Phyla canescens: multiple introductions into Australia as revealed by ISSR markers and nuclear ribosomal DNA internal transcribed spacers (ITS)

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Summary Lippia (*Phyla canescens* (Kunth) Greene: Verbenaceae) is a very invasive weed in wetlands and riparian zones in Australia and especially in the Murray Darling Basin. The use of chemicals as a control method is inappropriate in such environmentally sensitive areas, particularly the Macquarie Marshes and Gwydir Wetlands. Biological control will be the only option in many areas. We investigated genetic diversity in 12 populations of lippia from four different catchments in Australia, eight populations from the native range in South America and five populations from France where the species is non-native and invasive. Low levels of genetic diversity were detected within some Australian regions in contrast to the Argentinean and French populations. In the analyses the Australian material segregated with two disjunct regions in Argentina, suggesting that Australia has experienced multiple introductions of lippia.

Keywords Weeds, biogeography, biological control.

INTRODUCTION

Exotic species have caused considerable economic and environmental damage worldwide (Pimentel *et al.* 2001). The invasiveness of alien species and the ability of ecosystems to be invaded and colonised are among the main considerations for any weed control program (Dong *et al.* 2006). Understanding the genetic structure of populations of invasive weed species is important, especially for biological control programs involving host-specific pathogens (Burdon and Marshall 1981, Chapman *et al.* 2004).

Lippia (*Phyla canescens* Verbenaceae) is a serious weed of wetlands, riparian zones and floodplains, particularly in eastern Australia where many Ramsar wetlands are threatened by hydrological changes precipitated by soil-accreting lippia mats (Lucy *et al.* 1995, Earl 2003). A key area to investigate is the level of genetic diversity existing within and among infestations and whether novel alleles are appearing in introduced populations. The aim of this study is to evaluate the level of genetic diversity within and among the lippia infestations in Australia and to study the origin of Australian lippia in its native range in South America. The levels of genetic diversity in lippia may play a major role in determining the specificity of biological control agents. Medium to low levels of genetic diversity within and among lippia populations would increase the chances of biological control agents being effective in a range of environments.

Here we describe results on inter-population variability in lippia from Australia and two regions overseas.

MATERIALS AND METHODS

Collections of lippia were made from 12 populations in infested regions in Australia and from the native range of the species in South America. Samples were also obtained from non-native populations in France.

Silica gel dried samples were used for total genomic DNA isolation. In total, 20–50 mg of leaf tissue was crushed using a mixer mill. Genomic DNA was extracted using DNeasy Plant Minikit (QIAGENE, Inc.) following the manufacturer's instructions.

Inter-simple sequence repeat (ISSR) markers were used to estimate the level of genetic diversity within and among the populations of lippia. In total 12 primers were used to fingerprint individuals from all populations.

DNA sequences from internal transcribed spacer (ITS1 and ITS2) regions of ribosomal DNA were amplified in 25 µL volumes using standard polymerase chain reaction (PCR) protocols. ITS4i and ITS5 primers were used to amplify ITS regions. This resulted in ~700 base pairs (bp) of sequences. Resulting PCR products were purified by adding Exonuclease and Antarctic Phosphatase and incubating at 37°C for 15 min and 80°C for 15 min. Purified PCR products were sequenced using the ITS4i primer.

Obtained sequences were aligned using the online ClustalW tool from European Bioinformatics Institute (EBI). In total 592 bps were included in the analysis.

Population analyses As a dominant marker, ISSR bands were scored as present (1) or absent (0) and a data matrix was created. Data were analysed using GenAlex version 6 (Peakal and Smouse 2006) and genetic diversity indices were calculated for 18 populations (Table 1).

Biogeographic analyses The origin of Australian populations was investigated by conducting a phylogenetic analysis of nuclear ribosomal DNA internal transcribed spacers (ITS) sequences using PAUP* version 4.10b (Swofford 2002) with a sample from each region to determine relatedness. Five equally parsimonious trees were found by executing a heuristic search with gaps treated as missing data and characters unordered and weighted equally.

RESULTS

A total of 155 bands ranging from approximately 200 to 2000 bp were detected from 12 selected ISSR primers. The percentage of polymorphic loci (P) was as low as 16% in the Goondiwindi region and as high as 56% in the French populations. The percentage of polymorphic loci in Argentinean populations ranged from 18% to 52%. Analysis of molecular variance (AMOVA) (Figure 1) revealed that most of the genetic variation is distributed within populations. In the Principal Coordinate Analysis (PCA) two groups of the Argentinean populations were formed, with the Australian populations being represented in both (Figure 2). Therefore, it is reasonable to suggest that Australian populations have originated from at least two regions in Argentina.

The results of the phylogenetic analyses using ITS sequences also revealed that Australian populations of lippia group with two groups of populations from different geographic regions in South America. (Figure 3).

Other species of *Phyla* from South America form a non-resolved basal clade and are the subject of further studies.

DISCUSSION

The results indicate that some of the Australian populations of lippia show low levels of genetic diversity, whereas other populations have levels of diversity

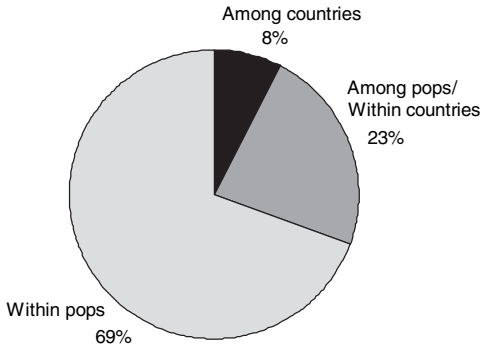

Figure 1. Partitioning of molecular variance in populations from Argentina, Australia and France.

Table 1. The number of observed (N_a) and effective alleles (N_e), Nei's genetic diversity (H), Shannon's information index (I) and percentage of polymorphic (P) loci. Argentina (Ar), Australia (Au) and France (Fr).

Population	N_a	N_e	H	I	P
Ar-BB	1.4286	1.2598	0.1507	0.2251	40.54%
Ar-BU	1.3056	1.1814	0.1064	0.1594	29.73%
Ar-H	1.3611	1.2354	0.1349	0.1993	35.14%
Ar-MR	1.5278	1.2987	0.1756	0.2657	51.35%
Ar-P	1.2414	1.2043	0.1101	0.1565	18.92%
Ar-RN	1.2857	1.2189	0.121	0.1747	21.62%
Ar-SM	1.3235	1.2386	0.1298	0.1877	29.73%
Ar-Tan	1.4706	1.3341	0.1876	0.2739	43.24%
Au-Goo	1.2	1.1652	0.0896	0.1278	16.22%
Au-K	1.3333	1.2136	0.1205	0.1781	32.43%
Au-RT	1.3333	1.2575	0.1383	0.1988	32.43%
Au-SR	1.3714	1.1834	0.1107	0.1709	35.14%
Au-Vic	1.2778	1.2076	0.1152	0.1669	27.03%
Fr-S1	1.5676	1.4515	0.2434	0.3489	56.76%
Fr-S2	1.3056	1.0236	0.1183	0.1746	29.73%
Fr-S3	1.4324	1.2935	0.1634	0.2393	43.24%
Fr-S4	1.3056	1.2088	0.1165	0.1706	29.73%
Fr-S5	1.2432	1.1747	0.0942	0.1363	24.32%
Total			$H_T = 0.1305$ $H_S = 0.1271$ $D_{ST} = 0.0034$ $G_{ST} = 0.0261$		

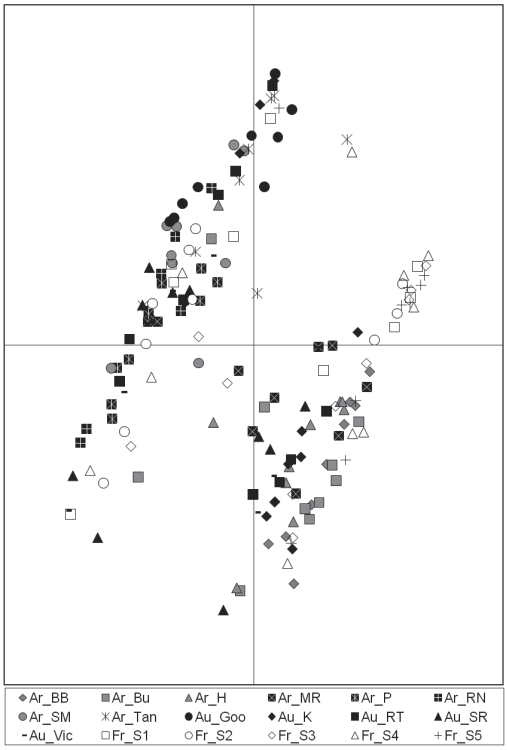

Figure 2. Principal coordinate analysis of lippia populations in Argentina (Ar), Australia (Au) and France (Fr).

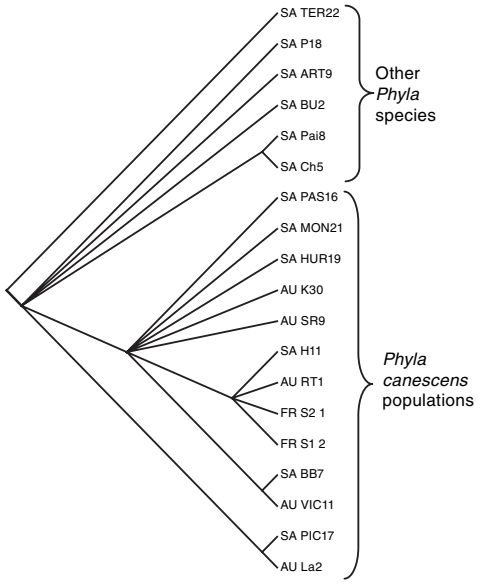

Figure 3. One of the five equally parsimonious cladograms. AU = Australia; FR = France; SA = South America.

comparable to that found in Argentina and France. In the PCA analysis the Australian populations were nested among Argentinean populations, suggesting that the Australian populations may have originated from these Argentinean populations or regions.

AMOVA showed that most of the variation is distributed within populations. This is supported by a low G_{ST} of 0.0261 which indicates that populations are genetically separated from one another. This also supports our view that Australian populations have originated from multiple introduction events.

Finally, results of phylogenetic analysis of ITS data again support the hypothesis that lippia has been introduced to Australia from multiple source populations.

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