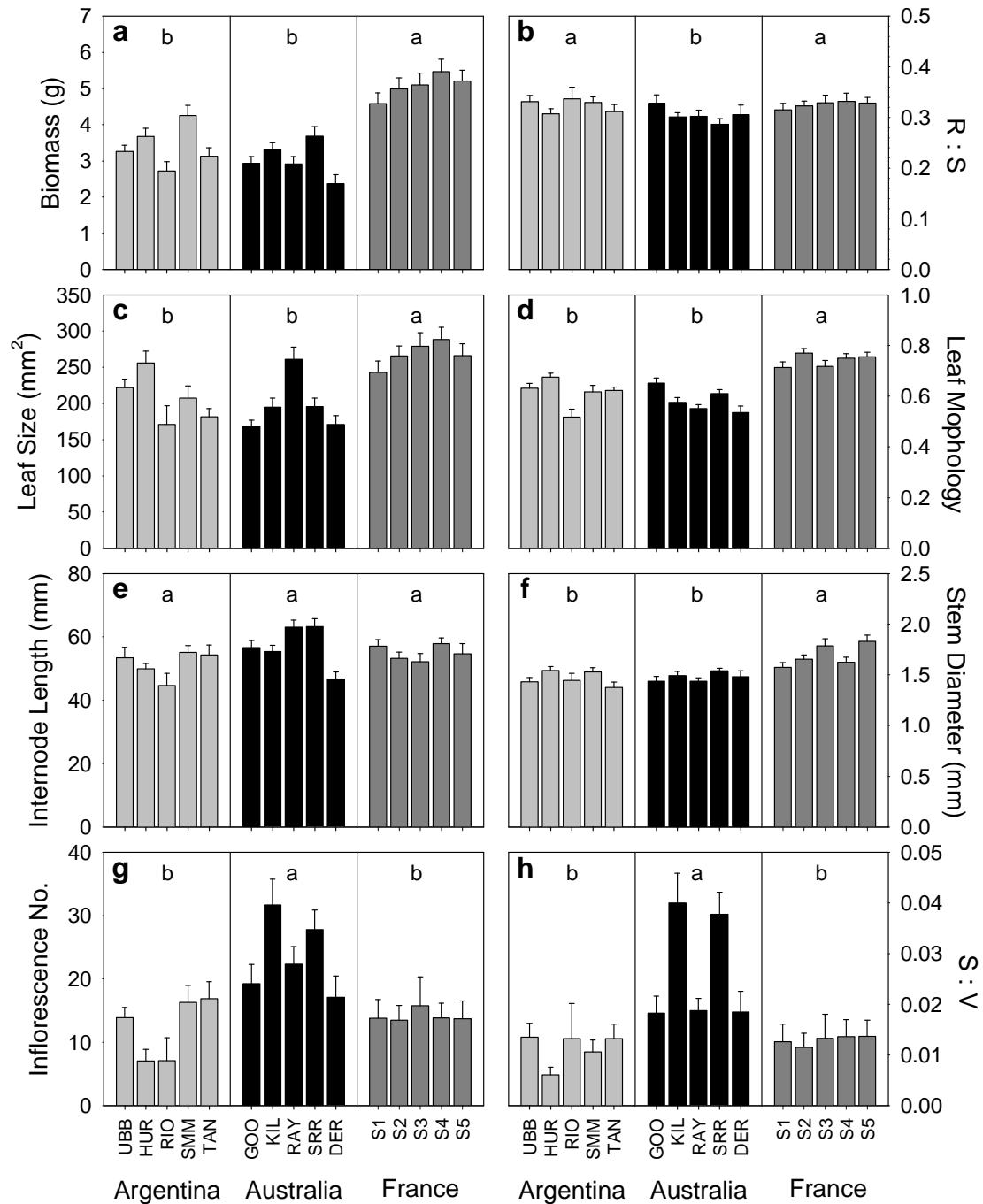
Figure 3. Principal coordinate analysis of *P. canescens* specimens based on ISSR loci (GenAlEx version 6, Australian National University, Canberra, Australia). Projections of individuals are shown on the factor-plane of the first, second and third principal coordinate. One of the first three principal coordinates that explained the greatest proportion of phenotypic variation was used to run a multiple regression analysis on each trait.
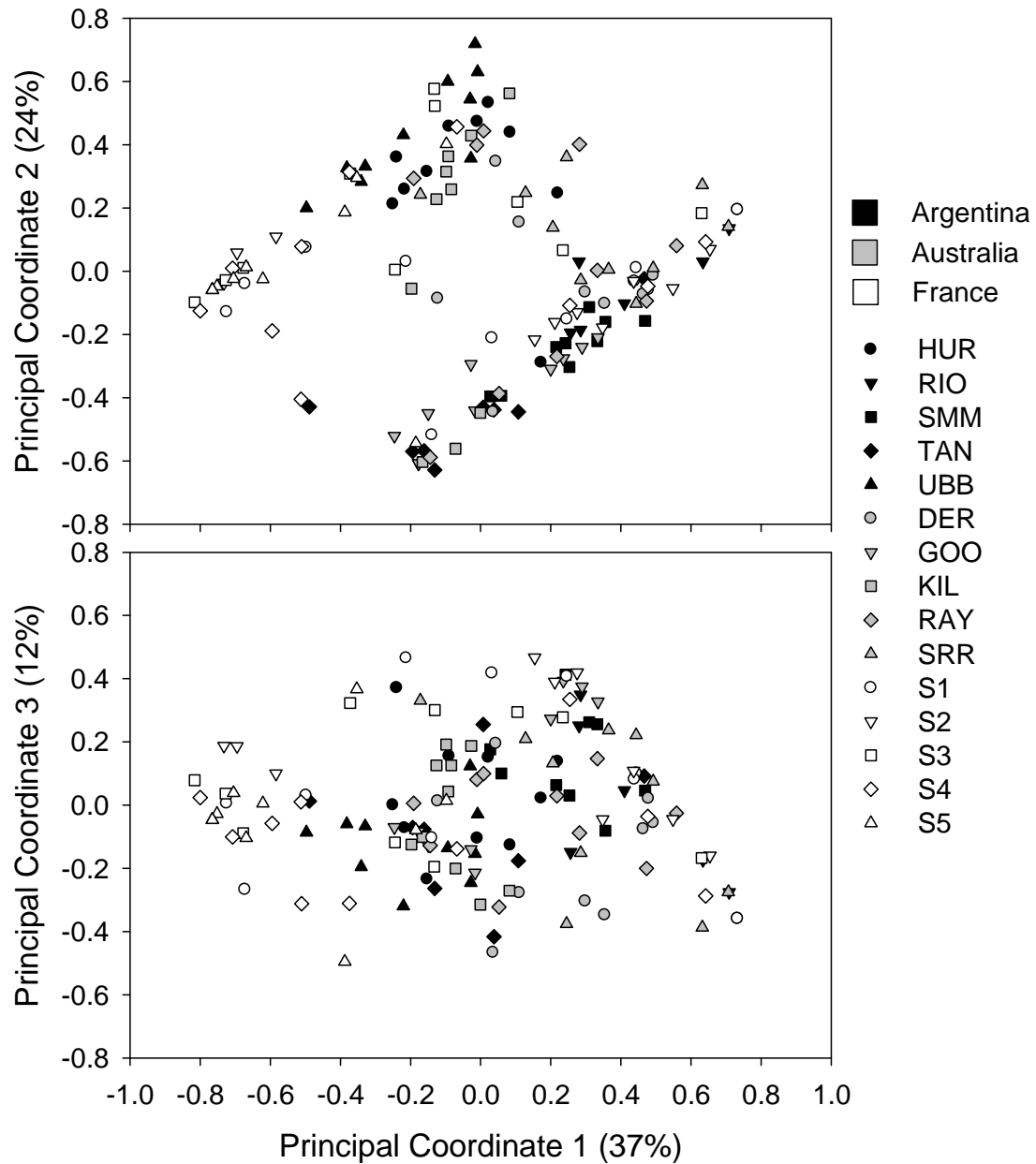
## References

Wildlife Conservation (WCS) and Center for International Earth Science Information Network (CIESIN), (2005). Last of the wild data Version 2 (LWD-2): Global human footprint dataset (HF).

Agence Méditerranéenne de l'Environnement, Agence Régionale pour l'Environnement Provence Alpes-Côte-d'Azur & Conservatoire Botanique National Méditerranéen de Porquerolles (2003). In. Plantes envahissantes de la région méditerranéenne. p. 48.

Coart E., Van Glabeke S., Petit R.J., Van Bockstaele E. & Roldan-Ruiz I. (2005). Range wide versus local patterns of genetic diversity in hornbeam (*Carpinus betulus* L.). *Conserv. Genet.*, 6, 259-273.

Conservatoire Régional des Espaces Naturels Languedoc-Roussillon (2006). Gestion d'une plante envahissante: *Lippia canescens*. *Journal du CEN-LR*, 5, 2-3.

Dilley M., Chen R.S., Deichmann U., Lerner-Lam A.L., Arnold M., Agwe J., Buys P., Kjekstad O., Lyon B. & Yetman G. (2005). Natural disaster hotpots: a global risk analysis. In: *Disaster management series*. The World Bank, Hazard Management Unit Washington, D.C., p. 145.

Earl J. (2003). The distribution and impacts of lippia (*Phyla canescens*) in the Murray Darling system. In. Murray Darling Basin Lippia Working Group, p. 91.

Goudet J. & Buchi L. (2006). The effects of dominance, regular inbreeding and sampling design on $Q_{st}$, an estimator of population differentiation for quantitative traits. *Genetics*, 172, 1337-1347.

Hijmans R.J., Cameron S.E., Parra J.L., Jones P.G. & Jarvis A. (2005). Very high resolution interpolated climate surfaces for global land areas. *Int. J. Climatol.*, 25, 1965-1978.

Leinonen T., O'Hara R.B., Cano J.M. & Merila J. (2008). Comparative studies of quantitative trait and neutral marker divergence: a meta-analysis. *J. Evol. Biol.*, 21, 1-17.

Lucy M., Powell E., McCosker R., Inglis G. & Richardson R. (1995). Lippia (*Phyla canescens*): a review of its economic and environmental impact on floodplain ecosystems in the Murray-Darling Basin. In. Queensland Department of Primary Industries Pittsworth Qld, p. 40.

O'Hara R.B. & Merila J. (2005). Bias and precision in $Q_{ST}$ estimates: Problems and some solutions. *Genetics*, 171, 1331-1339.

Olivier L., Galland J.P. & Maurin H. (eds.) (1995). *Livre Rouge de la flore menacée de France. Tome I : Espèces prioritaires.* SPN-IEGB /MNHN, DNP/Ministère Environnement, CBN Porquerolles, Paris.