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Population structure and genetic diversity of invasive *Phyla canescens*: implications for the evolutionary potential

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Abstract. Population bottlenecks during founder events tend to constrict the genetic diversity in introduced populations, thereby limiting their evolutionary potential and subsequent ability to adapt to their new environment. Paradoxically, rapid evolutionary changes have been recently found to be widespread in invasive species and have been proposed as a precursor to successful invasions. Information about population structure, introduction history and genetic diversity is essential for addressing this paradox and testing evolutionary hypotheses for any specific invasive species. In this study, we used microsatellite markers to investigate the genetic properties that may underpin the evolutionary potential of the invasive herb *Phyla canescens*, for which rapid, selection-driven evolution has already been demonstrated. Population structure and genetic diversity were compared between the native (South America) and two introduced ranges (eastern Australia and southern France). South American populations included all the 64 alleles found in the study and most individuals belonged to two distinct genetic clusters originating from northwest Argentina and central Argentina respectively. Invasive populations in eastern Australia and southern France were most closely linked to the central Argentine cluster. Microsatellite results also showed both a reduced genetic diversity at the population level, and the occurrence of a significant genetic bottleneck in many introduced populations. Our results suggest that *P. canescens* can undergo rapid, selection-driven evolution despite significant population bottlenecks and reductions in diversity. The question about the 'genetic paradox' for *P. canescens* therefore is how much genetic diversity is enough to underpin rapid evolution, or whether it is the type of diversity rather than the amount that is important.

Key words: biogeography; biological control; microsatellite; population bottleneck; rapid evolution; STRUCTURE software.

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Introduction

Biological invasions are a major threat to biodiversity and ecosystem services worldwide (Cohen and Carlton 1998, Mooney and Hobbs 2000), but mechanisms leading to successful invasion remain unclear (Richardson and Rejmánek 2011, Seastedt and Pyšek 2011). Introduced populations are generally expected to be genetically depauperate, owing to population bottlenecking during founding events (Roman and Darling 2007, Dlugosch and Parker 2008*a*). Low genetic diversity is likely to limit the evolutionary potential of introduced populations (but see Dlugosch and Parker 2008*b*), thereby decreasing their ability to adapt to the new environment (Dlugosch and Parker 2008*a*). This relationship between genetic diversity and population viability, in contrast to the success of invaders over native species, constitutes an apparent paradox in invasion biology (Roman and Darling 2007).

Rapid evolution may enable invasive species to better adapt to novel environments in the introduced range (Broennimann et al. 2007, Beaumont et al. 2009, Gallagher et al. 2010), accelerate their dispersal (Urban et al. 2008), or make them more competitive (Barney et al. 2009, Lachmuth et al. 2010), therefore potentially increasing their invasiveness. Recent studies have found that evolutionary changes such as larger size (Leger and Rice 2003, Buckley et al. 2010), faster growth rates (Siemann and Rogers 2001, Blair and Wolfe 2004, Guesewell et al. 2006), reduced defences (Rogers and Siemann 2004, Meyer et al. 2005), enhanced reproductive rates (Wolfe et al. 2004, Brown and Eckert 2005), and increased plasticity (Lavergne and Molofsky 2007, Cano et al. 2008) are widespread in successful invasive plant species (Whitney and Gabler 2008, Buswell et al. 2011), suggesting that rapid evolution is a crucial mechanism for invasion success (Maron et al. 2004, Lavergne and Molofsky 2007, Cano et al. 2008). This makes the paradox mentioned above even more puzzling. Genetic diversity and its structure within introduced populations, as well as a comparison of these patterns among native and introduced populations, provide valuable information for assessing the evolutionary potential of invasive species. This provides important insights into the nature of the paradox and helps to test evolutionary hypotheses used to explain successful invasions.

Neutral genetic diversity is helpful for assessing the evolutionary potential of invasive species. Many empirical studies have compared genetic diversity between the native and introduced populations (Dlugosch and Parker 2008*a*). Where very low genetic diversity was observed in introduced populations, successful invasion has generally been attributed to preadaptation (Clark et al. 2013) or the existence of generally advantageous genotypes (Parker et al. 2003, Le Roux et al. 2007). Recent studies have found that reduced genetic diversity in invasive populations is not as common as expected and increased genetic diversity has been found prior to the exponential range expansion of introduced populaitons in some case (Fennell et al. 2014). Several mechanisms, such as high propagule number during founding events and multiple introductions (Bossdorf et al. 2005, Novak and Mack 2005, Rosenthal et al. 2008, Pairon et al. 2010, Kelager et al. 2013), may offset the genetic effects of a population bottleneck during invasion and thereby improve the evolutionary potential of introduced populations (Bossdorf et al. 2005, Lavergne and Molofsky 2007, Roman and Darling 2007).

A species' native population structure and evolutionary history defines the genetic pool from which invasive populations were drawn, and subsequently the genetic composition that may be subject to selection pressures in the new range (Keller and Taylor 2008). Comparative studies of the genetic structure between the native and introduced ranges are helpful for finding the putative population source(s) of origin and reconstructing the invasion history (Williams et al. 2005, Prentis et al. 2009, Jenkins and Keller 2011, Clark et al. 2013, Kelager et al. 2013). This approach may help to determine the range of physiological and ecological tolerances of the invasive species, as well as the genetic pool subject to the new selection regime (Miura 2007), thereby providing important insights on the ability of a species to adapt to a new prevailing environmental pressure (Thompson et al. 2011). Such information is valuable for assessing the likelihood of adaptive evolution, and ultimately invasion success.

Phyla canescens (Kunth) Greene (common name: lippia, Verbenaceae) is a perennial herb native to South America, where it is widely distributed (Appendix A: Fig. A1) (Munir 1993). It was intentionally introduced into many countries in North America, Europe and Africa as an ornamental plant (Moldenke 1939, Boota 1979, Kennedy 1992). In Australia and France it has become invasive, mainly on floodplains and in pastures. In Australia, *P. canescens* was first recorded in the 1920s, probably having been introduced as an ornamental or low maintenance lawn. It is listed as a noxious weed in south-eastern Australia ("declared plant" in New South Wales and "significant weed" in Queensland) due to its detrimental economic and environmental impacts including excluding pasture grasses in grazing lands, threatening native rare plants, causing erosion and soil slumping along river banks, and damaging roads (Earl 2003, Whalley et al. 2011). In France, *P. canescens* was first reported to be cultivated in Paris in 1826 and became naturalised in southern France (Hyères, Var Department) in 1870. It is currently listed as an invasive plant species of concern in the Mediterranean region (Agence Méditerranéenne de l'Environnement et al. 2003), where it has been reported to form monocultures (Conservatoire Régional des Espaces Naturels Languedoc-Roussillon 2006), threatening critical conservation habitats (Olivier et al. 1995).

P. canescens has a dual reproductive strategy. In the presence of favourable moisture conditions, *P. canescens* flowers from spring to autumn and produces a prodigious number of seeds of considerable persistence (up to 25000 seeds m^{-2} with estimated longevity of 10+ years; McCosker 1994, Price et al. 2011). It can also propagate vegetatively from fragmented stem nodes (Xu et al. 2010*b*, 2012, Julien et al. 2012), which facilitates its expansion on floodplains (Price et al. 2011). Once established, *P. canescens* grow radially to form a dense, vegetative mat, which restricts germination of other species and produces a mono-specific ground cover (Whalley et al. 2011).

In a previous study, we compared phenotypic traits and the pattern of inter-simple sequence repeat (ISSR) genetic diversity of *P. canescens* between the native and introduced ranges (Xu et al. 2010*a*). ISSR data suggested significant genetic differentiation between northern and central Argentine populations and show that invasive populations are more closely linked to the latter (Xu et al. 2010*a*). Furthermore, invasive populations in Australia and France did not exhibit reduction of ISSR genetic diversity and displayed a selection-driven, post-invasion evolution (Xu et al. 2010*a*). However, as a dominant marker, ISSR does not differentiate dominant homozygotes from heterozygotes, thereby limiting the amount of information that can be obtained (Semagn et al. 2006, Allan and Max 2010). As codominant markers that contain many alleles, microsatellites are more likely to detect historic population bottlenecks and are better able to elicit population structure than dominant ISSR markers. Here, aiming at addressing three critical questions in relation to the evolutionary potential of *P. canescens*, we investigated the population structure and genetic diversity of *P. canescens* in South America, Australia and France, inferred from microsatellite markers. Specifically, we asked: how is *P. canescens* structured geographically across South America; what is the source(s) of introduced populations in Australia and France; and what level of population- and region-level (South American) genetic diversity was captured during founder events. Answers to these questions will provide insights into the genetic basis underpinning the evolutionary potential of *P. canescens* in introduced ranges. Expectations from our previous study lead to three hypothesis to be tested here: (1) that there is considerable genetic structure within the native populations, (2) that invasive populations are mainly linked to central Argentine populations, and (3) that invasive populations possess high genetic diversity and thereby evolutionary potential.

Material and Methods

Samples

The taxonomic placement of *P. canescens* and two closely related species (*P. reptans*, and *P. nodiflora*) is debatable. Some studies regard them as different species (Greene 1899, Kennedy 1992, Munir 1993). Here we adopted this taxonomic classification, which is supported by our nuclear ribosomal internal transcribed spacers (ITS) sequence data (Appendix B: Fig. B1). Populations were initially identified based on their morphol-

ogy (Julien and Sosa, personal communication), which included confirming that they were distinct from the morphologically similar *P. reptans*, which also occurs in northern Argentina. Collected samples were then confirmed through molecular sequencing. This included reference *P. reptans* samples from northern Argentina. Populations that were not placed in the *P. canescens* clade (Appendix B: Fig. B1) on the basis of ITS sequences were excluded from analysis. Most samples used in this study have been deposited in herbariums (Table 1). However, it is worthy to note that other studies placed *P. canescens*, *P. reptans* and *P. nodiflora* as varieties of *P. nodiflora* (Moldenke 1939, O'Leary and Mulgura 2011) based on morphology, despite the lack of supporting genetic evidence and of experimental work to confirm the capability of interbreeding between these species.

Sampling in South America was aimed at providing a representative picture of the distribution range of *P. canescens* to allow eliciting the broad-scale spatial genetic structure of *P. canescens* and identifying the putative sources of populations in France and Australia (Prentis et al. 2009). Specimens of *P. canescens* were obtained when undertaking related projects (Sosa et al. 2008, Xu et al. 2010*a*) and included samples from six areas throughout South America: central, northwest, and northeast Argentina, Chile, Peru and Venezuela. In the introduced range, *P. canescens* specimens were from eight sites in eastern Australia and five sites in southern France, where the impact of *P. canescens* is largest (Agence Méditerranéenne de l'Environnement et al. 2003, Crawford 2008), although the invasive status of these populations were not specifically assessed.

A total of 700 individual samples collected from South America (444), Australia (175), and France (81) were used in this study (Table 1). Individuals were at least 5m apart from each other at each site (1–30 per site). Low sample sizes at some sites (mainly in South America) reflected the rarity there. Leaves were desiccated using silica gel for DNA analysis.

Sites with 14 or more samples were treated as one population (analysis unit). A high proportion of South American sites had less than 14 samples. These were subsequently pooled as one population for each area (see above) to ensure sufficient sample size for genetic analysis (Table 1). Given the major geographic barriers (e.g., Andes Mountains) that define these areas, we regard this pooling approach as reasonable, even if individual specimens were sampled in a relatively wide geographical range in some cases (e.g., ~900 km in Chile). Further analysis with our data also supported this pooling approach (Appendix C). This resulted in a total of 19 South American populations (from 47 sites) for genetic analysis.

Microsatellite genotyping and data summarizing

Total genomic DNA of each individual was extracted from leaves using a DNeasy Plant Minikit (QIAGEN, Hilden, Germany) or the CTAB method. Nine nuclear microsatellite loci (Phc07, Phc12, Phc13, Phc15, Phc17, Phc18, Phc20, Phc21, Phc22) described by Fatima and Gross (Fatemi and Gross 2008) were used for genotyping each *P. canescens* population. We acknowledge that the loci used for further analysis is relatively modest, but that is a common challenge in many non-model systems. These markers are sufficient to reliably differentiate broad-scale spatial genetic structure of *P. canescen* because the estimates of genetic structure were not sensitive to loci number in our study (i.e., each individual loci provided consistent results in the estimates of genetic structure; Appendix C).

Each 25 μL amplification reaction contained 16 mM (NH4)2SO4, 67 mM Tris-HCl (pH 8.8), 0.01% Tween-20, 4 mM MgCl2, 0.4 mM of each dNTP, 0.5 unit *Taq* Polymerase, and 20 ng genomic DNA. The microsatellite PCR cycling profile consisted of: 10 minutes of initial denaturation at 95°C, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 59°C for 45 s, and extension at 72°C for 1 min; followed by eight cycles of denaturation at 95°C for 30 s, annealing at 53°C for 45 s and extension at 72°C for 1 min; with a final extension of 10 min at 72°C. The amplified products were resolved on 6% polyacrylamide sequencing gels and silver stained according to the protocol described by Bassam (Bassam et al. 1991).

Microsatellite data were checked for the presence of null alleles (Micro-Checker version 2.23; van Oosterhout et al. 2004) and genotypic linkage disequilibrium (GenePop version 4.0;

Table 1. Information of sampling sites.

Range, area, and site	Lat, long	Location	Herbarium deposition	Other information (vegetation, soil, etc.)
Native				
Venezuela				
VCL (7)†	10.00, −69.10	La Concordia, Lara, Central-Western Region	XAL	Urban, flat, brown clay loams
VPC (9)†	10.47, −68.05	Puerto Cabello, Carabobo, Central Region	XAL	Urban, flat, brown sandy loams, stony
Northwest Argentina				
PIC (25)	−23.40, −64.30	Route 34, 11km south of Pichanal, Salta Province	SI	NA
350 (16)	−23.70, −62.31	Route 81, km 1682, Formosa Province	SI	Chaco, roadside
VOL (24)	−23.92, −65.46	Volcan, Province of Jujuy	CANB	NA
BUR (19)	−26.51, −64.75	Conjunction of Route 36 and 304, 1 km south of Burruyacu, Tucuman province	CANB	Transitional area between Yungas (rain forest) and Chaco (dry plains)
TER (25)	−27.51, −64.89	Termas de Rio Hondo, Santiago del Estero Province	CANB	NA
ART (25)||	−27.99, −64.01	Route 9, Monte Redondo, Santigo del Estero Province	SI	NA
MON (23)||	−27.99, −64.01	Route 9, Monte Redondo, Santigo del Estero Province	SI	NA
405 (20)	−28.62, −65.10	Province Route 6, 6 km SW of Frías, Santiago del Estero Province	SI	NA
Northeast Argentina				
Ibar (3)‡	−25.20, −59.83	Ibarreta, Formosa Province	SI	Chaco, roadside
Marg (3)‡	−25.10, −58.96	Margarita Belen, Chaco Province	SI	Chaco, roadside
Palo (3)‡	−25.55, −59.31	Palo Santo, Formosa Province	SI	Chaco
Pira (3)‡	−25.76, −59.12	Pirane, Formosa Province	SI	Chaco, roadside
Vill (3)‡	−26.16, −59.35	Villa Dos Trece, Formosa Province	CANB	Chaco, roadside
Eduv (3)‡	−26.84, −59.05	La Eduvigic, Chaco Province	SI	Chaco roadside
Basa (2)‡	−27.90, −59.28	Basail, Chaco Province	SI	Chaco
Central Argentina				
PAS (16)	−33.30, −65.88	Paso de las Carretas dique, San Luis Province	SI	NA
GIL (4)§	−34.46, −59.51	San Andes de Giles, Buenos Aires Province	SI	Pampas
ESP (3)§	−34.56, −59.37	West Cabrera's father's farm, Las Espinas, 16 km from Mecedes, Buenos Aires Province	CANB	Pampas
Jun (2)§	−34.59, −61.01	Junin, Buenos Aires Province	SI	Pampas, urban area, disurbed
CHA (4)§	−34.63, −60.51	West of Chacabuco, Buenos Aires Province	SI	Pampas
LIN (4)§	−34.84, −61.50	Lincoln, Buenos Aires Province	CANB	Pampas
REA (1)§	−35.04, −64.26	Route 35, 3 km south of Realico, La Pampa Province	SI	Pampas
ROQ (2)§	−35.50, −64.10	Route 35, 5 km north of San Roque, La Pampa Province	CANB	Pampas
AME (3)§	−35.51, −63.00	Route 33 of America, Buenos Aires Province	SI	Pampas
ROS (2)§	−36.70, −64.28	South of Santa Rosa, La Pampa Province	CANB	Pampas
GUA (4)§	−36.90, −62.39	North of Guamini, Buenos Aires Province	SI	Pampas
JAC (4)§	−38.09, −63.34	Route 35, northwest Bahia Blanca, km 121, south of Jacinto Araoz, Buenos Aires Province	CANB	Pampas
HUR (30)	−34.58, −58.63	Near Hurlingham, Buenos Aires city northern suburbs, Buenos Aires Province	SI	Disturbed urban area
SMM (25)	−35.45, −58.79	San Miguel del Monte, Laguna de Monte, Buenos Aires Province	CANB	Wet Pampas
TAN (21)	−37.19, −59.05	Near Tandil, central Buenos Aries Province	CANB	Wet Pampas

Table 1. Continued.

Range, area, and site	Lat, long	Location	Herbarium deposition	Other information (vegetation, soil, etc.)
TRE (22)	−38.53, −60.51	Tres Arroyas, Buenos Aires Province	SI	NA
UBB (22)	−38.70, −62.25	Universidad National del Sur, Bahia Blanca, Buenos Aires Province	CANB	Pampas
Peru				
PAI (8)¶	−7.68, −79.33	Paijan, La Libertad Region	N/A	Cropland uncultivated, flat,brown sandy loams, stony
CAS (8)¶	−9.46, −78.32	Casma, Ancash Region	N/A	Irrigation system, pasture, flat, brown sandy loams
PAT (8)¶	−10.74, −77.77	Pativilca, Lima Province	N/A	Cropland uncultivated, flat, brown sandy loams
CH (9)¶	−11.53, −77.29	Chancay, Lima Province	N/A	Road formation, flat, brown sandy loam, stony
LIM (5)¶	−12.13, −77.03	Universidad Nacional Agraria, La Molilna, Lima Province	N/A	Urban, flat, brown sandy loams
Chile				
VAL (3)#	−28.60, −70.81	Huasco River bed, Route 5, 5 km west of Vallemar, Atacama Region	CANB	Stony river bed
LAS (4)#	−29.88, −61.26	Route 5, 2 km north of La Serena, Coquimbo Region	CANB	Dry coastal
PUP (3)#	−31.87, −71.40	River bed under Puento Pupio, Route D85 to illapel, 12 km west of Los Vilos, Coquimbo Region	CANB	Dry river bed in desert
QUI (3)#	−32.83, −71.48	Quinteros River bridge, 7 km south of Quinteros, Valparaiso Region	SI	Edge of bridge embankment
CVL (4)#	−33.82, −70.17	El Volcan, Santiago Region	SI	Dry village centre and roadsides
SEB (3)#	−33.52, −71.60	Avenue Capitan Carera Pinto, roadside between San Sebastian and Algorobo, Valparaiso Region	CANB	Dry sandy roadside
AUR (1)#	−37.25, −73.32	Arauco, Biobío Region	CANB	Footpath, dry sand
PED (3)#	−36.84, −73.11	San Pedro de la Paz, across the rivere south of Conception, Biobío Region	CANB	Urban grass verges around a car park, sandy soil
Invasive				
Australia				
MA (14)	−16.99, 145.42	Mareeba, State of Queensland	N/A	
KIL (24)	−26.97, 152.56	Wivenhoe Dam region near Kilcoy, State of Queensland	CANB	Reservoir area, pasture
SRR (26)	−27.33, 151.25	St Ruth's Reserve, Dalby, State of Queensland	CANB	Dark clay soil, mixed pasture and woodland
GOO (24)	−28.80, 150.07	"Limebon" Boggabilla, Goondiwindi, State of New South Wales	N/A	Dark sedimentary floodplain soil, annually inundated
G2 (20)	−29.62, 149.80	State of New South Wales	NE	NA
N1 (20)	−30.40, 149.89	State of New South Wales	NE	NA
WB (20)	−30.61, 147.69	Macquaire marshes, State of New South Wales	N/A	
RAY (27)	−32.78, 151.72	Heatherbae, Raymond Terrace, State of New South Wales	CANB	Dark sedimentary floodplain soil; annual inundated
France				
S1 (18)	43.27, 3.13	Nissan-lez-Ensérune (Domaine de la Plaine), Hérault Department	CANB	Pasture with cultivation history
S2 (15)	43.24, 3.17	Les Cabanes de Fleury (Le Bouquet), Aude Department	CANB	Pasture with cultivation history
S3 (19)	43.27, 3.14	Nissan-lez-Ensérune, Hérault Department	N/A	Pasture with cultivation history
S4 (15)	43.30, 3.07	Capestang (Le Viala), Hérault Department	N/A	Pasture with cultivation history

Table 1. Continued.

Range, area, and site	Lat, long	Location	Herbarium deposition	Other information (vegetation, soil, etc.)
S5 (14)	43.27, 3.07	Nissan-lez-Ensérune (Périès), Hérault Department	N/A	Pasture with cultivation history

Notes: Numbers in parenthesis are the number of individuals sampled. "Lat, long" is latitude and longitude. In each area within South America, populations with less than 14 individuals were pooled (marked with superscripts) for subsequent analysis. Abbreviation of herbariums in which samples were deposited: XAL, Herbario del insituto de ecologia, XALAPA, Vera Cruz, Mexico; SI, Instituto de Botánica Darwinion, Buenos Aires, Argentina; CANB Australia National Herbarium, Canberra, Australia; MOBOT, Missouri Botanical Garden Herbarium; NE, NCW Beadle Herbarium, University of New England, Armadale, Australia.

† Venezuela, pooled population.
‡ Northeast Argentine (NEA), pooled population.
§ Central Argentina miscellaneous (CAM), pooled population.
¶ Peru, pooled population.
# Chile, pooled population.
|| ART and MON are collected from two geographically close locations but are treated as two populations due to distinguishable morphology.

Rousset 2008). Conformance to Hardy-Weinberg equilibrium expectations was determined for all non-pooled populations with a *U* test for alternative hypothesis of heterozygote excess and deficiency at a $P = 0.01$ level (GenePop version 4.0; Rousset 2008). Summary data for microsatellite loci were calculated for each population, including the percentage of polymorphic loci (P%), the mean number of alleles per locus (A), the mean number of effective alleles per locus (A_e), the total number of private alleles (A_p) and observed heterozygosity (H_o) (calculated with GenAlEx; Peakall and Smouse 2006), Allelic richness per locus (R_s) and gene diversity (or expected heterozygosity, H_s; calculated with FSTAT 2.9; Goudet 2002). Region-level (South America, Australia and France) parameters were calculated by including all individuals within a region as one population. The statistical power of our microsatellite data was tested using POWSIM (version 4.1; Ryman and Palm 2006). Five levels of population differentiation ($F_{st} =$ 0.001, 0.0025, 0.005, 0.01, 0.025) were pre-defined with effective population sizes $N_e = 1000$ and $N_e = 10{,}000$ and the simulation was replicated 1000 times.

Population structure

The hierarchical partitioning of genetic variation among regions (South America, Australia and France), within regions and among populations, and within populations was assessed using analysis of molecular variance (AMOVA; Armstrong and De Lange 2005) based on the pairwise squared Euclidean distance among molecular loci (GenAlEx version 6.4; Peakall and Smouse 2006). We also calculated the level of genetic differentiation between regions by estimating F_{st} using FSTAT, with the statistical significance of F_{st} values determined by 1000 permutations among ranges. A Mantel procedure was used to test for a correlation between geographical and genetic distance (pairwise F_{st}) respectively for the three studied ranges. Because the relatively low number of samples per population (for some South American populations) and microsatellite loci may lead to overestimation of F_{st}, we conducted several analyses to test this (Appendix C).

A Bayesian-clustering method implemented in the program STRUCTURE version 2.3 (Pritchard et al. 2000) was used to estimate the number of genetic clusters (K). Preliminary simulations suggest that combining both native and invasive individuals resulted in assigning South American populations as the admixture of invasive populations, which is obviously inappropriate. Therefore, we separately resolved the structure within the native and invaded ranges.

We used the method described by Evanno et al. (Evanno et al. 2005) to determine the optimal K value in STRUCTURE clustering analysis. In brief, the optimal K was determined by ΔK, which is a measure of the second order rate of change in the likelihood of K and its maximum indicates the breakpoint in the slope of the distribution of lnP(D) for the different K values tested.

Preliminary simulations indicated a hierarchical structure in both the native and introduced ranges. Because Evanno et al.'s (2005) method detects only the uppermost level of genetic structure, a hierarchical clustering approach by Coulon et al. (2008) was applied. After the optimal K was determined for the first level of genetic structure, each individual was assigned to its inferred ancestry cluster ($q > 0.6$). Then, subsequent clustering analyses were run, respectively for each of the K partitioned groups. This process was repeated until the number of genetic clusters inferred was 1 for all of the K groups inferred in the proceeding round. The ΔK method is not appropriate when the true K is 1, so after each iteration we first examined plots of lnP(D) to test whether lnP(D) was maximum for $K = 1$ (Pritchard et al. 2010).

We chose the admixture model with independent allele frequencies and conducted 10 replicates of each run. The number of genetic groups was set from $K = 1$ to $K = 20$ for the first level of the hierarchical analysis and from $K = 1$ to $K = 10$ for other levels of the analysis. The location from which the samples were collected (classified into eight areas: Venezuela, Peru, Chile, northwest Argentina, northeast Argentina, central Argentina, Australia and France) was used to assist clustering. Each run started from a burn-in period of 100,000 Markov chain Monte Carlo (MCMC), followed by 50,000 iterations. These burn-in and run lengths were deemed sufficient because they resulted in clear convergence in α value and the likelihood, and results were the same as that of preliminary runs with a 10,000 burn-in period and MCMC replications. The outputs of STRUCTURE analyses were visualized using the software CLUMPP (Jakobsson and Rosenberg 2007) and DISTRUCT (Rosenberg 2004).

Identify putative sources of invasive individuals

We used two approaches to identify the putative population source(s) of the invasive individuals. First, we treated South American genetic clusters (identified by the approach mentioned above) as known origins and used the USEPOPINFO model within STRUCTURE to assign all invasive individuals to these South American clusters. In brief, all South American and invasive individuals were combined for one STRUCTURE analysis while all South American individuals were pre-defined as coming from particular clusters in the program (as potential origins). With this information, STRUCTURE then clustered the remaining (invasive) individuals to pre-specified native clusters to infer their origins. Second, the genetic relationships among native and introduced genetic clusters were determined by constructing a neighbor-joining (NJ) tree using Nei's genetic distance matrices based on microsatellite data (Phylip 3.69, Felsenstein 2004). An Australian *P. nodiflora* population genotyped with the same microsatellite markers was used as the outgroup and bootstrap analyses were performed for 1000 pseudoreplicates.

Testing population bottleneck

Three approaches were applied to evaluate the potential decrease in diversity from native to invasive populations. First, the population- and region-level (treating all individuals from South America, Australia or France respectively as a single population) values of R_s, H_o and H_s were compared among regions using 1000 permutations among regions (FSTAT 2.9; Goudet 2002). Secondly, we used the Wilcoxon sign rank test (a component of BOTTLENECK version 1.2; Piry et al. 1999) to detect any significant pattern of transient heterozygote excess under the two-phase model (TPM) and the stepwise mutation model (SMM; Cornuet and Luikart 1996). The Wilcoxon test has been recommended as the preferred method when relatively few loci are available. We allowed 95% single stepwise mutations and 5% multi-step mutations in the TPM, with a 12% variance among multiple steps, as recommended by Piry et al. (1999). Thirdly, the genotypic allele frequency distribution was examined based on the method described by Luikart et al. (1998) in BOTTLENECK version 1.2 (Piry et al. 1999), which postulates that recent bottlenecks should result in a relative decrease in rare alleles, creating a characteristic mode-shift distortion in the distribution of allele frequencies.

Results

Microsatellite

We amplified all nine microsatellite loci in all of the *P. canescens* populations. Locus Phc13 showed a very complex allele pattern that could

Table 2. Microsatellite genetic diversity indices of South American and invasive populations of *Phyla canescens*.

Country/population	Diversity indices								
	N	P%	*A*	A_e	A_p‡	R_s	H_o	H_s	F_{is}
South America									
Venezuela (2)†	16	100	3.500	2.447	3 (0)	3.367	0.667	0.556	−0.199
Argentina									
PIC	25	66.7	1.833	1.776	3 (0)	1.833	0.667	0.355	−0.879
350	16	83.3	3.667	2.261	8 (1)	3.550	0.521	0.485	−0.074
VOL	24	100	4.500	2.590	10 (0)	4.004	0.625	0.556	−0.125
BUR	19	100	5.833	3.052	10 (0)	5.061	0.614	0.641	0.041
TER	25	100	6.000	3.602	12 (0)	5.161	0.607	0.655	0.074
ART	25	100	5.667	3.221	13 (0)	4.798	0.613	0.622	0.014
MON	23	100	5.500	2.755	8 (0)	4.519	0.587	0.559	−0.051
405	20	100	5.333	3.158	10 (0)	4.690	0.683	0.656	−0.041
NEA (7)†	20	100	5.333	3.618	9 (0)	5.026	0.583	0.702	−0.036
PAS	16	100	4.333	2.619	3 (1)	4.127	0.583	0.563	0.169
CAM (11)†	33	100	6.500	3.350	8 (0)	5.117	0.664	0.660	−0.105
HUR	30	100	6.000	3.193	8 (1)	4.695	0.743	0.673	−0.006
SMM	25	100	5.167	2.971	5 (0)	4.554	0.647	0.654	−0.012
TAN	21	100	4.667	2.200	1 (0)	3.956	0.549	0.530	−0.037
TRE	22	100	3.500	2.667	2 (0)	3.238	0.629	0.594	−0.058
UBB	22	100	5.333	3.384	3 (0)	4.714	0.689	0.686	−0.005
Peru (5)†	38	100	4.333	2.340	2 (0)	3.642	0.327	0.462	0.293
Chile (8)†	24	100	6.333	3.504	8 (0)	5.435	0.570	0.666	0.143
Australia									
MA	14	100	2.333	1.930	0 (0)	2.235	0.857	0.482	−0.779
KIL	24	100	3.333	2.510	0 (1)	3.205	0.660	0.594	−0.110
SRR	26	100	4.500	2.883	0 (4)	3.943	0.673	0.630	−0.068
GOO	24	83.3	2.500	1.900	0 (0)	2.380	0.618	0.414	−0.492
G2	20	83.3	2.833	1.920	0 (0)	2.676	0.425	0.401	−0.060
N1	20	100	2.500	2.135	0 (0)	2.445	0.967	0.530	−0.823
WB	20	100	3.000	2.190	0 (2)	2.830	0.742	0.534	−0.389
RAY	27	100	2.500	1.950	0 (1)	2.344	0.794	0.453	−0.753
France									
S1	18	83.3	2.000	1.819	0 (1)	3.376	0.778	0.553	−0.306
S2	15	100	4.000	2.836	0 (0)	2.588	0.756	0.429	−0.633
S3	19	100	3.333	2.143	0 (2)	3.354	0.711	0.442	−0.490
S4	15	100	2.833	1.935	0 (1)	3.260	0.689	0.455	−0.489
S5	14	100	3.833	1.270	0 (2)	3.545	0.679	0.559	−0.359

Notes: Parameters include the number of individuals sampled per population (N), the percentage of polymorphic loci (P%), the mean number of alleles per locus (A), the mean number of effective alleles per locus (A_e), the number of private alleles (A_p), the mean allelic richness per locus (R_s, based on $n = 12$), observed heterozygosity (H_o) gene diversity (or expected heterozygosity, H_s), and inbreeding coefficient (F_{is}). H_o in bold are significantly higher than expected ($P < 0.01$), indicating a deviation from Hardy-Weinberg equilibrium.

† Pooled populations, with number of sites pooled given in the parenthesis (see Table 1).

‡ For private alleles, the first number is the number of alleles exclusive to a given region (Native, Australia, or France) in the considered population, while the number in parentheses is the number of alleles that are exclusive to the considered population in its region.

not be reliably genotyped using the silver stain approach. Locus Phc20 was not successfully amplified in a substantial proportion of samples, including all individuals collected from northwest Argentina and Peru and two Chilean sites (VAL and CVL). Micro-checker detected the presence of null alleles at locus Phc22. Consequently, these three loci were not used for further analysis. The remaining six loci showed no indication of scoring error due to stutter or large allele dropout in our Micro-checker analysis. Linkage disequilibrium was tested for each pair of loci in each non-pooled population. Significant correlation ($P < 0.01$) between loci was found in 60% of the populations. However, the linked loci differed among populations and there was no consistent linkage between any pair of loci across these populations, so these correlations are unlikely to have influenced our results. A global test across all loci showed that within non-pooled populations, all five French populations, five of the eight Australian populations but only one South American populations displayed significantly higher observed than expected heterozygosity (Genepop, $P < 0.01$; Table 2). This deviation from Hardy-Weinberg equilibrium in

Table 3. Analysis of molecular variance (AMOVA) between ranges of *Phyla canescens,* and within each region (South America, Australia and France).

Source of variation	df	Sum of squares	Variance components	Percent variation	Significance level
South America					
Among populations	18	510.3	0.570	24.3	***
Within populations	869	1545.8	1.779	75.7	...
Australia					
Among populations	7	148.6	0.452	22.7	***
Within populations	342	526.2	1.539	77.3	...
France					
Among populations	4	7.1	0.012	0.9	ns
Within populations	157	217.1	1.383	99.1	...
All three regions					
Among ranges	2	197.5	0.208	8.6	***
Among population	29	712.7	0.522	23.6	***
Within populations	1368	2305.2	1.685	67.8	...

Notes: Significance level of *F* statistics are shown (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

introduced populations is not surprising due to their non-equilibrium demographic nature. POWSIM simulation resulted in a 100% probability of our data detecting population differentiation corresponding to $F_{st} = 0.005–0.01$ (Appendix D: Table D1). This is sufficient to elicit broad-scale spatial genetic structure of *P. canescens* since our previous ISSR study suggested its F_{st} among populations was over 0.2 (Xu et al. 2010*a*).

All six loci used for genetic analysis were polymorphic in 27 out of the total 32 populations (Table 2). Four populations (350, GOO, G2, S1) had one monomorphic locus and one population (PIC) had two monomorphic loci. A total of 64 alleles were observed across the 700 individuals, with each locus having 6 to 16 alleles and an observed mean (*A*) of 1.8 to 6.5 per population. All 64 alleles were detected in South American populations, including the 36 alleles found in Australia and France.

Population structure in South America

Significant genetic structure was found within South America, with 24% of the total genetic diversity distributed among populations (AMOVA; Table 3). Geographic structure detected among Argentine populations was evidenced by a positive relationship between pairwise F_{st} and geographical distance ($R^2 = 0.23$, $P < 0.0001$; Fig. 1). In contrast, no correlation was detected between population pairs across the geographic barriers of the Andes Mountains and the Amazon Basin (Venezuela-Chile, Venezuela-Peru, Venezuela-Argentine, Chile-Peru, Chile-Argentine and Peru-Argentine populations; Fig. 1). Further analyses suggest that despite relatively low loci number and sample size (for some populations), the risk of overestimating genetic structure of *P. canescens* is nil (Appendix C).

Bayesian clustering analyses of microsatellite genotypes implemented in STRUCTURE indicated a hierarchical genetic population structure. When we used Evanno et al. (2005)'s ad hoc estimator of the number of clusters, ΔK peaked at $K = 2$ (Fig. 2a). The hierarchical clustering approach of Coulon et al. (2008) identified four levels of population genetic subdivision for South American individuals (Fig. 3a). The uppermost level separated Argentine individuals into two geographically distinct genetic clusters: northwest Argentina (orange) and central Argentina (blue-green), with a notable exception of MON and some limited mixing especially in population NEA (pooled from 7 sites in northeast Argentina; Fig. 4a). *P. canescens* from other South American countries were mixtures, predominantly of genotypes from northwest Argentina for Venezuelan individuals and central Argentina for Peruvian and Chilean individuals. The second level of subdivision separated Venezuela and PIC (red) from other northern Argentine individuals (orange), and Peruvian individuals (blue) from central Argentine individuals (blue-green). Chilean individuals were a mixture, predominantly from the Peruvian cluster (Fig. 4a). On the third level of subdivision, Venezuelan and PIC individuals were separated;

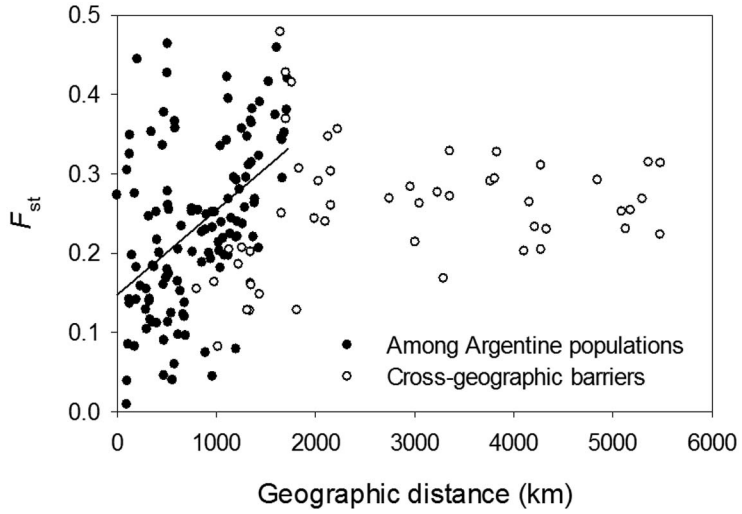

Fig. 1. The relationship between pairwise F_{st} values and pairwise geographical distance in South America. There is a positive relationship between pairwise F_{st} and pairwise geographical distance among Argentine populations ($y = 0.0001x + 0.1474$, $r^2 = 0.23$, $P < 0.0001$), but there is no significant relationship between population pairs across the two major geographic barriers (the Andes Mountain and the Amazon Basin) in South America ($P = 0.12$).

Peruvian and Chilean individuals were distinguished from each other; and north and central Argentine individuals were both split into three clusters without any clear geographic pattern. Finally, three Argentine genetic clusters identified by the third level subdivision could be further divided into two groups each (the fourth level subdivision; Fig. 3a).

Population structure in introduced ranges

In introduced ranges, significant genetic structure was found in Australia (23% variation among populations), whereas there was no differentiation among French populations (AMOVA; Table 3; also see F_{st} in Table 4). No correlation between pairwise F_{st} and geographic distance was observed for Australian and French populations (data not shown). Given the short distance among French populations, the low differentiation and absence of distance-F_{st} relationship is expected. Across the ranges, 68% of genetic variation within *P. canescens* was distributed within populations, while inter-range differences only contributed 9% of the variation (Table 3).

Introduced individuals were divided into three clusters on the uppermost level (Fig. 3b), respectively representing French populations (including a few KIL and SRR individuals), population RAY and some SRR individuals, and other Australian populations (Fig. 3b). French populations could not be further subdivided while a second level subdivision distinguished SRR and RAY individuals, and separated the other Australian individuals into two clusters. STRUCTURE could not further separate these genetic clusters.

Putative sources of invasive individuals

To identify the source of invasive individuals in Australia and France, we assigned invasive individuals to South American genetic clusters with STRUCTURE and clustered five invasive genetic clusters (identified in level 2 subdivision) with native genetic clusters in a neighbor-joining tree. STRUCTURE and NJ tree analysis using the uppermost level subdivision of South American individuals assigned all invasive individuals or clusters to the central Argentine cluster (Appendix E: Fig. E1). When the second subdivision of South American individuals was used, STRUCTURE assigned invasive individuals primarily to the central Argentina cluster but the Peruvian cluster also contributed to population SRR (Figs. 3c and 4d). The northwest Argentine and Venezuelan genetic clusters made little or no

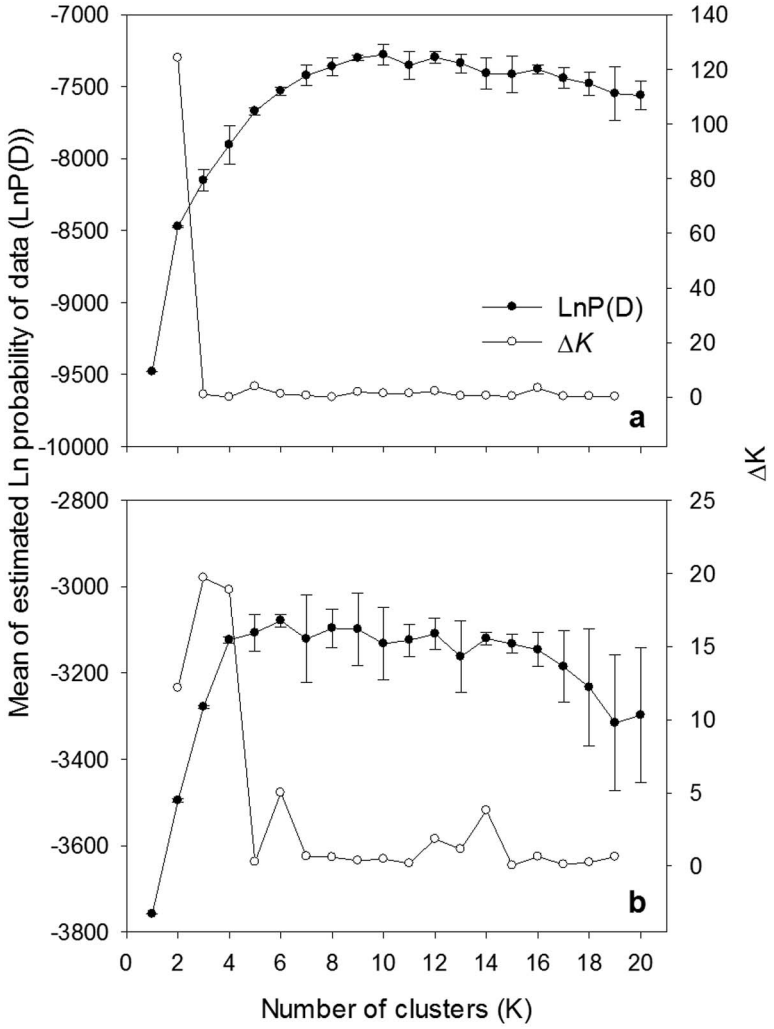

Fig. 2. Log-likelihood probability (LnP(D)) and Δ*K* (Evanno et al. 2005) of the number of inferred clusters (K) of (a) South American and (b) Australian and French individuals estimated using STRUCTURE (Pritchard et al. 2000).

contribution to invasive individuals. In the NJ tree analysis, invasive clusters were more closely grouped with the central Argentine cluster than with the Peruvian cluster (53% bootstrap support) and only distantly related to Venezuelan and northern Argentine clusters (Fig. 3d). Using the third and the fourth level subdivision of South American individuals generally could not reliably provide more precise information about the source of Australian and French individuals (Appendix C).

Population bottleneck

P. canescens displayed reduced genetic diversity in the introduced ranges when compared to both population- and region-level diversity in South America. In general, only a subset of South American diversity is represented in Australian and French populations (see *Microsatellite* above) and region-level R_s and H_s both decreased significantly. Loss of diversity was also observed at the population level. That is, the mean allelic richness (R_s) was significantly lower in Australia and France than in South America (permutation, $P < 0.01$; Table 4). South American populations

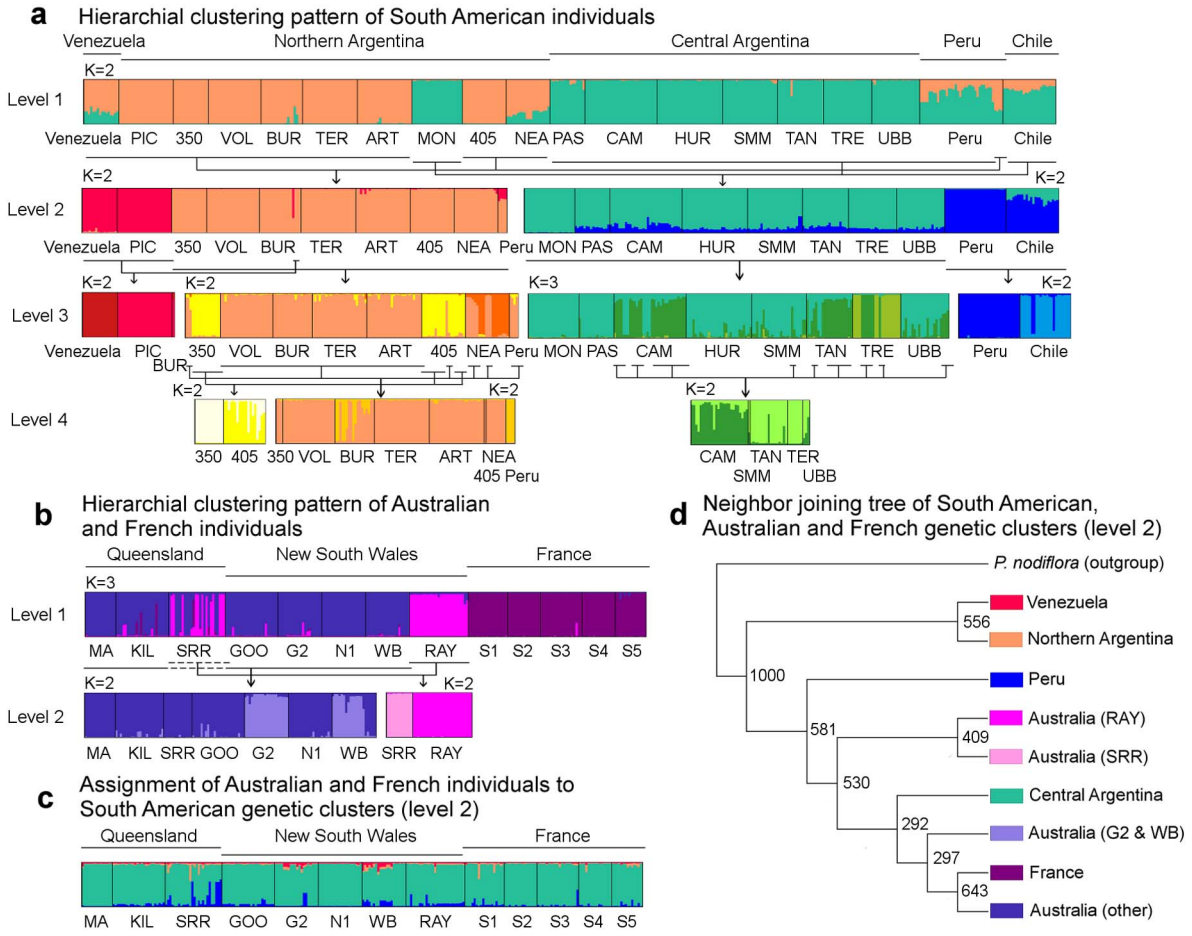

Fig. 3. Bayesian clustering of South American, Australian and French individuals in the software STRUCTURE (a, b) and identification of South American sources for invasive individuals (c, d). Hierarchical Bayesian clustering was used to determine the number of genetic groups (K) for South American (a) and invasive individuals (b). South American and invasive individuals were respectively clustered at four and two hierarchical levels. Arrows indicate subsequent hierarchical analysis. Populations are arranged (left to right) following the north-to-south direction for those collected east of the Andes (Venezuela and Argentina) and Australia. The sources of invasive individuals were identified by assigning invasive individuals to level-2 South American clusters (c) and neighbor-joining tree construction (d). Each individual included in a STRUCTURE analysis is represented by a vertical bar showing the degree of admixture. Bootstrap (1000) support of each node is shown for the neighbor-joining dendrogram.

possessed the highest H_s, followed by the Australian and French populations. The difference in H_s was insignificant when all three regions were compared together (permutation, $P = 0.09$), but pairwise comparisons showed H_s in South American populations was higher than in French populations (permutation, $P = 0.04$). Inbreeding coefficients (F_{is}) of South American populations were close to zero on average, and were significantly higher than that of the two introduced ranges (~−0.4, permutation, $P = 0.01$). The pattern also remains the same when introduced populations are compared with central Argentine clusters, to which they are most closely linked (data not shown).

The Wilcoxon sign rank test in BOTTLENECK detected a significant heterozygosity excess for three of the introduced populations (KIL, N1, S1)

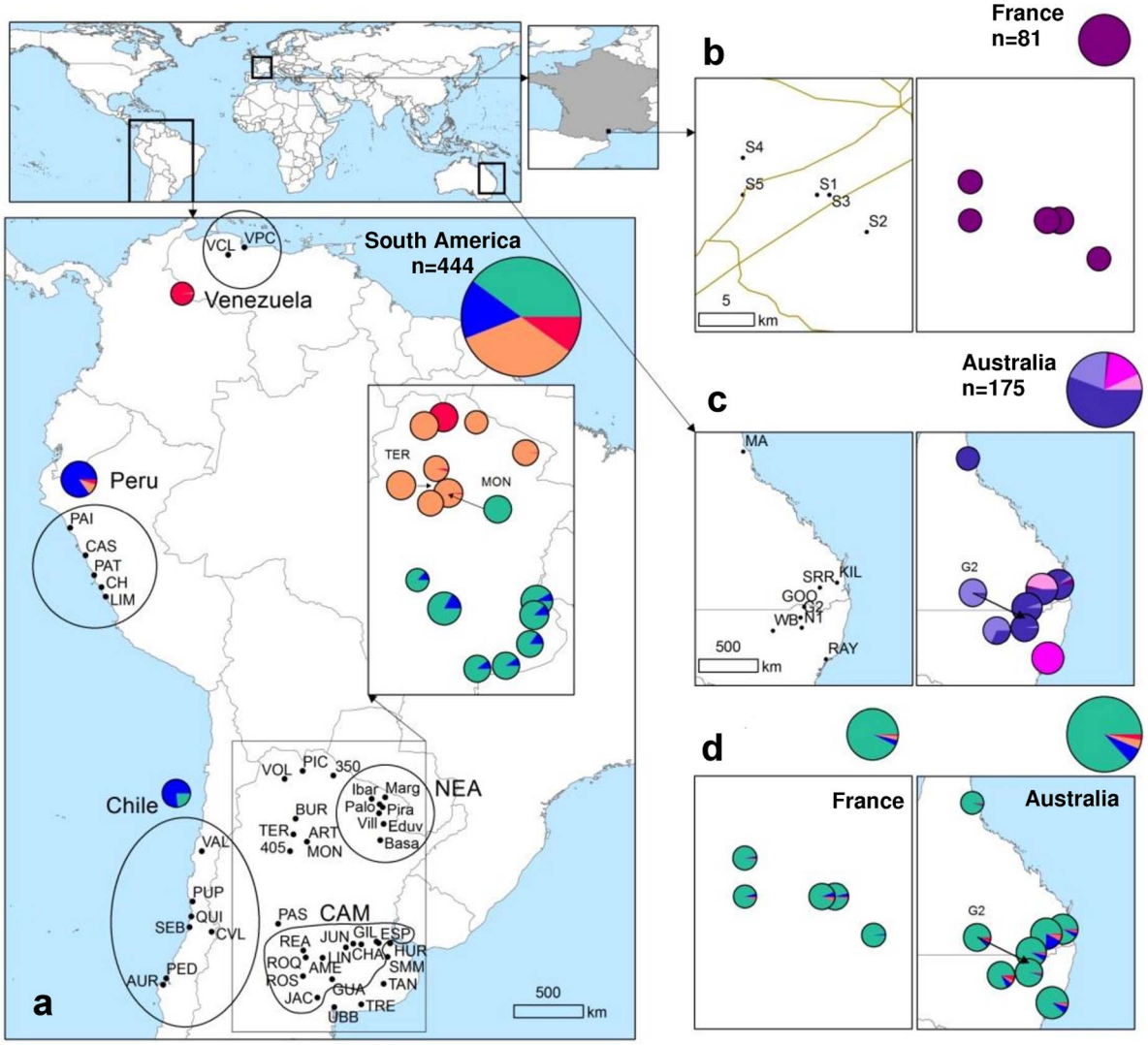

Fig. 4. The relative frequency of individuals assigned to each level-2 South American cluster summarized as pie diagrams, separately for populations collected from South America (a), Australia (b) and France (c) and for invasive individuals allocated to South American clusters (d) the size of pies are proportional to the number of individuals sampled and colors correspond to level-2 subdivisions in Fig. 3a.

under both the two-phase model and the stepwise mutation models (Table 5). Additionally, four introduced populations (MA, N1, RAY, S1) showed a significant mode-shift distortion of their allele frequencies, which is consistent with populations that have undergone a relatively recent genetic bottleneck. In South America, three populations showed a significant mode-shift distortion of their allele frequencies (PIC, PAS, TRE), and two of these also showed a significant heterozygosity excess under both mutation models in the Wilcoxon test (PIC, TRE). The data also suggest heterozygote deficiencies in two or four South American populations respectively under the TPM and SMM models.

Discussion

Our study suggests that *P. canescens* in South America includes two distinct genetic clusters, respectively from northwest Argentina and cen-

Table 4. Overall microsatellite genetic diversity indices for *Phyla canescens* on the population and region level.

Indices	*N* (population no.)	R_s	H_o	H_s	F_{is}	F_{st}
Population level						
South America	444 (19)	4.289[a]	0.603[a]	0.592[a]	−0.019[a]	0.244[a]
Australia	175 (8)	2.769[b]	0.710[a]	0.508[ab]	−0.398[b]	0.233[a]
France	81 (5)	3.224[b]	0.701[a]	0.486[b]	−0.441[b]	0.026[b]
Significance level		**	ns	ns	*	**
Region level						
South America	444 (1)	9.781[a]	0.603[a]	0.772[a]	0.207[a]	...
Australia	175 (1)	5.326[b]	0.710[a]	0.644[ab]	−0.133[ab]	...
France	81 (1)	4.986[b]	0.724[a]	0.496[b]	−0.251[b]	...
Significance level		**	ns	**	*	...

Notes: The parameters were compared among three regions and statistical significance levels are marked (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Results of paired comparisons are also shown; numbers followed by different letters are significantly different from each other. R_s, mean allele richness per locus; H_o, observed heterozygosity; H_s, gene diversity or expected heterozygosity; F_{is}, inbreeding coefficient; and F_{st}, among-population differentiation.

tral Argentina (supporting hypothesis 1), with the latter being the putative source of introduced populations in eastern Australia and southern France (supporting hypothesis 2), as well as elsewhere in South America. However, population-level genetic diversity was less in introduced populations than those from central Argentina, and significant population bottlenecking was detected for several introduced populations (rejecting hypothesis 3). The rapid evolution observed in Australia and France (Xu et al. 2010*a*) therefore occurred despite significant population bottlenecking and reduction of genetic diversity.

Genetic structure in the native range

Our study identified two distinct genetic clusters of *P. canescens* in South America and the differentiation between northwest Argentine and central Argentine clusters was also supported by morphological observations in the field (Sosa et al. 2008), although this requires more formal taxonomic analysis. This genetic differentiation may be attributable to the consequence of biogeographic isolation. The two clusters are separated by *c*. 500 km, with *P. canescens* being largely absent in between (Sosa et al. 2008). Biogeographic isolation may result from the architecture of the river system, as *P. canescens* is a floodplain species that is highly reliant on floods for dispersal and recruitment (Earl 2003, Macdonald et al. 2012). The northwest Argentine cluster largely occupies different watersheds (interior basins) from the central Argentina cluster (the La Plata Basin).

The only exception to biogeographic structuring in Argentina was a single northwest Argentine population (MON), which was assigned to the central Argentine cluster. This population was noted at the time as being morphologically distinct from other populations in northwest Argentina, including co-occurring ART (Julien and Sosa, *personal observations*). The lack of interbreeding with ART at the same site suggests that MON may represent a new, potentially human-assisted, introduction from central Argentina. Given that *P. canescens* has been used as an ornamental and its seed and stem fragments can be spread by vehicles and machinery (McCosker 1994), such human-assisted movement within South America is not unexpected. Alternatively, ART (northwest Argentine cluster) and MON (central Argentine clusters) might have adapted to different local photoperiods so that the lack of interbreeding may be caused by differences in flowering phenology. Tracking the persistence of MON would provide insights on the cause of the differentiation between the two native clusters. If this lineage persists, it would suggest that the central Argentina cluster is also preadapted to the drier and warmer climate of northwest Argentina and the differentiation could be caused by biogeographic barriers rather than climate.

Strong genetic structuring of *P. canescens* in South America and its ability to undergo rapid, selection-driven evolution in the phenotypes during invasion (Xu et al. 2010*a*) suggest that biogeographic differences in physiological requirements across South America are possible. For example, specimens from the coastal desert of Peru and Chile have unique leaf morphologies

Table 5. Results of BOTTLENECK tests (Wilcoxon and mode shift).

	Wilcoxon test (*P* value)				
	TPM		SMM		
Country/population	Def.	Exc.	Def.	Exc.	Mode shift
South America					
Venezuela (2)†	0.72	0.34	0.72	0.34	N
Argentina					
PIC	1.00	0.03	1.00	0.03	Y
350	0.31	0.89	0.31	0.89	N
VOL	0.34	0.72	0.28	0.78	N
BUR	0.06	0.96	0.04	0.98	N
TER	0.66	0.42	0.50	0.58	N
ART	0.08	0.95	0.08	0.95	N
MON	0.04	0.98	0.04	0.98	N
405	0.66	0.42	0.50	0.58	N
NEA (7)†	0.92	0.22	0.92	0.22	N
PAS	0.28	0.78	0.28	0.78	Y
CAM (11)†	0.04	0.98	0.02	0.98	N
HUR	0.28	0.78	0.28	0.78	N
SMM	0.50	0.58	0.50	0.58	N
TAN	0.06	0.96	0.04	0.98	N
TRE	0.98	0.02	0.98	0.04	Y
UBB	0.72	0.34	0.50	0.58	N
Peru (5)†	0.22	0.92	0.22	0.92	N
Chile (8)†	0.34	0.72	0.22	0.92	N
Australia					
MA	0.92	0.22	0.92	0.22	Y
KIL	0.98	0.02	0.96	0.05	N
SRR	0.72	0.34	0.66	0.42	N
GOO	0.89	0.31	0.89	0.31	N
G2	0.69	0.41	0.69	0.41	N
N1	1.00	0.01	0.99	0.02	Y
WB	0.96	0.06	0.96	0.06	N
RAY	0.96	0.06	0.96	0.06	Y
France					
S1	0.98	0.03	0.98	0.03	Y
S2	0.95	0.08	0.92	0.22	N
S3	0.78	0.28	0.78	0.28	N
S4	0.72	0.34	0.58	0.50	N
S5	0.28	0.78	0.28	0.78	N

Notes: For Wilcoxon test, *P* values of heterozygosity deficiency and excess are shown, with bold fonts highlight the cases with $P < 0.05$. For mode shift test, bold Y indicate shifted mode from normal L-shaped distribution of alleles.

† Pooled populations, with number of sites pooled given in the parenthesis (see annotate of Table 1).

(reduced, very linear and succulent, extremely grey-canescent; Kunth 1818, Boota 1979), which appears adaptive to this arid region (Kennedy 1992). However, *P. canescens* shows strong phenotypic plasticity in different growth condition (Xu et al. 2010*a*), microsite and season (Kennedy 1992). Thus, whether the genetic differentiation indicated by neutral microsatellite markers is associated with any adaptive functional traits to a specific local environment requires additional study. In particular, common garden experiments are needed to test whether the observed phenotypic differentiation among South American populations is heritable or due to plasticity.

P. canescens range expansion in South America

Genetic structuring of *P. canescens* across the major geographic barriers in South America (Murray et al. 2012), the Andes Mountains and the Amazon Basin, was weak with Venezuelan, Peruvian and Chilean individuals all identified as admixtures of the two Argentine clusters (O'Leary and Mulgura 2011). Chilean populations appeared to source from central Argentina, Peruvian mostly so, while Venezuelan populations were mainly from northern Argentina. The lack of differentiation at microsatellite loci suggests dispersal and naturalisation may have occurred relatively recently, and therefore may have been human-assisted. In particular, *P. canescens* has been observed in Peru at least since

1818 (Kunth 1818) and microsatellite data suggests that there have been multiple dispersal events across the Andes (respectively represented by the blue and blue-green clusters in Fig. 3a), potentially including specimens that already have complex genetic composition (e.g., artificially crossed ornamental plants). Herbarium and nursery records, together with high density sampling and more sensitive genetic tools (e.g., next-generation sequencing), may help clarify the anthropogenic role in the current phylogeography of *P. canescens* in South America.

Reduction of genetic diversity during the invasion of P. canescens

We found that invasive populations of *P. canescens* from both eastern Australia and southern France only include a subset of genetic diversity present in South America, mostly from central Argentina. This observation is consistent with our previous ISSR study (Xu et al. 2010*a*). Certainly this reduction of species-level genetic diversity is not unexpected for invasive populations, even for species that have undergone multiple introductions (Dlugosch and Parker 2008*a*).

Consistent with limited putative source populations, microsatellite results detected reduced allele richness and suggest historic population bottlenecks in introduced populations. This reduction in population-level genetic diversity (25–35% for R_s and 14–18 for H_s) was not observed in our previous ISSR study (Xu et al. 2010*a*), indicating that microsatellite markers are a more sensitive tool for detecting genetic diversity change in *P. canescens*. In introduced populations, we also detected significant heterozygote excess as a strong indication of population bottlenecking (Luikart et al. 1998). This is likely to be a conservative estimate of the level of bottlenecking due to the relatively limited number of loci and individuals sampled per population included in our study (Cornuet and Luikart 1996). These results highlight that *P. canescens* is capable of undergoing rapid evolution (Xu et al. 2010*a*) despite experiencing a significant population bottleneck. This is consistent with other invasive species that have undergone rapid evolution (Novak and Mack 2005, Dlugosch and Parker 2008*a*, Rollins et al. 2013). For example, a recent analysis found that population-level loss in genetic diversity in invasive plants that provided evidence of evolutionary changes (19%; expected heterozygosity; Rollins et al. 2013) was similar to the average across all invasive species (22.6% for species irrespective of evidence of adaptive change; Dlugosch and Parker 2008*a*). This suggests that neutral genetic diversity may not be as important as previously assumed for adaptive evolution of introduced species.

Our study demonstrates that rapid evolution occurred in *P. canescens* despite a substantial loss of population-level and species-level genetic diversity. This result has also been noted in a few but quite limited numbers of previous studies (Dlugosch and Parker 2008*a*, Harris et al. 2012, Rollins et al. 2013). For example, the invasive shrub *Hypericum canariense* demonstrated a rapid evolution in life history traits despite its establishment from a single, genetically depauperate founding population (Dlugosch and Parker 2008*b*). This ability may be an important mechanism for *P. canescens* and some other invasive plants to adapt to local environments in both the native and introduced ranges. To resolve the "genetic paradox" in invasion biology we therefore need to better understand the relationship between adaptive variation and neutral genetic diversity, quantify how much genetic diversity is sufficient to maintain the evolutionary potential of an invasive species, and assess the relative contribution of the type vs. the amount of genetic diversity to the evolutionary potential. Understanding these problems will provide deeper insights into the genetic basis underpinning the evolutionary potential of invasive species.

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Supplemental Material

Ecological Archives

Appendices A–E are available online: http://dx.doi.org/10.1890/ES14-00374.1.sm