Origins and diversity of exotic silverleaf nightshade (*Solanum elaeagnifolium*) present in Australia as determined by sequence analysis of a chloroplast intergenic spacer region

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Summary *Solanum elaeagnifolium* Cav. (silverleaf nightshade) is a native perennial of the Americas present in Australia as a Weed of National Significance. Knowledge of the genetic diversity and origins of silverleaf nightshade in Australia may inform future programs that propose using geographic source specific biocontrols to mitigate the weed.

Here we sequenced the *trnS-trnG* intergenic spacer in chloroplast DNA (cpDNA) from native and introduced populations of silverleaf nightshade to identify haplotypes representative of cpDNA lineages in the species distribution. We used network analysis to infer a genealogy among identified haplotypes and compared the array of haplotypes detected in Australia to those found elsewhere.

Eleven silverleaf nightshade haplotypes were identified and parsimoniously arranged as a shallow star-like genealogy. Two of five haplotypes in Australia were also in the United States of America, Mexico and South Africa. A third haplotype prevalent in Australia and South Africa was absent elsewhere but previously reported from Paraguay. Presence of two haplotypes unique to Australia indicated our sample was insufficient to identify all cpDNA diversity in the native distribution. Haplotype diversity in Australia was similar to that elsewhere and showed no signs of diminished genetic diversity expected if foundered by few colonisers.

We argue silverleaf nightshade in Australia originated from genetically diverse populations in North and South America. Silverleaf nightshade in Australia is likely at a late successional stage of colonisation, containing a variety of genetic backgrounds that may respond differently to various biocontrols. Our future work will explore additional genealogies to pinpoint geographic origins of silverleaf nightshade in Australia.

Keywords Genetic diversity, cpDNA, *trnS-trnG*, haplotype network, Weed of National Significance.

INTRODUCTION

Solanum elaeagnifolium (silverleaf nightshade) is a native perennial of the Americas that has emerged as a noxious exotic weed affecting agriculture in Australia. Silverleaf nightshade in Australia was first observed at Bingara (New South Wales) in 1901, but subsequently spread to other states and is now recognised as a Weed of National Significance. The source origin(s) of silverleaf nightshade in Australia is unknown. Recent analyses using dominant genetic markers indicates at least two geographically overlapping silverleaf nightshade gene pools may be present in Australia (Zhu *et al*. 2013) suggesting silverleaf nightshade entered Australia from either a single genetically diverse source, or multiple sources of varied genetic background.

More precise knowledge of the origins of an introduced weed can significantly inform future programs that propose using geographic source specific biocontrols to mitigate an exotic weed presence (Goolsby *et al*. 2006). Genealogical analyses of single and or multigene sequences at linked polymorphic chloroplast DNA (cpDNA) gene regions have proved useful for identifying both the diversity and origins of invasive species (Goolsby *et al*. 2006, Prentis *et al*. 2009). This approach has potential application for identifying the diversity and origins of invasive *Solanum* weeds (Zhang *et al*. 2013).

In this study, we sequenced and identified nucleotide polymorphisms present among silverleaf nightshade at a maternally inherited cpDNA intergenic spacer region. We used a parsimony network analysis to infer genealogical relationships among identified haplotypes in the native and introduced populations, and as a means to determine the likely genetic source origins of silverleaf nightshade present in Australia. We also identified the diversity of haplotype lineages present among silverleaf nightshade sampled from introduced populations in Australia and South Africa, and from the species native range in the Americas.

MATERIALS AND METHODS

Silverleaf nightshade native to the Americas were sampled (N = 84) from multiple sites in the United States of America, Mexico and Argentina. Silverleaf nightshade introduced to Australia (N = 131) and South Africa (N = 20) were also sampled. The Australian sample included preserved material from taxonomically identified voucher specimens (N = 4) provided by the Australian National Herbarium. All silverleaf nightshade samples were catalogued with unique specimen identifiers for DNA analyses. DNA was extracted from <1 mg of leaf from each specimen using a Corbett Research 1820 X-tractor Gene robotic system and protocols reported in Gopurenko *et al*. (2013). Sample PCRs (15 µL) targeted amplification of the $trnS_{GCU} - trnG_{UCC}$ intergenic spacer region present in cpDNA using primers previously reported by Hamilton (1999). Apart from primers used, all PCR and sequencing procedures followed that reported in Gopurenko *et al*. (2013). Bidirectional AB1 trace sequences were quality checked and assembled using Lasergene SeqMan Pro ver. 8.1.0(3) (DNASTAR Inc., Madison, Wisconsin). CpDNA haplotypes present among silverleaf nightshade sequences were identified using FABOX ver. 1.35 (Villesen 2007). Summary sequence statistics (uncorrected percent sequence distances) among cpDNA haplotypes were generated using MEGA version 5 (Tamura *et al*. 2011). Haplotype (h) and nucleotide (π) diversity (Nei 1987) at sample regions in the Americas (United States of America, Mexico and Argentina) and introduced populations (Australia and South Africa) were estimated using ARLEQUIN ver. 3.5.1.2 (Excoffier and Lisher 2010). Genealogical relationships among silverleaf nightshade haplotypes were estimated as a 95% parsimony network using TCS ver. 1.21 (Clement *et al.* 2000) assuming equal weighting among mutations and including indels as a 'fifth' character in analysis. Ambiguous connections within the network were resolved by assuming criteria as per Pfenninger and Posada (2002).

RESULTS AND DISCUSSION

We identified 11 cpDNA haplotypes among the sampled silverleaf nightshade, five of which were present in Australia (Figure 1). Closest matches at GenBank to all eleven haplotype sequences were exclusively to *Solanum elaeagnifolium* (>99% sequence similarity) confirming species identity of the samples. Levels of mutational polymorphism among the 11 haplotypes were minimal, with average and maximum sequence

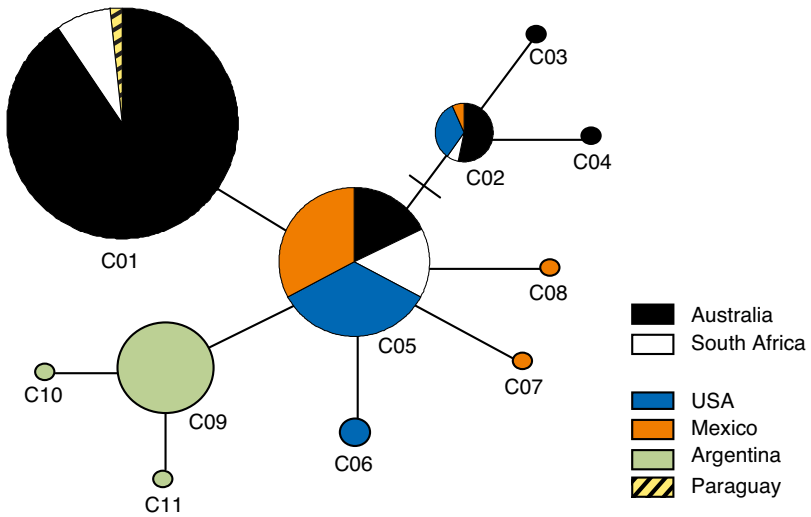

Figure 1. Parsimony (95%) network of genealogical relationships among silverleaf nightshade chloroplast DNA haplotypes sampled from native and introduced regions. Lines connecting eleven haplotypes labelled C01–C11 represent single mutations; crossbars on connecting lines indicate additional mutations. The size of each haplotype is indicative of its relative frequency observed in this sample. Haplotypes are shaded as per their frequency of occurrence in native populations in the Americas (United States of America (USA), Mexico and Argentina) and introduced populations in Australia and South Africa. Sequence identical to haplotype C01 observed in silverleaf nightshade from Paraguay (Levin *et al*. 2006) was obtained from GenBank accession AY998411.

differences among haplotypes observed as 0.428% and 0.774% respectively. The 95% parsimony network (Figure 1) joined all haplotypes as a shallow star-like network with the most recent common ancestral haplotype (C05) in the network observed in all populations except those in Argentina. All haplotypes observed in Argentina (C09; C10; C11) were unique to that country indicating some degree of isolation of the silverleaf nightshade populations in Argentina from the North American populations. In contrast, only a few low frequency haplotypes were unique to either Mexico or the United States of America; greater sampling effort is required to determine if these rare haplotypes have broader regional distribution in the Americas.

The ancestral cpDNA haplotype (C05) and one derived haplotype (C02) prevalent among silverleaf nightshade in North America were also found among silverleaf nightshade in Australia and South Africa (Figure 1). Most silverleaf nightshade in Australia (79%) and many in South Africa (45%) have a derived haplotype (C01) that was previously reported (Levin *et al*. 2006) as present in Paraguay South America, but not detected among silverleaf nightshade samples from the Americas in the current study. Surprisingly we detected two derived haplotypes present among silverleaf nightshade in Australia (C03 and C04) that were not detected elsewhere indicating our sample of silverleaf nightshade from the Americas was insufficient to identify all diversity present in the species native distribution.

Levels of haplotype diversity in Australia were high and similar to that observed in the native range in the Americas and at an introduced population in South Africa (Table 1). The high genetic diversity of silverleaf nightshade in the introduced populations present in Australia and South Africa suggests founders to these countries were diverse and not affected by any prolonged population bottleneck events; although successive and frequent founder events from multiple source populations would also promote the high levels of diversity evidenced here.

We argue the assemblage of cpDNA lineages present in Australia and South Africa originated from silverleaf nightshade populations in North and South America. The high genetic diversity of the exotic populations relative to that seen in the native distribution provides some evidence the exotic populations examined here are at late stages of population succession, rather than early stages of colonisation. This is similar to that evidenced at the exotic and invasive weed, *Echium plantagineum* L., also established in Australia (Burdon and Brown 1986). If the cpDNA diversity observed among Australian silverleaf nightshade is also reflective of the species overall genomic diversity, then it is likely silverleaf nightshade in Australia may differ in response to various biocontrols, particularly if resistance to biocontrols is linked to a diverse array of segregated alleles that are geographically widespread.

Our future work will expand sampling and explore nuclear genealogies integratively with the cpDNA work presented here in an attempt to pinpoint the geographic source origins of silverleaf nightshade in Australia.

Table 1. Genetic diversity at silverleaf nightshade populations native to the Americas and introduced to Australia and South Africa. Sample size (N), number of cpDNA haplotypes observed (N_H); haplotype (h) and nucleotide diversity (π) statistics.

Population	N	N_H	h	π
USA	30	3	0.393	0.0011
Mexico	25	4	0.230	0.0005
Argentina	29	3	0.136	0.0002
the Americas	84	8	0.611	0.0013
Australia	131	5	0.358	0.0013
South Africa	20	3	0.578	0.0011

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