Introduction of Paterson's curse (*Echium plantagineum*) to Australia – unravelling the story by DNA sequence analysis

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Summary Paterson's curse (*Echium plantagineum* L.) is an annual/biennial species in the family Boraginaceae. Native to N.W. Africa, the Iberian Peninsula and Atlantic Western Europe, it is a noxious weed in Australia and has spread over 30 million ha resulting in >A$250 million annual losses to agricultural industries (Anon. 2009). Determination of the pathway of introduction is critical to gain further insight into the invasion process including the sources of propagules.

Two hypotheses regarding the origin of Australian populations were formulated: 1) introduction as an ornamental via seed importation from England; 2) multiple introductions via South Africa from the Iberian Peninsula and UK.

In this study, 131 Australian *E. plantagineum* samples were analysed using one nuclear gene region (ITS) and three chloroplast gene regions (*trn*L intron, *trn*L-*trn*F spacer and *trn*H-*psb*A) and compared with successfully sequenced samples from the Iberian Peninsula (N = 43), UK (N = 15), South Africa (N = 24) and the USA (N = 4). A high level of genetic diversity was found in Australia, which suggests a complex history of introduction of Australian *E. plantagineum*. The most abundant haplotype in Australia was shared with the Iberian Peninsula and UK populations, which suggests that they are both critical source populations for Australian *E. plantagineum*.

Importantly, however, 10 of 12 haplotypes found in Australia were represented in South Africa, indicating its potentially important historical role as an intermediate source population for *E. plantagineum* introductions. The Australian *E. plantagineum* population was genetically most similar to the UK population and significantly different from other populations, which supports UK *E. plantagineum* as potential founding population and the Iberian Peninsula and South African populations as additional sources of genetic diversity.

Keywords Genetic diversity, invasive weed, introduction history, DNA barcoding.

INTRODUCTION

Paterson's curse (*Echium plantagineum* L.) is a noxious weed in Australia. It produces toxic pyrrolizidine alkaloids in the shoots (Skoneczny *et al.* 2015, Weston *et al.* 2013) and bioactive shikonins in the root (Weston *et al.* 2011, Zhu *et al.* 2016a). It competes with pasture grasses and is often toxic to grazing livestock. *Echium plantagineum* now infests more than 30 million ha and causes over A$250 million in annual losses (Anon. 2009).

Determining the path of introduction of an invasive species is critical in comprehending the plant invasion process, managing current infestations, and preventing future introductions. *Echium plantagineum* is native to N.W. Africa, the Iberian Peninsula and Atlantic Western and was first recorded in 1843 in the Camden Gardens, Sydney as an ornamental obtained from the UK (Piggin 1977).

This record, for decades, has been considered the first introduction event (Piggin 1982). However, considering the introduction of Merino sheep from the native range of *E. plantagineum* in the late 1800s, Zhu *et al.* (2016b) hypothesized that *E. plantagineum* was introduced to Australia much earlier than 1843, as an accidental by-product of livestock shipments to the country.

Molecular approaches such as DNA sequence analysis have been widely used for genetic structure and introduction history studies (Shaik *et al.* 2015). However, information on the genetic background of *E. plantagineum* is very limited. Burdon and Brown (1986) reported a similar level of genetic diversity of Australian *E. plantagineum* compared to the species in the native ranges using isozyme markers. A nuclear gene region (ITS) and three chloroplast regions (*trn*H-*psb*A spacer, *trn*L intron and *trn*L-*trn*F spacer) were recently investigated to separate two closely related species: *E. plantagineum* and *E. vulgare* (Zhu *et al.* 2014). These gene regions were further used to investigate genetic diversity and structure of both species in Australia (Zhu *et al.* 2016b). Region-specific haplotypes were detected, which supports the theory of multiple introduction events of *E. plantagineum* in Australia (Zhu *et al.* 2016b).

In this study, four previously explored gene regions (Zhu *et al.* 2014) were used to investigate the introduction history of Australian *E. plantagineum* by comparing the genetic diversity of populations from the native Iberian Peninsula population to Australia, South Africa and the UK. We hypothesized that, *E. plantagineum* was historically sourced directly from the UK and the Iberian Peninsula, as well as indirectly through South Africa.

MATERIALS AND METHODS

Leaf tissue was sampled from both the field and preserved herbarium specimens, provided by the Australian National Herbarium and the Natural History Museum, London UK. Geographic distribution of sampled plants comprised: the Iberian Peninsula (N = 53), Australia (N = 131), South Africa (N = 25), the UK (N = 40) and the USA (N = 4).

DNA extraction, PCR, sequencing, alignment and network analysis were performed as described by Zhu *et al.* (2013). Samples were sequenced for one nuclear region (ITS) and three chloroplastic regions (*trn*H-*psb*A spacer, *trn*L intron and *trn*L-*trn*F spacer).

RESULTS

Only two alleles were found in the ITS region. The level of polymorphism in the ITS region was not sufficient for genotype investigation comparing the native and invasive ranges and was disregarded for further analysis.

By contrast, the chloroplastic regions are more polymorphic than the nuclear region. Only samples with all three chloroplastic regions successfully sequenced were used in further analysis, including; 43, 131, 24, 15 and 4 samples from the Iberian Peninsula, Australia, South Africa, the UK and the USA, respectively (Table 1). Low PCR success in UK samples probably because of DNA degradation due to long term preservation of herbarium specimens. Analysed regions were represented by 8, 12, 11, 4 and 2 haplotypes, respectively (Table 1). Levels of nucleotide (π) and haplotype (h) diversity at the cpDNA regions were similar among the Iberian Peninsula ($\pi = 0.0016$; $h = 0.7409$), Australia ($\pi = 0.0020$; $h = 0.7657$) and South Africa ($\pi = 0.0023$; $h = 0.8913$) (Table 2). In contrast, *E. plantagineum* from the UK was genetically much less diverse compared to other populations ($\pi = 0.0010$; $h = 0.3714$).

Ten out of 12 haplotypes in Australia were recovered in South Africa despite a relatively small sample size (N = 24, in South Africa; Table 1 and Figure 1). However, the most common haplotype (Hap 4) in Australia was absent in South Africa, but present in the UK as 80% of haplotypes encountered, despite a small sample size in the UK. No significant difference was found between UK and Australian populations of *E. plantagineum* using a population pairwise F_{ST} test. In contrast, significant pairwise differences were found between all other *E. plantagineum* populations (Table 3).

Table 1. *Echium plantagineum* haplotypes found in the Iberian Peninsula (native range), Australia (Aus), South Africa (SAf), UK and USA. *n*: number of samples successfully sequenced at the three chloroplastic regions. * indicates the presence of particular haplotype.

Region	*n*	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14
Native	43		*	*	*		*	*	*			*		*	
Aus	131	*	*	*	*	*	*	*	*	*	*	*	*		
SAf	24	*	*	*		*	*	*	*	*		*	*		*
UK	15		*		*	*	*								
USA	4		*		*										

DISCUSSION

High levels of chloroplast genetic diversity evident in *E. plantagineum* sampled from its native range in the Iberian Peninsula, and from invasive populations in South Africa and Australia, suggest a complex history of introduction of this species. Interestingly, most haplotypes detected in Australia were also present in the invasive South Africa population as opposed to the native population in the Iberian Peninsula (Table 1 and Figure 1), indicating either un-sampled diversity is present in the Iberian Peninsula, or was lost from that region after introduction of the species into South Africa. Seven and 10 out of 12 Australian haplotypes were found in the Iberian Peninsula and South Africa, respectively, which suggests that the Iberian Peninsula and South Africa were likely important sources of *E. plantagineum* introduction to Australia.

Table 2. Nucleotide (π) and haplotype (h) diversity of *E. plantagineum* populations from the Iberian Peninsula (native range), Australia, South Africa, the UK and the USA.

Populations	π	h
Native	0.0016 ± 0.0011	0.7409 ± 0.0565
Australia	0.0020 ± 0.0012	0.7657 ± 0.0297
South Africa	0.0023 ± 0.0014	0.8913 ± 0.0395
UK	0.0010 ± 0.0008	0.3714 ± 0.1532
USA	0.0006 ± 0.0007	0.6667 ± 0.2041

Table 3. Pairwise comparison (shown as F_{ST} value) of genetic structure between *E. plantagineum* populations.

Populations	Australia	UK	South Africa
Australia			
UK	0.03		
South Africa	0.16***	0.32***	
Native	0.21***	0.38***	0.06*

Statistical significance indicated as *** P <0.001 and * P <0.05. USA samples were excluded in this figure due to limited sample size.

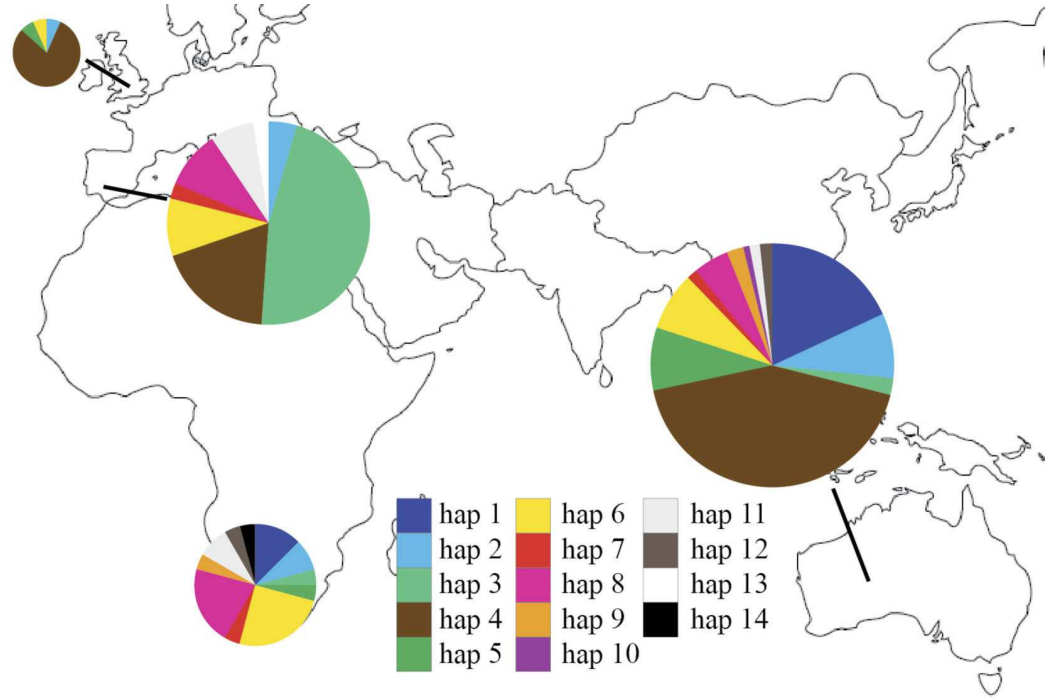

Figure 1. Distribution of haplotypes of *E. plantagineum* in its native range (Iberian Peninsula), Australia, South Africa and the UK. The USA samples were excluded in this figure due to limited sample size.

Interestingly, although *E. plantagineum* has been cultivated since 1658 in the UK (Kloot 1982), it showed a very limited sequence variation. Four haplotypes were found in the UK with one haplotype predominating (Hap 4, Figure 1), while the other three haplotypes were represented by only a single specimen. The predominant Hap 4 in the UK is also the most frequent and widely distributed haplotype in Australia (Figure 1), indicating a portion of the *E. plantagineum* diversity in Australia was most likely sourced either directly as an ornamental import from the UK, and/or indirectly from native populations in the Iberian Peninsula where the haplotype is also evident. Historically, climate conditions in the UK have generally been unsuitable for the establishment of *E. plantagineum* (Dr. Mark Spencer, pers. comm.).

The limited diversity evident in UK samples suggests adaptive selection for a restricted range of haplotypes tolerant of UK climate. Alternatively, population bottleneck processes associated with invasion history may also have limited the diversity present in the UK. All herbarium specimens collected from the UK evaluated in this study were relatively old (from 1845–1959). Established populations of this species in the UK are now generally restricted to the Channel Islands and Cornwall. It would be useful to monitor current genetic composition in the UK.

The high level of genetic diversity in South Africa suggests either multiple introductions of *E. plantagineum* or single introduction of multiple genotypes to this region. However, introduction of 11 haplotypes at one point in time is unlikely, especially considering the presence of several rare haplotypes from the native range (Hap 2, 9, 11, 12 and 14). The South African samples investigated in this study were collected from localities near Cape Town, which was the most important port connecting Europe to the Far East, before the opening of the Suez Canal in 1869. Since 1488, shipments of agricultural products from the UK and the Iberian Peninsula travelling to Asia and Australia (onwards from 1788) travelled to Cape Town to replenish their supplies before crossing the Indian Ocean. Centuries of trade likely enabled the introduction of less common haplotypes to South Africa from Europe with seed contamination of agricultural products leading to the high level of genetic diversity observed in South Africa. Similarly, introductions via South Africa to Australia were also noted in other invasive species such as capeweed (Scott 1990) and redlegged earth mite (Ridsdill-Smith 1997). Surprisingly, the most common Hap 4 in the UK and Australia was not observed in South Africa. This may be associated with the limited sampling size or lack of this genotype in South Africa.

Colonial British settlement of Australia after 1788 allowed the export of produce to Australia from Europe via South Africa. Serial accidental introductions of *E. plantagineum* from South Africa to Australia would therefore likely have increased the probability of export of *E. plantagineum* genetic diversity in South Africa to Australia.

In conclusion, the genetic diversity of invasive *E. plantagineum* in Australia was likely derived from deliberate introduction of plants as ornamental subjects from the UK in 1840s or the accidental introduction via agricultural products from the UK since 1788. In addition, Australian introductions of *E. plantagineum* were reinforced by trade shipping and accidental importations of seeds from genetically diverse, invasive *E. plantagineum* populations in South Africa, which supplements additional genetic diversity in Australia. Considering the large number of ships arriving in Australia via South Africa from UK, the first introduction event of *E. plantagineum* to Australia could also potentially have occurred much earlier than that first recorded in 1843.

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