Worldwide phylogeography of the globally invasive plant: *Jatropha gossypiifolia*

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Summary The introduction history of many invasive plant species, disseminated globally as ornamental plants, is poorly known. In particular, there is limited understanding of the role of genetic diversity, and single versus multiple sources of introduction in facilitating invasion success. We used chloroplast microsatellites (cpSSRs) to establish (i) patterns of phylogeographic structure in the native and introduced range of *Jatropha gossypiifolia*, and (ii) the origin(s) of introductions and the level of genetic diversity present in native and introduced populations. *Jatropha gossypiifolia* exhibited limited phylogeographic structure in its native range best explained by contemporary movement associated with the ornamental plant trade. Multiple introductions from diverse source locations and no reduction in genetic diversity was found in the introduced range of *J. gossypiifolia*.

Keywords Biological invasion, genetic diversity, introduction history, *Jatropha gossypiifolia*, ornamental plant, phylogeography.

INTRODUCTION

Bellyache bush (*Jatropha gossypiifolia* L.) is a perennial deciduous member of the Euphorbiaceae family. This species is a native of Central and South America. Its original range is not known (Howard 1989), but the current range extends from Florida USA, Mexico and Central America through the Caribbean islands to southern Brazil and Paraguay. This species now has a pantropical distribution and is recorded as an invasive weed in Australia, South East Asia, coastal areas of western Africa, India, Pacific Islands, in parts of South America and the Antilles Islands (Holm et al. 1979). In Australia, *J. gossypiifolia* can be found in dry tropical regions of Queensland, the Northern Territory and Western Australia (Bebawi *et al*. 2007). This species is currently considered one of the world's worst tropical weeds, due to its propensity for spread, ability to compete with and displace existing pasture species and toxicity to livestock.

Reconstructing introduction histories of introduced species is generally a difficult task, as introduction events may occur over large temporal and spatial scales (Puth and Post 2005). The history of spread of *J. gossypiifolia* throughout pantropical regions of the world is poorly known. The limited data on the introduction history of *J. gossypiifolia* indicates that it was transported globally prior to the late 1800s and for many decades after as an ornamental and/or medicinal plant. The number and geographic location of original source populations of these introductions however, are not known. Therefore it is not feasible to use historical data, due to a lack of relevant records, to confidently reconstruct the invasion history of this species. In spite of this, no effort has been made to use molecular genetic techniques to elucidate the geographic source(s) of *J. gossypiifolia* in Australia or overseas.

The primary goal of this study was to determine the geographical origin(s) of non-native populations of this invasive species, both in Australia and around the world, using molecular genetic techniques. To this end, we first determined the level of haplotypic diversity and phylogeographic structure across the native range of this species. In addition, we assessed patterns of haplotypic diversity among specimens collected from the introduced range, and matched the genetic profile of these specimens to those from the native range to resolve the geographic origin(s) of these populations.

MATERIALS AND METHODS

The majority of samples were obtained from the herbarium collection of *J. gossypiifolia* at the Missouri Botanical Gardens. Other specimens were collected from the CSIRO Mexican Field Station, Queensland Herbarium, Noumea Herbarium, and field collectors throughout Australia and India. In total 252 individual samples were collected.

DNA was extracted from samples using a Nucleospin 96 Plant Kit (MN). A 10–20 mg segment of dry

leaf material was pulverised to a fine powder using a beadmill, after which the Nucleospin 96 Plant Kit extraction protocol was followed. DNA was eluted in 100 µL of elution buffer and was quantified by running on an agarose electrophoresis gel with a quantifying DNA ladder of known concentration.

Ten cpSSR markers (ccmp1 – ccmp10, Weising and Gardner 1999), were assessed for their ability to amplify DNA fragments in *J. gossypiifolia*. PCR amplifications were performed in a final reaction volume of 10 µL containing ~20 ng of template DNA, 1x Amplitaq gold reaction buffer, 0.2mM of each dNTP, 2.5mM $MgCl_2$, 0.5µM each forward and reverse primer, and 1 U Amplitaq gold DNA polymerase (Applied Biosystems). The addition of 1% (ccmp 2 and 4) or 2% (ccmp 6, 7 and 10) PVP40 (polyvinylpyrrolidone) greatly improved amplification success of herbarium samples. PCR was carried out with an initial denaturation step of 94°C for 5 min, followed by 35 cycles of 94°C for 20 s, 50°C for 20 s, 72°C for 20 s, and a final extension at 72°C for 20 min. Forward primers of the 10 loci were labelled with a FAM, PET, NED or VIC florescent dye. Amplified products were combined with internal size standard LIZ 500 (-250) (Applied Biosystems), separated using an ABI 3730 capillary sequencer (Applied Biosystems), and scored using genemapper software version 4 (Applied Biosystems).

Population diversity indices were calculated for both the native and introduced range and included, number of haplotypes (*N*), number of private haplotypes (*N*p), and Nei's haplotype diversity ($HE = [n/(n-1)][1-\sum^{n}_{i=1} p_i^2]$), where, *n* is the number of individuals analysed and *pi* is the frequency of the *i*-th haplotype in a population (Nei 1987).

Genetic relationships among haplotypes were estimated using a distance matrix of the absolute size difference metric (D_{AD}), carried out in the program MICROSAT.

RESULTS

Six of the 10 cpSSR markers were successfully amplified from *J. gossypiifolia* DNA, and five of these (ccmp2, ccmp4, ccmp6, ccmp7 and ccmp10) were polymorphic (Table 1). Locus ccmp10 displayed inconsistent peak morphology and non-specific banding patterns, and was subsequently discarded from further analyses. Haplotypes were generated by combining polymorphisms across the four cpSSR loci, since they are assumed to be genetically linked. Across the four cpSSR loci 25 alleles were identified and varied between two and nine alleles at each locus (average 6.25 alleles per locus). This resulted in 33 distinct haplotypes among the specimens. Samples from both the native and introduced ranges of *J. gossypiifolia* were characterised by high haplotype diversity (native range, $H_E = 0.88 \pm 0.016$; introduced range, $H_E = 0.85 \pm 0.032$).

Native bellyache bush haplotypes were divided into three haplotype groups. Haplotype group 1 was found only in Bolivia, Peru and Colombia; while haplotype groups 2 and 3 had a cosmopolitan distribution throughout Central America, the Caribbean and South America. A lack of phylogeographic structure was observed in the Australian range of bellyache bush, with any haplotypes occurring throughout the entire Australian range.

Australian infestations of *J. gossypiifolia* were found to be introduced from diverse sources, including three geographically restricted haplotypes from Honduras, Costa Rica and Brazil as well as a number of widely distributed cosmopolitan haplotypes. African and Asian specimens were most commonly related to haplotypes of South American origin, particularly Brazil and Bolivia, or haplotypes that ranged from Brazil or Bolivia into Central America. In fact, with the exception of Oceania, where limited sampling found only haplotype 20 in New Caledonia, most introduced infestations of *J. gossypiifolia* were derived from multiple sources (e.g. Australia, Africa and Asia).

DISCUSSION

Limited phylogeographic structure was observed in the native range of *J. gossypiifolia*. The wide distribution of many haplotypes from different haplotype groups most likely reflects the recent movement of *J. gossypiifolia*, associated with the ornamental or medicinal plant trade. Many haplotypes and some haplotype groups have large discontinuities in their distributions, and a number of isolated occurrences are associated with urban areas where they are presumed

Table 1. cpSSR loci used to examine genetic variation and the number of introductions of *Jatropha gossypiifolia* in this study.

Locus	Dye	Primers	Size (BP)
ccmp2	FAM	GATCCCGGACGTAATCCTG	204
		ATCGTACCGAGGGTTCGGAAT	
ccmp4	PET	AATGCTGAATCGAYGACCTA	127
		CCAAAATATTBGGAGGACTCT	
ccmp6	FAM	CGATGCATATGTAGAAAGCC	116
		CATTACGTGCGACTATCTCC	
ccmp7	NED	CAACATATACCACTGTCAAG	130
		ACATCATTATTGTATACTCTTTC	

garden escapees. As *J. gossypiifolia* is commonly grown as an ornamental or medicinal plant in South America and the Antilles Islands (Holm *et al*. 1979), this hypothesis would seem most plausible, although long-distance dispersal may have also contributed to absence of strong genetic structure in *J. gossypiifolia*. Given that natural seed dispersal of this species is very limited, the long-distance dispersal hypothesis would seem unlikely.

More rigorous sampling is required to confirm either hypothesis due to some limitations to the current data set. The geographic spread and representation of native samples was constrained by the availability of herbarium specimens, with some regions over represented (e.g. Costa Rica n = 11) and others under represented (e.g. Guatemala, n = 1). Many haplotypes were attributed to single specimens, and this limitation could be overcome by a more comprehensive sampling regime across the native range providing more complete information concerning the geographic extent of haplotype groups.

This study demonstrated that multiple introductions of diverse haplotypes from throughout the native range had occurred in the exotic range of *J. gossypiifolia*. The introduction of multiple haplotypes and genetic diversity from numerous source locations of *J. gossypiifolia* may explain the considerable morphological variation seen in Australia (Bebawi et al. 2007).

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