Invasive Fireweed in Australia: Exploring the Invasion Dynamics of Senecio madagascariensis using Population Genetics

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Abstract

Senecio madagascariensis (fireweed) is an herbaceous flowering plant native to South Africa and known to be invasive in Australia, Argentina, Brazil, Hawaii and Japan. Fireweed first appeared in the Australian herbarium record in 1918 but little is known about its invasion dynamics. This thesis presents detailed molecular genetic analyses of *S. madagascariensis* in Australia and native South Africa, as well as broad biogeographic analysis of *S. madagascariensis* invasions from around the world. The thesis aims were to elucidate geographic source, mode of introduction, spread dynamics, hybridisation outcomes with an Australian congener, and potential for adaption to the Australian environment.

Using nuclear and chloroplast microsatellites, populations from across the range in Australia and in the KwaZulu Natal province of South Africa were genotyped. Additionally, chloroplast microsatellites were used to genotype all *S. madagascariensis* specimens held in the Queensland Herbarium, National Herbarium of Victoria and National Herbarium of New South Wales, and from contemporary populations in all other known invasive ranges of the species across the world. Amplified fragment length polymorphisms (AFLPs) were used to study the outcome of hybridisation between *S. madagascariensis* and a *S. pinnatifolius* 'dune variant' in natural populations, and to look for evidence of potential selection acting on the genome.

Results indicate that *S. madagascariensis* was introduced at least twice to Australia and has resulted in sequential bottlenecks due to seeding of new invasion foci from material within Australia. Global *S. madagascariensis* invasions have limited diversity and are consistent with secondary invasions originating in Australia. Hybridisation

between *S. madagascariensis* and *S. pinnatifolius* 'dune variant' occurs in natural populations at low levels but does not appear to result adult hybrid plants at least in the populations studied here. Two AFLP loci were highlighted by both population level outlier analyses, and individual level regression analyses against environmental variable data, providing evidence for potential recent selection on the genome and an indication of putative selective agents.

In conclusion, this thesis explores the invasion history and contemporary invasion dynamics of one of Eastern Australia's worst weeds, *S. madagascariensis*. Findings highlight the ongoing need for appropriate biosecurity measures to limit accidental founding of further invasion foci and flag Australia as a potential bridgehead for *S. madagascariensis* invasions worldwide. Location of putative source populations points to areas which may prove fruitful for locating suitable biological control agents. The native *S. pinnatifolius* is unlikely to introgress with *S. madagascariensis* but risks displacement in native environments where the two occur together. Evidence of potential recent selection on the genome associated with rainfall and light availability could indicate that *S. madagascariensis* is adapting to Australian conditions, making further encroachment on the range of *S. pinnatifolius* more likely.

Originality Statement

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Eleanor Elizabeth Dormontt and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Dormontt EE, lowe AJ, Prentis PJ. 2011. Is rapid adaptive evolution important in successful invasions. In: Richardson DM ed. *Fifty Years of Invasion Ecology: The Legacy of Charles Elton.* 1st ed., Blackwell Publishing Ltd. (John Wiley & Sons, Inc)

Prentis PJ, Wilson JRU, Dormontt EE, Richardson DM, Lowe AJ. 2008. Adaptive evolution in invasive species. *Trends in Plant Science,* **13**: 288-294. (Elsevier Ltd)

Wilson JRU, Dormontt EE, Prentis PJ, Lowe AJ, Richardson DM. 2009a. Biogeographic concepts define invasion biology. *Trends in Ecology & Evolution,* **24**: 586-586. (Elsevier Inc)

Wilson JRU, Dormontt EE, Prentis PJ, Lowe AJ, Richardson DM. 2009b. Something in the way you move: dispersal pathways affect invasion success. *Trends in Ecology & Evolution,* **24**: 136-144. (Elsevier Inc)

Dedication

I dedicate this thesis to my late Great Grandfather Walter Rothsay Adams. Although I never had the chance to know him personally as an adult, I feel a strong connection through my father and I think my love of all things scientific is somehow in the genes from his side. One of the few pictures of us together is of me as a toddler in the garden, with Grandad patiently explaining something or other to me about the plants. I also like to think he would be the one member of my family with the intestinal fortitude to read this whole thesis, perhaps even with interest! Although other friends and family may pleasantly surprise me yet. It is with great thanks to all of them that I complete this mammoth undertaking. Without their unswerving support and pathological faith in my abilities, I would not be the person I am today or have achieved so much. Read on at your own risk…

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To my parents, thanks for everything (including getting divorced) – you've done a great job and I love you both very much. To Martina, my 'other mother', you have inspired me through your hard work and dedication, a truly wonderful role model. And finally to my children Ashley and Zachery, young as you are, I hope you will one day read this (even if you don't get much further than these acknowledgements) and be inspired to know that if I can do this – you can do anything.

Ok enough nostalgia... on to the good stuff...

CHAPTER 1

Introduction, including excerpt from:

Is rapid adaptive evolution important in successful invasions?

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***Fifty Years of Invasion Ecology: The Legacy of Charles Elton*, 1st edition.**
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Statement of Authorship

Is rapid adaptive evolution important in successful invasions?

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Dormontt, E. E. (Candidate)
Contributed to initial manuscript conceptualisation, acted as lead author in drafting initial manuscript, coordinated addressing reviewers' comments, prepared figures and tables.

I hereby certify that the statement of contribution is accurate

Signature:..... Date: 28/03/2013

Lowe, A. J.
Contributed to initial manuscript conceptualisation, and commented on and edited subsequent manuscript drafts.

I hereby certify that the statement of contribution is accurate and consent to the inclusion of this manuscript in Eleanor Dormontt's PhD thesis.

Signature:..... Date: 28/03/2013

Prentis, P. J.
Contributed to initial manuscript conceptualisation and initial drafting, commented on and edited subsequent manuscript drafts.

I hereby certify that the statement of contribution is accurate and consent to the inclusion of this manuscript in Eleanor Dormontt's PhD thesis.

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The following sections (1.1-1.4) are taken from:

Dormontt EE, Lowe AJ, Prentis PJ. 2011. Is rapid adaptive evolution important in successful invasions? In: Richardson DM ed. *Fifty Years of Invasion Ecology: The Legacy of Charles Elton.* 1st edition, Blackwell Publishing Ltd.

The full book chapter as published can be found in Appendix 1. Minor edits have been made to improve flow and consistency. The remainder of Chapter 1 as presented here in this thesis is an unpublished review of *Senecio madagascariensis* invasion in Australia, identification of knowledge gaps that will be addressed by the research presented in this thesis, and a contextual statement. The contextual statement broadly summarise the purpose, content and conclusions of the various chapters that make up the remainder of this thesis, and describes how each chapter relates to other chapters and the thesis as a whole. Specific background details and relevant literature pertinent to each study are described and reviewed in each chapter and so are not repeated in the contextual statement.

1.1 Ecological and evolutionary explanations of invasion success

Ecological explanations of invasion success have dominated the literature on biological invasions since Elton's seminal publication (Elton, 1958). Traditionally this focus has primarily been on traits of the exotic organism or recipient environment that make invasion more likely (e.g. growth rate and leaf area (Grotkopp and Rejmanek, 2007); species richness, reviewed in Fridley et al. (2007). Other theories concentrate on the unique interactions that can arise between environment and invader, such as enemy release (reviewed Liu and Stiling, 2006) and novel weapons (see, for example, Thorpe et al. 2009). Introduction dynamics such as propagule pressure (Simberloff, 2009), residence time (Wilson et al., 2007) and human use (Thuiller et

al., 2006), have also been identified as important ecological factors facilitating successful invasions. The possible evolutionary determinants of invasion success have received much less attention (Callaway and Maron, 2006), but the past few years have seen a surge in interest, with publications considering evolutionary processes now more common than those with a purely ecological perspective (Fig. 1).

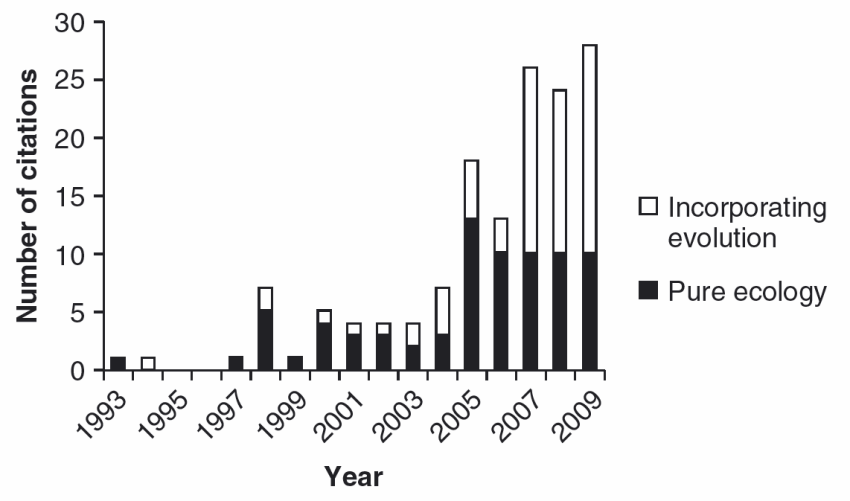

Figure 1. Citations returned from the ISI Web of Science, search topic = invasion - biology AND ecology NOT evolution, (referred to here as 'pure ecology '); and topic = invasion - biology AND evolution (referred to here as 'incorporating evolution').

Part of the reason for this delay in realizing the potential relevance of evolutionary mechanisms to invasions (despite some early recognition, for example Baker, 1965) is that invasion dynamics were typically considered likely to constrain rather than promote adaptive evolution. For example, introduction of a few propagules from a single area would likely result in a genetic bottleneck in the invasive range. The ensuing small population would likely be more inbred, have lower genetic diversity, have a more uniform susceptibility to pathogens, and have limited (or non-existent) gene flow with native populations. In fact, many of these characteristics are those generally associated with rare, endangered species. Empirical evidence supports one or more of these assumptions in a variety of invasive taxa (see, for example,

Amsellem et al., 2000, Amsellem et al., 2001, Bailey et al., 2009, Prentis et al., 2009, Schmid-Hempel et al., 2007, Zhang et al., 2010; reviewed studies in Dlugosch and Parker, 2008a), supporting the notion that many species succeed in their new environment without need for adaptive evolution. However, there are a growing number of case studies that do not conform to this pattern (see, for example, Chun et al., 2010, Marrs et al., 2008a; reviewed studies in Roman and Darling, 2007 and Dlugosch and Parker, 2008a), or exhibit evidence for rapid adaptive evolution despite these constraints (see, for example, Dlugosch and Parker, 2008b). This new evidence, combined with a conceptual shift towards viewing biological invasions as both quantitatively and qualitatively different to natural colonizations (Ricciardi, 2007, Wilson et al., 2009), has led to greater consideration of the importance of evolutionary processes in facilitating successful invasions.

In the remainder of this chapter we look at two of the main ecological explanations for invasion success, propagule pressure and enemy release. Excellent reviews on these hypotheses already exist (see, for example Orians and Ward, 2010, Simberloff, 2009) so it is not our intention to cover old ground; rather, it is to summarize some of the recent literature and highlight how these ecological features can promote conditions conducive to adaptive evolution. We then examine what the genetic mechanisms behind rapid adaptive evolution might be and highlight the empirical regimes required to distinguish between alternate hypotheses.

1.2 Propagule pressure and rapid adaptive evolution

Support for the importance of propagule pressure in biological invasions

One of the most influential parameters of invasion success appears to be propagule pressure, which is the number and size of introductions to the recipient environment

(reviewed in Simberloff, 2009). In studies examining the relative impacts of various parameters thought to influence invasion success, propagule pressure has consistently been identified as the primary determinant. For example, propagule pressure exerts a greater influence than source population latitude (Maron, 2006), abiotic conditions (Von Holle and Simberloff, 2005) and species richness of the recipient environment (Eriksson et al., 2006, Von Holle and Simberloff, 2005). As propagule pressure can fluctuate both temporally and geographically for a single species, invasion success can appear unpredictable unless propagule pressure is considered (Lockwood et al., 2005). Models seeking to explain abundance and distribution of invasive species that incorporate propagule pressure have been consistently more successful than those that have not (Richardson and Pyšek, 2006), and any attempts to predict future invasions must similarly allow for this variable (Rouget and Richardson, 2003). In fact, Colautti et al. (2006) suggest that propagule pressure should be the null model for all biological invasions.

How propagule pressure can promote conditions conducive to rapid adaptive evolution

The relationship between propagule pressure and invasion success is not necessarily linear, i.e. more propagules more often, does not always equate to optimal propagule pressure. For example, patch occupancy modelling of sexual diploid organisms by Travis et al. (2005) revealed a parabolic relationship between propagule pressure and invasion success for species poorly adapted to the environment; an intermediate propagule pressure aided establishment and facilitated rapid adaptive evolution to the local environment.

Optimal propagule pressure can promote conditions conducive to rapid adaptive evolution in several different ways. Theoretically, propagule pressure could reduce

mate limitation in outcrossing species, ultimately relaxing Baker's law (Baker, 1955, Stebbins, 1957) and increasing the opportunities for obligate outcrossers to establish invasive populations. A continual flow of propagules into a novel environment can also relieve inbreeding depression and limit genetic drift, as well as increase genetic diversity (Simberloff, 2009). In this way, diversity within introduced populations can reach levels comparable to native populations (Table 1), or if propagules arrive from multiple differentiated source populations, can even exceed that which is observed in the native range (see, for example, Lavergne and Molofsky, 2007). The prominence of multiple source populations in biological invasions (Table 1, Bossdorf et al., 2005, Dlugosch and Parker, 2008a, Roman and Darling, 2007, Wilson et al., 2009) has really only come to light since the advent of molecular techniques able to distinguish putative source origin. However, the importance of multiple sources is still under some debate, the increased diversity brought about by multiple sources can certainly theoretically contribute to rapid adaptive evolution (Prentis et al., 2008) but a causal association has not been demonstrated (Dlugosch and Parker, 2008a). Simberloff (2009) reviewed 14 studies in which propagule pressure had been positively associated with increased genetic diversity, three of which were also able to demonstrate rapid evolution (Lavergne and Molofsky, 2007, Roman, 2006, Saltonstall, 2002) but were unable to distinguish between neutral and adaptive processes.

Species	Common name	Family	Native range	Invasive range(s) studied
Aegilops triuncialis	Barbed goatgrass	Poaceae	Eurasia	California
Alliaria petiolata	Garlic mustard	Brassicaceae	Europe	North America
Ambrosia artemisiifolia	Annual ragweed	Asteraceae	North America	France
Apera spica-venti	Silky bentgrass	Poaceae	Europe	Canada
Avena barbata	Slender oat	Poaceae	Mediterranean Basin	California
Brachypodium sylvaticum	False brome	Poaceae	Eurasia	North America
Bromus mollis	Soft brome	Poaceae	England	Australia
Bromus tectorum	Cheatgrass	Poaceae	Eurasia	North America
Bryophyllum delagoense	Mother of millions	Crassulaceae	Madagascar	Australia
Capsella bursa-pastoris	Shepherd's purse	Brassicaceae	Europe	North America
Centaurea diffusa	Diffuse knapweed	Asteraceae	Eurasia	North America
Centaurea stoebe micranthos	Spotted knapweed	Asteraceae	Eurasia	North America
Chondrilla juncea	Skeleton weed	Asteraceae	Turkey	Australia
Clidemia hirta	Soapbush	Melastomataceae	Costa Rica	Hawaii
Cytisus scoparius	Scotch broom	Fabaceae	Europe	Australia; California; New Zealand; Chile
Echium plantagineum	Paterson's curse	Boraginaceae	Europe	New South Wales
Epipactis helleborine	Broad leaved Helleborine	Orchidaceae	Europe	Canada

Molecular marker(s)	Native breeding system	Genetic diversity	Inferred number of sources and admixture	References
nSSR	Predominant inbreeder	–	Multiple without admixture	Meimberg et al. 2006
nISSR; nSSR	Predominant inbreeder	=/–	Multiple without admixture	Meekins et al. 2001; Durka et al. 2005
nSSR	Predominant outcrosser	=	Multiple with admixture	Genton et al. 2005
Allozymes	Obligate outcrosser	=	Multiple	Warwick et al. 1987
Allozymes	Predominant inbreeder	=/–	n/a	Clegg & Allard 1972; Garcia et al. 1989
nSSR; cpDNA	Mixed	–	Multiple with admixture	Rosenthal et al. 2008
Allozymes	Predominant inbreeder	=	n/a	Brown & Marshall 1981
Allozymes	Predominant inbreeder	+	Multiple	Novak et al. 1991; Novak & Mack 1993
nSSR	Mixed? + Vegetative	–	n/a	Hannan-Jones et al. 2005
Allozymes	Predominant inbreeder	=	Multiple	Neuffer, & Hurka 1999
cpDNA; nSSR	Obligate outcrosser	=/–	Multiple with admixture	Hufbauer & Sforza 2008; Marrs et al. 2008b
cpDNA; nSSR	Obligate outcrosser	=	Multiple with admixture	Hufbauer & Sforza 2008; Marrs et al. 2008a
Allozymes	Obligate inbreeder (apomict)	–	n/a	Chaboudez 1994.
Allozymes	Mixed	+	n/a	DeWalt & Hamrick 2004
cpSSR; nSSR	Predominant outcrosser	=	Multiple with admixture	Kang et al. 2007
Allozymes	Predominant outcrosser	=	n/a	Burdon & Brown 1986
Allozymes; cpDNA *trnL* intron PCR-RFLP	Predominant outcrosser	=/+	n/a	Squirrell et al. 2001a,b

Continued

Species	Common name	Family	Native range	Invasive range(s) studied
Erigeron annuus	Daisy fleabane	Asteraceae	North America	Europe
Heracleum mantegazzianum	Giant hogweed	Apiaceae	South West Asia	Europe
Heracleum persicum	Persian hogweed	Apiaceae	South West Asia	Europe
Heracleum sosnowskyi	Sosnowskyi's hogweed	Apiaceae	South West Asia	Europe
Hirschfeldia incana	Hoary mustard	Brassicaceae	Southern Europe	UK
Hypericum canariense	Canary Island St. John's wort	Clusiaceae	Europe	North America
Hypericum perforatum	St. John's wort	Clusiaceae	Canary Islands	California; Hawaii
Ligustrum robustum	Tree privet	Oleaceae	Sri Lanka; India	Mascarene Islands
Olea europaea cuspidata	Wild olive	Oleaceae	Africa; Asia	Eastern Australia; Hawaii
Olea europaea europaea	Cultivated olive	Oleaceae	Mediterranean Basin	South Australia
Pennisetum setaceum	Fountain grass	Poaceae	Egypt	Namibia; South Africa; Hawaii; California
Phalaris arundinacea	Reed canarygrass	Poaceae	Europe	North America
Phyla canescens	Lippia	Verbenaceae	South America	Australia; France
Pueraria lobata	Kudzu	Fabaceae	China	USA
Rubus alceifolius	Giant bramble	Rosaceae	South East Asia	La Reunion; Mayotte; Mauritius; Madagascar; Queensland
Schinus terebinthifolius	Brazilian pepper tree	Anacardiaceae	South America	Florida
Silene latifolia	White campion	Caryophyllaceae	Europe	North America
Silene vulgaris	Bladder campion	Caryophyllaceae	Europe	North America
Spartina alterniflora	Smooth cordgrass	Poaceae	North American Atlantic/Gulf coast	North American Pacific coast
Trifolium hirtum	Rose clover	Fabaceae	Turkey	California

Molecular marker(s)	Native breeding system	Genetic diversity	Inferred number of sources and admixture	References
RAPD	Predominant inbreeder (apomict)	–	n/a	Edwards et al. 2006
AFLP	Mixed	n/a	Multiple	Jahodova et al. 2007
AFLP	Mixed	n/a	Multiple	Jahodova et al. 2007
AFLP	Mixed	n/a	Multiple	Jahodova et al. 2007
RAPD	Predominant outcrosser	=	Multiple	Lee et al. 2004.
AFLP; ITS	Mixed	–	Single	Dlugosch & Parker 2008b
AFLP	Predominant inbreeder (apomict)	=	Multiple	Maron et al. 2004
cpRFLP; RAPD	Predominant outcrosser	=	n/a	Milne & Abbott 2004
nSSR; ITS; ptDNA	Obligate outcrosser	–	Single	Besnard et al. 2007
nSSR; ITS; ptDNA	Obligate outcrosser	=	Multiple with admixture	Besnard et al. 2007
ITS; nSSR; nISSR	Obligate inbreeder (apomict)	=	n/a	Le Roux et al. 2007
Allozymes	Predominant outcrosser	+	Multiple with admixture	Lavergne & Molofsky 2007
nISSR; ITS	Predominant outcrosser + Vegetative	n/a	Multiple	Fatemi et al. 2008; Xu et al. 2010
nISSR	Predominant outcrosser + Vegetative	=	Multiple	Sun et al. 2005
AFLP	Mixed	–	n/a	Amsellem et al. 2000, 2001
cpDNA; nSSR	Obligate outcrosser	=	Multiple with admixture	Williams et al. 2005
cpDNA	Obligate outcrosser	–	Multiple with admixture	Taylor & Keller 2007
cpDNA	Mixed	=	n/a	Taylor & Keller 2007
cpDNA; nSSR	Predominant outcrosser + Vegetative	=	Multiple with admixture	Blum et al. 2007
	Mixed	=/+	n/a	Molina-Freaner & Jain 1992

1.3 Enemy release and rapid adaptive evolution

Evidence for enemy release in biological invasions

The enemy release hypothesis (ERH) (reviewed by Colautti et al., 2004, Keane and Crawley, 2002, Liu and Stiling, 2006, Orians and Ward, 2010) theorizes that exotic species escape the negative effects of their natural enemies when in the introduced range (Darwin, 1859, Elton, 1958). Although this argument is intuitive, the positive effect of enemy release in the introduced range depends upon the extent to which abundance in the native range is controlled by natural enemies (Hierro et al., 2005). A recent review of studies which compare herbivore damage in native and introduced ranges revealed that roughly half found reduced damage in the introduced range, the reminder of studies showed no significant difference; indicating that for herbivores at least, the assumptions of ERH do not always hold true (Bossdorf et al., 2005). On the other hand, a comparison of invasive and non-invasive naturalized species in Ontario, New York and Massachusetts found 96% more herbivore damage on the non-invasives (Cappuccino and Carpenter, 2005). These results suggest that although changes in herbivore load between native and introduced ranges may not always occur, preferential herbivory is likely an important regulator of plant community dynamics. Aside from herbivory, a study on 473 naturalized plant species in the USA found 84% fewer fungal and 24% fewer viral pathogens in the exotic compared with the native range, and that those plants experiencing the greatest levels of pathogen release were reported most widely as noxious weeds (Mitchell and Power, 2003).

Enemy release, much like propagule pressure, can fluctuate in both space and time. Siemann et al. (2006) found that herbivore damage and tree performance were lowest in the areas of first introduction of the Chinese tallow tree (*Sapium sebiferum*), suggesting that herbivore release is important in establishment, but that insect

herbivory accumulates over time. Conversely, certain habitats appear resistant to colonization in the native range but not in the exotic; for example soapbrush (*Clidemia hirta*) is excluded from native Costa Rican forest areas by herbivores and fungal pathogens not present in the introduced range and hence it has become an invasive forest species in Hawaii (DeWalt et al., 2004); see also Callaway and Rout (2011).

Enemy release seems to be important in some invasions but not others. For example, differences between soil biota in the native and introduced ranges had no effect on growth and survival of marram grass (*Ammophila arenaria*) (Beckstead and Parker, 2003). Instead Eppinga et al. (2006) suggest that *A. Arenaria* may dominate by accumulating local pathogens, which in turn have a stronger negative effect on the surrounding native plants than on *A. arenaria* itself. Another possible explanation for ERH 's lack of general applicability is that plants adapted to grow in high resource environments are more likely to be limited by herbivores, whereas low resource adapted plants are better defended due to the comparatively higher cost of herbivory (Blumenthal, 2006). Therefore, although higher resource plants will experience a greater release from herbivore enemies upon introduction, low resource-adapted, well-defended plants may eventually produce a stronger evolutionary response to the reduction in natural enemy threat (Blumenthal, 2006).

Plant enemies can be specialist or generalist and there is good evidence that ERH does not apply equally to both types. A recent study of *Senecio jacobaea* in its introduced and native ranges showed that invasive populations had reduced defence against specialist herbivores as predicted by ERH, but conversely had increased protection against generalist herbivores (Joshi and Vrieling, 2005). Similarly, although finding an overall reduction in herbivores in the introduced range, Liu and

Stiling (2006) revealed that the reduction was predominantly in specialists and those feeding on reproductive parts. In fact, there is good evidence that exotics are often preferentially chosen by native generalist herbivores (Parker and Hay, 2005) supporting the idea of biotic resistance to invasive species (Elton, 1958). A fascinating application of this theory has been used to explain the comparative success of Old World versus New World invasive plants: In a meta-analysis of studies covering over 100 invasive species, Parker et al. (2006) concluded that plants were particularly susceptible to novel generalist herbivores. The authors note that during European colonization of the New World, Old World generalist herbivores such as cattle, replaced the New World native generalist fauna. Old world invasive plants may therefore thrive by following their native natural enemies, not escaping them (Parker et al., 2006).

Can enemy release facilitate rapid adaptive evolution?

Based on the assumptions of the ERH, Blossey and Notzold (1995) put forward their theory of 'evolution of increased competitive ability ' (EICA), which states that in the absence of herbivores, natural selection will favour those genotypes with increased resource allocation to competitive ability. This hypothesis generates two testable predictions, namely that individual plants from the invasive range will allocate more resources to growth and/or reproduction than those from the native range, and that specialist herbivores will favour plants from the introduced range over those from the native range as they possess fewer defensive mechanisms (Blossey and Notzold, 1995). The original study found supporting evidence for both predictions in the purple loosestrife (*Lythrum salicaria*) (Blossey and Notzold, 1995), but work since has provided mixed support (see Bossdorf et al., 2004, Hierro et al., 2005, Orians and Ward, 2010 for reviews). In particular studies tend to find support for one assumption but not the other, for example the Tansy Ragwort *Senecio jacobaea* has increased

growth and reproduction, and decreased specialist herbivore defences in the invasive range in accordance with EICA, but actually has increased general herbivore defence and greater herbivore tolerance (Joshi and Vrieling, 2005, Stastny et al., 2005). Similarly the giant goldenrod *Solidago gigantea* showed reduced herbivory tolerance in the invasive range but this did not translate to better performance in the absence of herbivores (Meyer et al., 2005). *Solidago gigantea* seems to invest more resources into rhizome production than flowers in the invasive range (Meyer and Hull-Sanders, 2008). Although no changes in chemical defences could be identified, a generalist herbivore from the native range performed significantly better when grown on invasive plants, suggesting that some defence has been lost in the invaded range (Hull-Sanders et al., 2007).

A neat study that has provided recent support for EICA looked at phytochemical shifts in the invasive wild parsnip *Pastinaca sativa* by analysing herbarium records (Zangerl and Berenbaum, 2005). Levels of various furanocoumarins were initially low in the introduced range but increase dramatically at the same time as their specialist herbivore, the parsnip webworm *Depressaria pastinacella*, was accidentally introduced into the invasive range (Zangerl and Berenbaum, 2005). However, the associated assumption of increased vigour was not investigated so does not provide evidence for all aspects of EICA; in fact it could be argued that the observed shifts in resource allocation were merely a plastic rather than evolutionary response.

Several studies have shown increased defence in the invasive range which is contrary to the predictions of EICA (see, for example, Muller and Martens, 2005, Wikstrom et al., 2006) and in *Alliaria petiolata* , evolution of *reduced* competitive ability has been proposed based on the theory that if competition is lower in the invasive range, selection will favour reduced competitive ability if it has an associated

fitness cost (Bossdorf et al., 2004). The conflicting evidence for EICA may be illustrative of the complex and variable mechanisms that are associated with plant invasions but a confounding factor may be the lack of control for sampling bias and maternal effects in many common garden studies (Bossdorf et al., 2005, Colautti et al., 2009).

The EICA hypothesis has arguably received most attention and empirical scrutiny, but is by no means the only possible evolutionary outcome of changes in enemy pressures in the invaded range. Orians and Ward (2010) put forward a set of testable evolutionary hypotheses based on the ecological conditions of the novel environment, of which EICA is but one of nine. Predicted evolutionary outcomes include increased or reduced defence allocation; changes in growth rate; allocation of defences more towards generalist or specialist enemies; and shifts between reliance on induced and constitutive defence traits (Orians and Ward, 2010). This framework provides an excellent basis for future work on the effects of changing enemy loads on adaptive evolution in invasive species and could potentially be effectively combined with genetic and genomic approaches (as discussed in Chapter 8) to reliably detect the genetic bases of any rapid adaptive evolution in plant defences.

1.4 Rapid adaptive evolution in invasive species

Support for the role of rapid adaptive evolution in biological invasions

Rapid adaptive evolution in introduced populations is proposed to have played a major role in some successful invasions (Maron et al., 2004, Phillips et al., 2006, Prentis et al., 2008, Whitney and Gabler, 2008). When species are introduced to biogeographical regions where they did not evolve, they may encounter a suite of novel environmental conditions and selection regimes. Under these new selection

regimes, genetically based phenotypic changes might affect individual fitness, the establishment of introduced populations and the spread of invasive populations across the landscape (Colautti et al., 2010, Maron et al., 2004, Whitney and Gabler, 2008, Xu et al., 2010). Consequently, rapid adaptive evolution in response to altered selection regimes could be an important determinant in the establishment, proliferation and spread of invasive species (Hendry et al., 2007, Prentis et al., 2008). Many studies of invasive species have found evidence of rapid phenotypic change between their native and introduced range, such as increased phenotypic plasticity, changes in body shape and size, and changes in breeding systems (Barrett et al., 2008, Huey et al., 2000, Lavergne and Molofsky, 2007, Barrett, 2011), but few studies have documented whether selection was the mechanism responsible for these phenotypic shifts (Barrett et al., 2008, Keller and Taylor, 2008).

So what is the evidence for rapid adaptive evolution as a mechanism promoting invasion success? Direct evidence for adaptive evolution promoting biological invasions is currently limited, but some excellent experiments have demonstrated the role of selection on ecologically relevant traits after the colonization of novel environments via human-mediated invasions. As evolutionary change in introduced populations can occur through neutral or adaptive processes, it is important to determine when adaptive evolution is responsible for observed changes. To establish that phenotypic evolution in introduced populations is the result of adaptation, it is necessary to statistically control for neutral processes that can also generate phenotypic change (reviewed in Keller and Taylor, 2008). In their seminal paper Maron et al. (2004) controlled for colonization history and determined that clinal variation observed in the introduced range of St. John's wort (*Hypericum perforatum*) evolved through adaptive processes. Another study controlling for neutral evolution has found that both adaptive evolution and colonization history have influenced the

generation of phenotypic clines in two introduced species of *Silene* in North America (Keller et al., 2009). Recently, Xu et al. (2010) demonstrated that adaptive evolution was responsible for phenotypic divergence between the native range of *Phyla canescens* and two different invaded regions. Although these excellent studies highlight that adaptive evolution can drive phenotypic divergence within and between the ranges of invasive species, they do not elucidate the type of genetic variation underlying ecologically relevant traits.

Several different mechanisms have been implicated in generating the genetic variation underlying rapid adaptive evolution and the colonization of new habitats (Barrett and Schluter, 2008, Colosimo et al., 2005, Prentis et al., 2008, Whitney et al., 2006). Although the exact source of genetic variation underlying traits important to successful invasions remains uncharacterized, in the following pages we contrast standing genetic variation with genetic novelty resulting from hybridisation, and discuss whether these processes might promote adaptive evolution during the invasion process. Understanding whether standing genetic variation, genetic novelty or both contribute to evolution in invasive species has wide implications in the field of invasion biology. This key information is important for predicting future invasions; identifying genes underlying ecologically relevant traits in invasive populations; and designing strategies to better manage the import of species to decrease the potential for rapid evolution in invasive populations.

Adaptation from standing genetic variation or new mutation

Genetically based phenotypic changes that allow species to adapt to new environments can arise from new mutations or standing genetic variation, which is defined as the presence of more than one allele at a locus in a population. Understanding whether standing genetic variation or new mutation is responsible for

rapid evolution in invasive species is currently an important question in invasion biology. We predict that most rapid adaptive evolution in invasive species should occur from standing genetic variation because favourable alleles are immediately available for selection to act upon and usually occur at a greater frequency within populations than new mutations (Barrett and Schluter, 2008, Prentis et al., 2010). Furthermore, as invasive populations often face new environments, neutral or even deleterious alleles in the native range may become advantageous in the introduced range. An elegant experimental demonstration supporting this prediction comes from the natural invasion of the Caribbean islands and Central America by the Brazilian water hyacinth (*Eichhornia paniculata*). In this species, recessive modifier genes that promote selfing occur at low frequencies in outcrossing source populations but fail to spread, possibly as reliable pollination services are available or because of the genetic costs associated with inbreeding depression. Selfing has evolved in the Caribbean islands where modifier genes have increased in frequency, possibly as a result of unreliable pollination services in this new environment (reviewed in Barrett et al., 2008). Rapid morphological change resulting from adaptation from standing genetic variation has also been documented in repeated natural invasions of novel freshwater environments by marine stickleback fishes (Colosimo et al., 2005). Both these examples demonstrate that rapid adaptive evolution can occur from standing genetic variation during the colonization of a novel habitat.

If sufficient genetic variation exists in introduced populations, adaptation from standing genetic variation could dominate rapid evolution during range expansion. This may be a particularly important mechanism of rapid evolution for invasive species that are exposed to ecogeographic variation during range expansion. Clines have been observed in the introduced range of several invasive species, including some striking examples in *Drosophila* and flowering plants (Huey et al., 2000, Keller

et al., 2009). However, the relative roles of introduction history, demographic processes and selection on generating clines in invasive species have rarely been tested in most studies (but see Colautti et al., 2010, Huey et al., 2000, Keller et al., 2009). In fact, Colautti et al. (2010) present strong evidence for adaptation from standing genetic variation for ecologically relevant traits during the northward expansion of purple loosestrife (*Lythrum salicaria*) in North America. Although this study provides compelling evidence of local adaptation to environmental conditions it also demonstrated that adaptation has been constrained at the northern limits of the introduced range because of a dearth of genetic variation for particular combinations of traits. Therefore adaptation from standing genetic variation in invasive populations may be limited if genetic constraints prevent selection from improving particular combinations of traits simultaneously. In such cases populations occupying marginal habitats at range extremes may suffer from a reduction in population growth, unless alleles that are beneficial in these environments arise by new mutation.

New beneficial mutations may also provide phenotypic variation for selection to act upon during colonization of new environments or range expansion in invasive species. Evidence for their role in the rapid adaptive evolution of invasive species, however, is currently lacking. Some of the best examples of adaptation through new mutation come from studies of microbes (Rainey and Travisano, 1998) and the evolution of resistance in pest species (Wootton et al., 2002). A recent study has found that adaptation from new beneficial mutations has enabled a species of bacteria to colonize a novel fluctuating environment through the evolution of bet-hedging genotypes that persist because of rapid phenotype switching (Beaumont et al., 2009). Although this example provides strong evidence for the colonization of a novel environment by adaptation from new mutation, we lack sufficient data to make

confident conclusions about the role of new beneficial mutations in adaptive evolution of invasive species.

Current population genetic theory and data (reviewed in Barrett and Schluter, 2008) has made it possible to distinguish between adaptation from standing genetic variation and adaptation from new mutation, because they leave different molecular signatures in the genome (Hermisson and Pennings, 2005). By applying this genetic theory to population genomic data, such as restriction-site-associated DNA (RAD) markers (Miller et al., 2007), generated by recently developed sequencing technologies, including 454, Illumina and Solid sequencing, it will become possible to decipher whether rapid adaptive evolution in invasive species occurs mainly through selection on standing genetic variation or new mutation (see Chapter 8).

Adaptation from genetic mixing

Genetic mixing resulting from hybridisation between different species (interspecific) and between different source populations of a single species (admixture) has been hypothesized to stimulate invasiveness in plants (see Abbott, 1992, Ellstrand and Schierenbeck, 2000). Evidence for admixture or hybridisation as a stimulus for invasion in specific taxa is increasing with many examples of highly invasive intra- and inter-specific hybrids, including lizards, toads and plants (see Abbott, 1992, Estoup et al., 2001, Ellstrand and Schierenbeck, 2000, Kolbe et al., 2008). However, some recent studies are also showing that the general role of hybridisation as a stimulus for invasiveness may be over estimated (Dlugosch and Parker, 2008b, Whitney et al., 2009). A recent study (Whitney et al., 2009) examined if the number of hybrids and the number of invasive species in 256 plant families were correlated. This relationship would be expected to develop if hybridisation leads to invasiveness, because the number of invasive species should be greater in hybrid prone plant

families. This study found that the present data did not support this hypothesis. Hybrids may not always be successful invaders because many hybrids may be largely sterile or unviable (Prentis et al., 2007), and because admixture after multiple introductions can lead to a mosaic of maladaptation, where trait values from one population might be better suited to another (Dlugosch and Parker, 2008a). Therefore, we believe it is premature to generalize that hybridisation is a stimulus for invasion, but nevertheless there are specific cases where hybridisation has been associated with invasiveness which deserve consideration.

Invasive plants of hybrid origin may have increased fitness due to heterosis or later - generation recombination of parental genotypes (Prentis et al. 2008). Excluding asexual and allopolyploid invaders (Ellstrand & Schierenbeck 2000), heterosis is likely to be transient in outcrossing species because of recombination and reductions in heterozygosity in later-generation hybrids (Baack & Rieseberg 2007). Therefore, we predict that recombination will be more important than heterosis in the rapid evolution of invasive species. Recombination in hybrids can generate novel combinations of traits upon which selection can act to produce a phenotype that is better suited to its new environmental conditions (Rieseberg et al. 2003; Kolbe et al. 2008). Specifically, recombination can cause genetically based phenotypic changes in invasive species through two main processes: adaptive trait introgression and transgressive segregation.

Adaptive trait introgression involves the transfer of beneficial alleles that increase fitness, between divergent populations or species. Consequently, adaptive trait introgression could be an important mechanism for evolution in admixed populations resulting from multiple introductions or after hybridisation between different species. For example, adaptive introgression has transferred abiotic stress resistance and

herbivore-resistant traits from *Helianthus debilis* into *Helianthus annuus* ssp. *annuus* , and formed a stabilized hybrid lineage, *H* . *annuus* ssp. *texanus* (Whitney et al. 2006, 2010). This new hybrid lineage has higher fitness than *H* . *annuus* ssp. *annuus* in the new environments it encounters in central and southern Texas (Whitney et al. 2006, 2010). Although this example highlights the potential for adaptive introgression to promote evolution in an invasive species, we need more data before concluding this is an important process for promoting biological invasions.

Transgressive segregation is the formation of traits that are novel or extreme relative to those of either parental line in hybrid progeny (Rieseberg et al. 1999). This phenomenon has been reported to be a common feature of plant hybrids and has been implicated as a key component in the success of some hybrid species (Arnold & Hodges 1995). Recent experiments have demonstrated that extreme phenotypes resulting from transgressive segregation can aid the colonization of novel environments in hybrid sunflower species (Lexer et al. 2003). These studies have also demonstrated selection on the extreme phenotypes, which act to increase fitness of the hybrid species in desert environments compared with either parental species (Rieseberg et al. 2003). These results indicate that transgressive segregation can produce phenotypic novelty that could facilitate biological invasions, but reciprocal transplant experiments are needed in each case to determine whether extreme traits are under selection in the introduced range of invasive species.

Testing whether hybridisation is actually a stimulus for invasion will be an important question in future research. To answer this question, researchers will first need to demonstrate whether or not invasive species are of hybrid origin. This will require genetic markers and population genetic analyses to determine that introduced species exhibit mixed ancestry from either genetically divergent populations in the

native range or between two different species. Secondly, researchers will need to demonstrate the increased fitness of hybrids in the invasive range compared with their progenitors. Such studies will require either reciprocal transplant experiments; or the use of long - term selection experiments, where different species or multiple introductions are grown in sympatry and allowed to hybridise. After several generations, the resulting populations can be genotyped to determine the ancestry of individuals and their relative fitness in these environments.

The remainder of this chapter (sections 2 and 3) introduce the focal species *Senecio madagascariensis* Poir and identify the knowledge gaps that will be addressed by the research presented in this thesis.

2. *Senecio madagascariensis* in Australia

Introduction history

Australian fireweed *Senecio madagascariensis* is an economically important agricultural weed and an invasive species from South Africa (Radford et al., 2000). The first recorded fireweed herbarium specimen was in 1918 at Raymond Terrace New South Wales (Radford, 1997) and is thought to have been introduced from the ballast of ships trading between Europe and Australia via South Africa (Sindel et al., 1998). Originally prominent in the Hunter Valley, fireweed was transported to north coast New South Wales in crop seed *circa* 1940 (Sindel, 1986). Although recognised as a weed in New South Wales in the 1960s (Sindel et al., 1998), fireweed did not become invasive *per se* until the 1980s when populations exploded after a long drought (Sindel and Michael, 1988).

Economic impact

The cost of fireweed management to New South Wales farmers has been estimated at $2.7 million per year on average, although costs can be up $5.4 million in particularly bad years (Page and Lacey, 2006). This high cost led to a biocontrol effort between 1989-1994 costing approximately $377,000 and ultimately failing to locate an appropriately host-specific agent (Page and Lacey, 2006).

Taxonomy

Australian fireweed has been confirmed as *Senecio madagascariensis* Poir. of the family Asteraceae and originates from South Africa (Scott et al., 1998, Radford et al., 2000). Similarities with the closely related *S. pinnatifolius* (previously *S. lautus*) have historically confused identification and it was only after Hilliard's (1977) treatment of Asteraceae in Natal that Australian fireweed was positively identified as *S. madagascariensis* and separated from the *S. pinnatifolius* complex (Sindel et al., 1998). This has been supported by morphological comparisons (Thompson, 2005, Radford, 1997) as well as cytological studies finding chromosome counts of $2n = 20$ for Madagascan and Australian fireweed compared to $2n = 40$ for *S. pinnatifolius* (Radford et al., 1995).

Description

Australian fireweed has been described by various authors including Thompson (2005), Sindel et al. (1998), Hilliard (1977) and Radford (1997), the following summary description includes information taken from these sources but is not exhaustive.

Senecio madagascariensis is a generally erect, herbaceous plant growing to around 0.6 m, with a taproot and many fibrous roots growing between 10 and 20 cm deep in the soil. The leaves are bright green, alternate and variable growing up to 12 x 2.5 cm in size but are often smaller. The inflorescences are heterogamous and radiate, with few to many on bracteates peduncles, arranged in open corymbose panicles, terminal or auxillary. It has approximately 20-21 phyllaries and 8-12 calycular bracts (often purple-tipped). Florets are coloured canary yellow, approximately 100-120 in total, of which 12-13 are ray florets. Fruits are achenes, 1.4-2.2 mm long, cylindrical with bristles in longitudinal bands. The pappus is white and 3.5-6.5 mm long.

Fireweed is a short lived perennial plant which most commonly behaves as an annual displaying high plasticity with respect to live cycle. Seed dormancy is negligible but can be induced by extreme temperatures. Seedlings develop quickly, producing flowers after 6-10 weeks and flower intensity peaks in spring and autumn. The average plant has been estimated to produce around 9000 viable seeds in its lifetime; this combined with wind dispersal is thought to contribute to fireweed's rapid spread (Sindel, 1986). Fireweed is an obligate outcrosser (Ali, 1966; Radford, 1997) and in Australia, flowers are pollinated predominantly by the introduced European honey bee *Apis mellifera*, and various species of Syrphidae (White, 2007). Evidence supporting the role of enemy release in fireweed has been mixed; it suffers proportionally less herbivory and is visited by less diverse and less abundant herbivores than the closely related native congerer *S. pinnatifolius* (White, 2007), yet Harvey (2012) found herbivore loads increased over time but this did not equate to enemy release at early stages of fireweed invasion.

Distribution

Sindel et al. (1998) reviewed the distribution of fireweed in Australia and reported particularly abundant areas in the Richmond, Manning and Hunter Valleys, in the County of Cumberland and between Wollongong and Berry. Fireweed was present to lesser extent along the entire New South Wales coastline and into the tablelands. In Queensland fireweed was prevalent in coastal pastures around Brisbane with single plants being found in Gympie. In 2007, two isolated patches of *S. madagascariensis* were identified as growing in far north Queensland. *Senecio madagascariensis* is also known to be naturalised in Japan, Hawaii, and parts of Africa and South America (Cruz et al., 2010, Kinoshita et al., 1999, Le Roux et al., 2006, Tracanna and Catullo, 1987, Invasive species compendium, www.cabi.org/isc).

3. Knowledge gaps

The introduction history of *S. madagascariensis* to Australia is not well understood beyond its first occurrence, presumably sometime before the first herbarium specimen was collected in 1918. No large scale population genetic projects have been undertaken on the species in Australia, nor in the identified likely source area of KwaZulu-Natal in South Africa (Radford et al., 2000). The herbarium record also remains a wealth of untapped information on *S. madagascariensis*, both in terms of understanding spread by traditional mapping techniques and as a genetic resource. There have been a growing number of studies utilising herbarium specimens for population genetic research (e.g. Chun et al., 2009, Provan et al., 2008). A combined study of both contemporary and historical collections could provide unprecedented information on the course of the *S. madagascariensis* invasion through time. This kind of study would allow comparisons of genetic diversity between native and

invasive populations and would have the potential to identify the location in time as well as space of separate introduction events.

On a global scale, Australia has been suggested as a potential source for other invasions of *S. madagascariensis* worldwide (e.g. Hawaii, Le Roux et al., 2006). To date there have been no studies looking at individuals from across multiple invasive ranges of *S. madagascariensis* and comparing them to native individuals. A population genetic study utilising the same markers on individuals from around the world would enable comparisons of diversity and could shed light on whether Australia, or the native range, is a more likely source of other global invasions of *S. madagascariensis*.

Hybridisation between *S. madagascariensis* and *S. pinnatifolius* has been confirmed between the tableland variety of *S. pinnatifolius* by Prentis et al. (2007). Previous researchers have reported finding putative hybrid adults between fireweed and the dune variety of *S. pinnatifolius* (White, 2008, Scott, 1994), a result that has yet to be confirmed by larger-scale population genetic analysis. Given that *S. madagascariensis* has spread rapidly in Australia, hybridisation could have provided a mechanism by which adaptive genes were acquired through introgression. Studying the outcome of hybridisation between fireweed and the dune variety of *S. pinnatifolius* will determine whether introgression is likely to have played a significant role in contributing to the invasive success of *S. madagascariensis* in Australia.

Fireweed is reported to have undergone a population “explosion” in the 1980s after a significant period of drought (Sindel and Michael, 1988). It is possible that some adaptive shift occurred prior to this event allowing *S. madagascariensis* to thrive. A

first step to identifying adaptive shifts can be the use of genome scans to look for signatures of selection. By assessing samples from the across the invaded range and applying a variety of techniques designed to identify loci under selection, it should be possible to determine putative candidate loci for further study into contemporary evolution in this species. And, additionally, the environmental factors which may be exerting selective pressure.

4. Contextual statement

This thesis has been written as a selection of manuscripts, either published, or in preparation for submission for publication. Chapters 1, 2, 5 and 8 are comprised of (or include material from) review papers on topics highly relevant to the subject of the thesis and written and published during candidature. On two of these reviews (Chapters 2 and 5) the candidate is not first author, however they are included here in the body of the thesis due to the large contributions made by the candidate, including composition of large sections, and the particular relevance of the subject content. Alongside the presented review chapter there are four data chapters. The format of these chapters is as draft manuscripts, with tables, figures and supplementary material presented at the end of each chapter. To improve thesis flow, where reference is made to other chapters, this is in the form of “see Chapter 3”, a convention that will obviously require revision during final preparation of manuscripts for journal submission. Data papers have been provisionally formatted to align with the submission requirements of potential journals, hence spelling and referencing conventions do differ between chapters. A list of all publications completed during candidature can be found in Appendix 2. One paper (Harris et al., 2012) is presented in full in Appendix 3, as it is particularly relevant to the work

undertaken here, just on different species. The following paragraphs summarise the remainder of chapters and explain their context with reference to the entire thesis.

Chapter 2 is a review paper looking at the current literature on dispersal pathways in both natural range expansions and biological invasions. This paper synthesizes literature from across a wide variety of research areas such as early plant and animal domestication, post-glacial recolonisation, global biotic exchanges, island biogeography, and invasion biology. Six dispersal pathways are recognised and described to represent the continuum of extra-range dispersal from leading edge (where individuals at the edge of a species' range can disperse into adjacent suitable habitats) to cultivation (where individuals are moved often in great numbers over great distances by humans, and receive care to aid establishment).

It is well known that human mediated introductions have increased in frequency in modern times, thanks to increased global transport networks. However, this review also highlights how human mediated introductions are changing qualitatively over time and now tend to be characterised by movement of multiple individuals on multiple occasions from multiple sources to multiple locations. The associated reduction in simultaneous movement of coevolved natural enemies, along with high genetic diversity and reduced mate limitation can enhance the potential for rapid adaptation in invasive species.

Part 2 of this chapter is an update 'letters response' article which clarifies the rationale behind defining 'invasions' as those species introductions mediated by humans.

Overall Chapter 2 describes the importance of understanding introduction history and invasion pathways, setting the scene for data Chapters 3 and 4 which investigate the invasion history of *Senecio madagascariensis* in Australia and across four other global introductions of the species. This review is also closely tied to a second review in Chapter 5, which looks at the potential mechanisms for adaptive evolution in invasive species, and the proceeding data Chapters 6 and 7 which examine two of these mechanisms in *S. madagascariensis* in Australia.

Chapter 3 combines molecular analysis of both contemporary and historical (herbarium) collections of *S. madagascariensis* in Australia and compares these to contemporary collections from South Africa where the species is native. This research is the first to use a combination of molecular data from contemporary and historical collections, traditional spread mapping from herbarium records and anecdotal evidence to reconstruct invasion history and dispersal pathways for *S. madagascariensis*. The novel approach of utilizing DNA analysis of herbarium records allowed invasion history to be characterized both spatially and temporally, revealing insights into introduction scenarios and changes in genetic diversity that would not have been accessible through study of contemporary collections only.

Chapter 3 builds on the theoretical framework of dispersal pathways put forward in Chapter 2, using *S. madagascariensis* as a model to investigate the invasion history of this economically important weed in Australia. Results from this chapter are the foundation for further studies on the species which rely on an understanding of population demography, genetic diversity and knowledge of potential number and locations of introduction events.

Chapter 4 expands upon the results of Chapter 3 by including a global dataset of *S. madagascariensis* introductions and analysing them for chloroplast haplotype diversity. Results indicate low levels of diversity in all invasions compared to the native range and are consistent with secondary invasions originating from Australia. This chapter also highlights the utility of historical specimen data as several *S. madagascariensis* individuals in Hawaii possessed a globally rare haplotype only found in the native range among contemporary collections. However, Australian herbarium records also contained this haplotype, maintaining support for Australia acting as a source for secondary invasions of *S. madagascariensis* world wide.

Chapter 5 reviews the mechanism by which biological invasions can undergo rapid adaptive evolution. Traditionally evolutionary change is considered to occur over very long periods of time and to arise from random genetic mutations that then undergo natural selection. This article reviews a wide range of literature regarding evolutionary change, particularly focusing on mechanisms that can promote rapid evolution through standing genetic variation (without the requirement for random favourable mutations). These mechanisms have been shown to effect evolutionary change over relatively few generations and certainly within the generational turnover often seen in invasive species' lag phase.

The article reviews four types of evolutionary change that can effect biological invasions and considers the likelihood of promotion or limitation of adaptive evolution in association with these mechanisms. Specifically, the review considers genetic bottlenecks, hybridisation (both within and between species), polyploidy and stress induced modification of the genome. This chapter provides the theoretical framework underpinning the rationale behind data chapters 6 and 7, whilst developing the ideas

put forward in chapter 2 regarding the potential consequences of human mediated extra-range dispersal.

Both chapters 6 and 7 investigate the potential for adaptive evolution in *S. madagascariensis* in Australia. Chapter 6 examines the outcome of hybridisation with the native congener *S. pinnatifolius* using AFLPs in both adults collected in the field and from open pollinated progeny arrays. As explored in the preceding review (Chapter 5) hybridisation can facilitate introgression of adaptive genes between species and promote the evolution of invasiveness through the transmission of genes conferring locally adapted traits. I studied hybridisation outcomes in allopatric populations of the two species as well as areas where the two species grow in close proximity. Results confirmed that hybridisation between the species does occur but at low frequencies. Despite successful hybrid seed set, no adult hybrids were identified. Results are discussed in relation the likelihood of introgression between the two species.

Chapter 7 utilises genome scans in combination with a spatial analysis method to identify candidate loci under selection in *S. madagascariensis* in Australia, as well as putative environmental variables that might be exerting selective pressure. Building on the review of potential mechanisms for adaptive evolution in invasive species (Chapter 5), work in this chapter compared two different sampling strategies and three different methods for identifying loci under selection. One loci was identified as a candidate for selection in every method and showed significant association with a variety of rainfall parameters. Another loci was identified by two of the three methods and was associated with light availability. Results are discussed with regards to possible biological relevance of the associations identified; possible alternative

explanations for the observed results and future research methodologies which could help further investigate selection in the *S. madagascariensis* genome in Australia.

The final chapter (8) presents a summary discussion of the entire thesis, demonstrating the contribution of this work to the body of scientific knowledge. Here I also discuss challenges encountered in the production of this work and highlight areas where additional research would improve our overall understanding of the issues addressed. The chapter concludes with the final part of the published book chapter which appears here in Chapter 1: a summary of proposed research methodologies which when applied to invasive species, would have the power to identify both the genotypic and phenotypic basis of adaptive evolution. This final section sets out a methodological framework that would be an ideal next step for *S. madagascariensis* research.

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CHAPTER 2(i)

Something in the way you move: dispersal pathways affect invasion success

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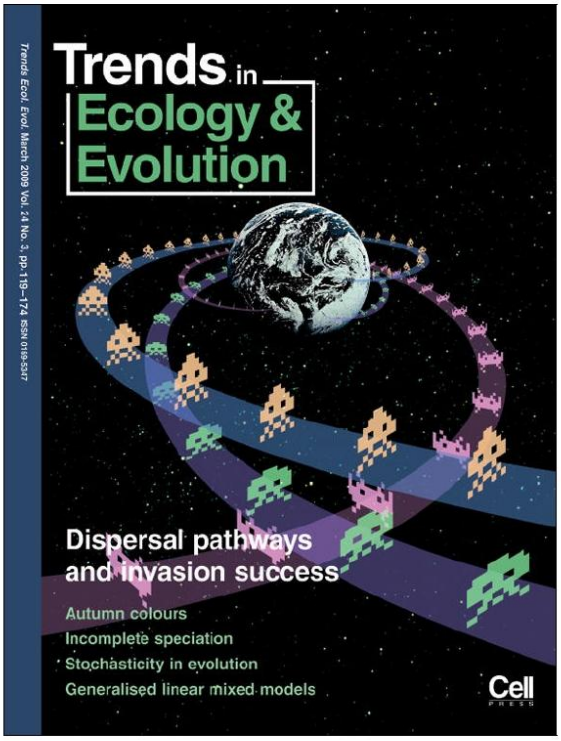

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CHAPTER 2(ii)

Biogeographic concepts define invasion biology

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CHAPTER 3

Sequential genetic bottlenecks in time and space: Reconstructing invasions from contemporary and historical collections

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Sequential genetic bottlenecks in time and space: Reconstructing invasions from contemporary and historical collections

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Abstract

Changes in genetic diversity over the course of biological invasions potentially affect invasiveness and susceptibility to control measures, yet how diversity develops over the course of invasions is rarely considered. Herbarium accession data can offer a useful historical perspective and have been used to track the spread of invasions through time and space. Nevertheless, few studies have utilised this resource for genetic analysis to reconstruct a more complete picture of historical invasion dynamics, including the date and location of separate introduction events and how these relate to changes in genetic diversity over time. In this study, we combined nuclear and chloroplast microsatellite analyses of contemporary and historical collections of *Senecio madagascariensis*, a globally invasive weed first introduced to Australia c. 1918 from its native South Africa. Analysis of nuclear microsatellites together with temporal spread data inferred from herbarium vouchers, revealed an initial introduction to south-eastern Australia which later founded a second population in mid-eastern Australia. Genetic diversity in the original invasive population was lower than in the native range, but higher than in the subsequent invasion, indicating

sequential founder effects. In the invasive range, chloroplast data revealed an increase in genetic diversity over time as new haplotypes appeared, probably as the result of subsequent introduction(s) to Australia from the native range during the latter half of the 20th century. Our work demonstrates how molecular studies of contemporary and historical field collections can be combined to reconstruct a more complete picture of the invasion history of introduced taxa. Further, our study indicates that a survey of contemporary genetic structure only (as undertaken for the majority of invasive species studies) is unable to assess changes in genetic diversity over time and can prevent identification of source populations and occurrence of multiple introductions.

Introduction

Biological invasions can comprise populations that differ in their levels of diversity [1] and invasiveness [2], and invasions by the same species in different areas can be the result of either single or multiple introduction events [3]. Invasive populations can vary in how aggressively they invade and how they respond to control measures [4] and these characteristics can change over time as a result of bottlenecks and multiple introductions [5]. Treating biological invasions as potentially comprised of discrete and evolving populations is therefore important both conceptually, for our understanding of the mechanisms behind successful colonisation, and practically, for our capacity to accurately predict and appropriately respond to invasive species.

Biological invasions are generally accompanied by changes in genetic diversity, most usually a reduction in within population diversity in the invasive range [6]. However, multiple introductions are very common in human-mediated introductions [7,8,9,10,11], and can augment genetic diversity [12], increase propagule pressure

and reduce mate limitation [10]. High genetic diversity can be beneficial on both ecological and evolutionary timescales: in the short term, high diversity has been shown to improve colonisation success [13]. In the longer term, admixture between disparate source populations can reduce inbreeding depression [11] and increase fitness [14], as well as produce novel gene combinations and increase evolutionary potential [15,16]. Conversely, multiple introductions can sometimes result in a 'mosaic of maladaptation' [6], where populations would be better adapted to different locales but are limited by the spatially stochastic nature of their introduction and restricted gene flow.

Herbarium collections harbour a wealth of information on plant invasions but remain underutilised; critical questions in invasion biology surround processes acting in the lag phase of introductions, where species are present in a new environment but not yet invasive [17]. A major problem in studying lag phase processes is that once a species has become invasive, the opportunity to study lag phase dynamics in real time has passed. Herbarium material is therefore an important resource as it can document a species' history throughout the lag phase period. Herbarium records are often used to plot the spread of invasive species through time, (e.g. [18,19,20]) and other aspects of the invasion process investigated include enemy release [21], change in morphological traits over time [22], and genetic diversity and introduction sources [23]. By combining the temporal information from herbarium records with modern advances in DNA extraction and genetic analysis, we can now study the genetic composition of invasions through time. These techniques have recently been used successfully to detect cryptic invasions [24] and document the accumulation of genetic diversity over the course of an invasion [25]. This approach could also help clarify the role of genetic diversity and multiple introductions in successful invasions and how these processes relate to lag phase.

Senecio madagascariensis is a diploid herbaceous perennial plant native to South Africa and invasive in several countries, including Australia. The first herbarium record for the species in Australia was lodged in 1918 and collections exist to the present day. The species is classified as a noxious weed in the state of New South Wales and is estimated to cost farmers an average of AU$2.7 million per year [26]. Although recognised as a weed in New South Wales in the 1960s [27], *S. madagascariensis* was present in Australia for approximately 70 years before a population explosion in the 1980s [28], constituting a considerable lag phase. Recent work has identified a reduction in molecular transducer gene expression (often associated with response to biotic stimuli) in Australian *S. madagascariensis* compared to material from South Africa [29]. This finding suggests evolutionary changes might have occurred in invasive populations of *S. madagascariensis* during lag phase and subsequently aided the rapid spread observed during the 1980s [29]. Specifically, a reduction in expression of genes involved in response to biotic stimuli could be indicative of enemy release in the invasive range and potentially the evolution of increased competitive ability (EICA) [30]. An alternative explanation might be that a more invasive strain of *S. madagascariensis* was subsequently introduced around the time of lag-phase break, and was then able to spread more effectively than the resident *S. madagascariensis* genotypes present at that time. This scenario has recently been supported in the European invasion of *S. inaequidens*, where historical and molecular data were combined to reveal that a 70 year lag-phase in Bremen, Germany, was broken by the arrival of a different invasion route that 'overran' the more slowly expanding resident population [31]. Understanding the spatial, temporal and genotypic dynamics of *S. madagascariensis* over the course of the Australian invasion will increase our understanding of the circumstances surrounding its break from lag phase.

Our study combines traditional herbarium record mapping with genetic analyses of both historical and contemporary collections of *S. madagascariensis* in Australia, and an analysis of genetic variation in contemporary samples from its native range in South Africa. Specifically we ask a) is the Australian invasion comprised of a single panmictic, or multiple independent populations; b) does genetic diversity in the native range differ significantly from that of the Australian population(s) and have changes in diversity occurred over the course of the invasion; and, c) have there been multiple introductions and can source populations be located?

Methods

Study species

Senecio madagascariensis Poir. (Asteraceae) is an herbaceous plant growing to around 0.6 m with green leaves and bright yellow inflorescences. Flowering occurs predominantly in spring and autumn and flowers are insect pollinated. The species is a diploid (2*n* = 20), obligate outcrosser and its seeds are wind dispersed. *Senecio madagascariensis* is native to South Africa and Madagascar, where it is widespread. It also has limited native populations in Swaziland and Mozambique (Invasive species compendium, www.cabi.org/isc). *Senecio madagascariensis* is thought to have been introduced to Australia from the dry ballast of ships trading between Europe and Australia via South Africa [27]. Originally prominent in the New South Wales (NSW) Hunter Valley (the first herbarium specimen was found in 1918 at S 32° 43', E 151° 45'), anecdotal evidence points to the transportation of *S. madagascariensis* to north coast NSW in crop seed *c.* 1940 [32] (the first north coast NSW herbarium specimen was found in 1948 at S 28° 49', E 153° 16'). Currently, *S. madagascariensis* is present all along the coast of NSW and into south-

east Queensland. Plants at two sites in Far North Queensland (FNQ) have also been recently identified as *S. madagascariensis*, confirming the predictions identifying FNQ as climatically suitable for the species [33].

Contemporary field collections

The most likely native provenance of *S. madagascariensis* in Australia has been narrowed down to South Africa by ITS1 sequence data comparisons [34] and further to the KwaZulu-Natal province (KZN) by morphological and isozyme data [35]. We therefore concentrated our sampling on KZN (11 sites). We also sampled from the Eastern Cape and Western Cape provinces (four populations) as these were highlighted as more distantly related to Australian fireweed [35]. A representative voucher specimen was lodged from each South African site to confirm species identity (this can be particularly challenging in South Africa where many similar *Senecio* species co-exist). Only one population of the four collected outside of KZN was included in the final analysis due to misidentification in the field and polyploidy (see results). In Australia, 20 sites were sampled across the known distribution of *S. madagascariensis.* As misidentification is less likely in Australia (the native *S. pinnatifolius* is superficially similar but easy to distinguish based on bract number), a single voucher specimen from Halfway Creek was lodged to confirm identity. All sites included in the study had their voucher specimen confirmed as *S. madagascariensis* by a taxonomist (see acknowledgements). Voucher details are listed in Table S1, Supporting Information. Fresh leaf samples from ~20 plants ≥ 5 m apart at each site were collected (Table 1). Leaves were immediately placed on silica gel and stored separately until DNA extraction.

Herbarium collections

Electronic records were obtained from all Australian herbaria for *S. madagascariensis* and collated into a single database. The coordinate points for each observation were checked against Google Earth™ v4.1 (Google Inc.) and all duplicate records were removed. The density of herbarium collections was visualised using the DENSITY tool in ArcMap™ v10.0 (ESRI). Physical sampling of herbarium vouchers was undertaken for all *S. madagascariensis* accessions kept at the Queensland Herbarium, National Herbarium of Victoria and National Herbarium of New South Wales. Duplicate records containing different plants collected at the same time from the same location were included in order to capture as much potential diversity as possible (n = 247 sampled and DNA extracted, n = 221 successfully genotyped at all loci). A small leaf sample was taken from each record and stored at room temperature until DNA extraction.

Microsatellite genotyping

DNA extraction was carried out using the Machery-Nagel Nucleospin Plant II Kit with the PL2/PL3 buffer system. Primers for nine previously published nuclear microsatellite loci [36] were used to screen all native and invasive contemporary collections of *S. madagascariensis*. Previous trials using nuclear microsatellites with DNA extracted from herbarium voucher specimens achieved <10 % successful amplification (unpublished data), possibly due to low copy number of nuclear DNA compared to chloroplast DNA which produced >90 % successful amplifications. Nuclear microsatellite analyses were therefore restricted to contemporary collections only. PCR reactions (10 μL) were prepared with ~20 ng of template DNA, 1x reaction buffer, 0.2 mM each dNTP, 2.5 mM $MgCl_2$, 0.4 μM of each primer, and 0.02 U Amplitaq Gold® (Applied Biosystems). PCR reactions were carried out with an initial denaturation step of 94 ºC for 2 min, 35 cycles of 94 ºC for 1 min, T_a ºC for 1 mins, 72

ºC for 1 min 30 s, and a final extension at 72 ºC for 30 min. See Table S2, Supporting Information for annealing temperatures (T_a). Products were separated using the ABI 3730 DNA analyzer (Applied Biosystems) with the GeneScan™ –500 LIZ® size standard. Fragments were scored using Genemapper® Software v4.0 (Applied Biosystems).

Ten previously published chloroplast microsatellite primer pairs [37] were assessed for polymorphism using one individual from each of the native sites sampled. PCR reactions (10 μL) were prepared with ~20 ng of template DNA, 1x reaction buffer, 0.2 mM each dNTP, 2.5 mM $MgCl_2$, 0.5 μM of each primer, and 1 U IMMOLASE™ DNA polymerase (Bioline). Reactions were carried out with an initial denaturation step of 94 ºC for 5 min, 30 cycles of 94 ºC for 20 s, 50 ºC for 20 s, 72 ºC for 20 s, and a final extension at 72 ºC for 30 min. Products were analysed and scored as above. All contemporary South African samples and all Australian herbarium accessions were screened (Table 2) with three identified polymorphic loci. Details of all loci included in the final analyses are listed in Table S2, Supporting Information. PCR reactions were repeated for ~10% of samples in order to calculate error rates.

Data analysis

All microsatellite loci were assessed for suitability (Document S1, Supporting Information). Genetic clusters in HW were determined using the program STRUCTURE v2.3.3 [38] using the admixture model. Each run consisted of a burn-in period of 100,000 Markov Chain Monte Carlo (MCMC) repetitions, followed by 1,000,000 MCMC repetitions. Possible numbers of discrete populations (*K*) were set from one to the maximum number of sites sampled. Each value of *K* had five separate runs to allow detection of any spurious results. The program was run for all sites combined (South Africa and Australia) and separately for the native (South African) and

invasive (Australian) ranges. The most likely value of K was determined using STRUCTURE HARVESTER v0.56.4 [39] and taken as the value of K at which ΔK is maximal [40].

Appropriate measures of population differentiation have been a recent contentious issue in the literature (e.g.[41,42,43,44,45]). Meirmans and Hedrick [44] suggest reporting F_{ST} along with F'_{ST} or D_{est}, and we have chosen to report all three statistics to maximise future comparability of our results with other studies. F_{ST} and F'_{ST} were calculated using FSTAT v2.9.3.2 [46], the later in combination with RECODEDATA v0.1 [47] which creates a dataset that maximises possible F_{ST} values, enabling the F_{ST} value obtained for the original data to be scaled to its theoretical maximum. The program SMOGD v1.2.5 [48] was used to determine D_{est}, the particular algorithm used is unable to use groups of individuals with missing data for an entire locus, so three Australian populations were excluded from the calculation (Table 1). Isolation by distance (IBD) was measured using a Mantel test with 9,999 permutations between pairwise F_{ST} values and geographic distance.

Observed heterozygosity (H_o), unbiased expected heterozygosity (H_e), mean number of alleles per locus (A) and the inbreeding coefficient (F_{IS}) were calculated using GENALEX v6.4 [49]. Allelic richness (A_r) and private allelic richness (A_{pr}) [50] were calculated using ADZE v1.0 [51]. Differences between normally distributed datasets of equal variance, for populations as determined by genetic clusters in STRUCTURE, were investigated with one-way ANOVAs and further defined by Tukey's HSD tests. Range-wide comparisons (native vs. invasive) were analysed with Mann-Whitney U tests [52].

For the chloroplast microsatellite dataset, each unique combination of alleles was defined as a separate haplotype. Counts were made of total number of haplotypes and private haplotypes in a particular area. Simpson's diversity index (*H*) was determined in CONTRIB v1.02 [53] using the following equation:

$H = n / n - 1(1 - \sum_i x_i^2)$ where x_i is the estimated frequency of the *i*th haplotype in the population when a sample of *n* individuals is drawn at random [54]. Haplotypic richness (R_h) was calculated in CONTRIB v1.02 [53] and rarefied to sample size $n = 11$. Haplotype data were divided into three groups based on collection date (1918 - 1957, 1958 - 1987 and 1988 – 2007) to visualise and assess changes over time. *H* and R_h were determined from the chloroplast data for the genetic clusters in Australia (i.e. populations identified from the nuclear microsatellite data set) at 1987 (cumulatively, as sample sizes were too small to analyse collections up to 1957 separately ($n = 6$ (P1); $n = 3$ (P2) in 1957) and at 2007 non-cumulatively (only records collected between 1988 and 2007).

We tested various introduction hypotheses (Table S3, Supporting Information) using Resampling Stats Add-In for Excel v4.0 (statistics.com), in each case we used 10000 repeat samples to obtain estimated *P* values. For specific simulation details see results. For one simulation we used the 'Calculate Geodesic Distance Between Points' model for ArcGIS v10.0 (ESRI) available from the 'Geoprocessing Model and Script Tool Gallery' (resources.arcgis.com) to calculate the distance between each herbarium specimen location and all major ports in the study area (major ports were chosen based on the assignment of 'medium', 'large' or 'very large' on worldportsource.com) (Table S4, Supporting Information).

To assess potential source populations in South Africa, the proportion of haplotypes found in Australia in 1957 were compared with those found in contemporary native

sites using an extension of the Fisher exact test [55]. A median-joining network [56] was constructed using length differences for the chloroplast microsatellite dataset in NETWORK v4.6.0.0 [57].

Results

Invasive populations

All nine nuclear microsatellite loci were evaluated and seven deemed appropriate for use in further analyses (Document S1, Supporting Information). Samples from one South African site (Hluhluwe, KZN; Table S1, Supporting Information) were obviously polyploid based on $n > 2$ alleles present for several loci. As *S. madagascariensis* in Australia is exclusively diploid [58], this population was excluded from further analyses. Two distinct Australian populations (i.e. genetic clusters) were determined by STRUCTURE ($K = 2$) on the basis of nuclear microsatellites when data from Australia only was considered. When South African material was included in the analysis, the same Australian clusters were defined and all South African material clustered together independently ($K = 3$). The Australian clusters roughly equate to a south-eastern population (P1) ranging between Eden and Crescent Head in New South Wales (NSW), and a mid-eastern population (P2) ranging from Halfway Creek in NSW to Mount Glorious in Queensland (QLD) (Fig.1). When considered independently, South Africa was partitioned into two populations ($K = 2$) with Boesmansriviermond from the Eastern Cape representing a distinct cluster and all other sites from KwaZulu Natal comprised of individuals assigning to both clusters (Fig. S1, Supporting Information). Significant isolation by distance (IBD) was evident in South Africa with inclusion of Boesmansriviermond ($r = 0.81$, $P = 0.039$), but was not significant when Boesmansriviermond was excluded ($r = 0.07$, $P = 0.364$).

Invasion spread mapping from herbarium records in Australia shows an original invasion focus at Raymond Terrace, lower north coast NSW in 1918, with a second invasion focus seeded around 1948 at Lismore in northern NSW. The invasion continued to spread from these two foci up to the present day (Fig.1). Fifty eight percent of all alleles were found in both South Africa and Australia, 33% were unique to South Africa and 9% unique to Australia. Within Australia, 38% and 4% of alleles were unique to P1 and P2 respectively. Ten alleles (4% of the global total) were found in P1 and nowhere else, one allele (0.4% of the global total) was found exclusively in P2. Significant IBD was detected across Australia as a whole (r = 0.35, P = 0.003), but this pattern was not evident when P1 (r = −0.03, P= 0.507) and P2 (r = −0.17, P = 0.418) were considered independently.

Of the two sites from Far North Queensland (FNQ) included in the study, Malanda clustered with P1 whilst Herberton clustered with P2, despite these sites being only ~20 km apart (Fig. 1). These two sites are ~1300 km from the next closest plants in QLD and so are effectively isolated from the main invasion. These very large geographic distances mean that these two sites can have no gene flow with their parent populations and constitute new invasion foci in Australia; as such they have been excluded from population-based analyses of P1 and P2 but included in Australia wide calculations.

Genetic diversity comparisons

All measures of diversity based on the nuclear microsatellites were highest in South Africa, followed by P1 then P2 (Table 1). Allelic richness (A_r) and observed heterozygosity (H_o) were suitable for one-way ANOVA tests and significant differences between populations were found (A_r, $F_{2, 26}$ = 42.11, P < 0.001; H_o, $F_{2, 26}$ = 10.37, P < 0.001). South Africa had significantly higher A_r, than both P1 (P < 0.05)

and P2 ($P < 0.001$), and that P1 had significantly higher A_r, than P2 ($P < 0.001$). Both South Africa and P1 had a significantly higher H_o than P2 ($P < 0.001$ and $P < 0.01$ respectively). There was no significant difference in H_o between South Africa and P1. Comparing the native and invasive areas as a whole, South Africa had significantly higher levels of genetic diversity than Australia in all metrics (Mann-Whitney U test: A, $U = 211.5$, $P < 0.001$; A_r, $U = 191.0$, $P < 0.001$; A_{pr}, $U = 201.0$, $P < 0.001$; H_o, $U = 167.5$, $P < 0.05$; H_e, $U = 203.5$, $P < 0.001$).

Differentiation (as measured by F_{ST}) was lowest in South Africa (0.044), slightly higher in P1 (0.049) and higher again in P2 (0.081). The largest value of F_{ST} was obtained from Australia as a whole, and globally (both 0.100). Other differentiation statistics are reported for comparison (Table 1). Inbreeding (as measured by F_{IS}) was suitable for one-way ANOVA which detected significant differences between populations ($F_{2, 26} = 3.92$, $P < 0.05$); P2 had significantly higher F_{IS} than P1 ($P < 0.05$) with values in South Africa falling between those of P1 and P2 and not significantly different from either. A Mann-Whitney U test found no significant difference in inbreeding between Australia and South Africa ($U = 118.0$, $P = 0.760$).

Individual herbarium specimens were grouped into two populations based on whether they were found within the geographic range of P1 or P2. Of the global total sampled in this study, eight chloroplast haplotypes (57 %) were unique to South Africa. Two haplotypes (14%) were unique to Australia. These two unique haplotypes occurred only once each in P2 (near Warkon, QLD and Theodore, QLD) and not in P1 at all. P1 included one haplotype not present in P2. Australia and South Africa were both dominated by two closely related haplotypes, A and C; a rarer haplotype H, found in P1 in Australia, was only found in adjacent inland sites in South Africa (Table 2, Fig. 2). Haplotype data for the native range was analysed at the site level,

and for South Africa as a whole (Fig.2). Both H and R_h were greatest in South Africa, followed by P1 then P2 (Table 2).

Both H and R_h showed the same trend over time (Fig. 3) with P1 showing an increase in diversity whilst diversity in P2 decreases. We simulated random sampling of nine individuals (without replacement) from South Africa (representing the number of herbarium specimens collected in Australia by 1957) and tested the probability of obtaining larger differences in haplotypic proportions between South Africa and this sample, than were observed in our dataset. The difference was not significant (P = 0.633). A similar simulation based on 83 individuals (representing the number of herbarium specimens collected in Australia by 1987) was significant (P < 0.01) indicating that the haplotype composition in Australia at that time no longer constituted a random sample of those found in the native area studied.

Introduction scenarios

Two new haplotypes appear in the herbarium record in 1975 (haplotype G) and 1983 (haplotype H). To test whether the absence of these haplotypes in the herbarium record prior to 1975 was potentially a sampling effect, we simulated the random resampling (with replacement) of 32 individuals (number of herbarium records collected prior to the appearance of haplotype G in 1975) from a pool of 223 (total number of herbarium records genotyped in Australia) and asked how often the sample did not contain either haplotype G or H. The simulation obtained a P value of 0.019 indicating that the absence of these haplotypes in the early herbarium records is unlikely to be a sampling effect. Both haplotypes appear for the first time in the herbarium record <35 km from the port of Sydney. To test whether this proximity of new haplotypes to the same major port could be attributed to random sampling, we simulated sampling one from a pool of the first 31 different locations recorded in

Australia (the number of different herbarium record locations when the first individual with haplotype G was recorded). We also sampled a second individual from a pool of the first 49 different locations recorded in Australia (the number of different herbarium record locations when the first individual with haplotype H was recorded). We compared the distances from major ports of these two samples and recorded if they both fell within 35 km of the same port. We obtained a *P* value of 0.012, indicating that it is unlikely that the proximity of these samples to a major port in Australia is the result of random sampling from the herbarium record. Haplotype frequencies found in Australia in 1957 were most similar to those in contemporary populations at Tinley Manor and Durban. Haplotype frequencies were significantly different between Australia in 1957 and Vryheid ($P < 0.01$), Denny Dalton ($P < 0.001$) and Pennington ($P < 0.001$).

Discussion

By combining a detailed microsatellite study of contemporary and historical collections with spread data from herbarium records, we find consistent evidence that an initial introduction to Australia of invasive *Senecio madagascariensis* from South Africa, founded a population in lower north coast New South Wales (NSW). This initial invasion then founded a secondary, genetically depauperate population in northern NSW. Temporal analysis in Australia indicates that despite a reduction in genetic diversity when compared to the native range, diversity within Australia has increased over time, probably as a result of subsequent introduction from the native range. The probable timing of additional introduction(s) (some time prior to 1983) is shortly before the reported end of lag phase of *S. madagascariensis* in 1988 [28]. It thus remains plausible that *S. madagascariensis* emerged from lag-phase to become invasive due to the introduction of additional material from the native range.

Additional introductions could have triggered invasiveness either by means of increased standing genetic diversity on which selection could act, or by the introduction of better 'pre-adapted' genotypes more readily able to spread in the Australian environment. Nevertheless, our results do not rule out other explanations for the success of *S. madagascariensis* in Australia, such as enemy release ([29] but see [59].

Bayesian analysis of population structure based on the multilocus nuclear microsatellite data found evidence for these two distinct populations in Australia (the initial population 'P1' and subsequent population 'P2') with additional sites in Far North Queensland founded from each of these populations. This division is supported by significant isolation by distance across Australia as a whole but not within P1 or P2; greater differentiation across Australia than in South Africa, P1 or P2; and by the spread of herbarium records (Fig. 1). Population P2 appears to comprise a subset of the genetic diversity found in P1 with very low occurrence of private alleles and private haplotypes (Table 1, 2), supporting the role of P1 as the primary source of the secondary invasion at P2. This scenario is also consistent with anecdotal evidence that *S. madagascariensis* was transported in crop seed to north coast NSW in the 1940s [32].

Reduction in the genetic diversity of *S. madagascariensis*, first upon introduction to Australia (P1) and subsequently upon establishment of a secondary invasion (P2), does not appear to have hindered its spread. Levels of diversity observed for *S. madagascariensis* in Hawaii [60] were comparable to those obtained from Australia and are less than our estimates for the native range. These results conform to the general trend of reduced diversity in biological invasions [6]. A species' capacity to thrive across a broad range of environmental conditions despite limited

genetic diversity could be due to high levels of phenotypic plasticity [8], changes in gene expression [29] or diversity may simply be 'high enough' (despite a founder effect) for populations to adapt to varying local conditions. Alternatively, the invasive range may impose only weak selection pressures allowing relatively genetically depauperate populations to thrive.

Here we report three different measures F_{ST}, F'_{ST} and D_{est} to describe population differentiation. F_{ST} values obtained were low (maximum = 0.1) which would generally be interpreted as weak population differentiation. However, F_{ST} is sensitive to within population variation and where this differs between regions (such as here, between native and invaded ranges with different diversity levels) problems develop in accurately interpreting F_{ST} results. The use of F'_{ST} circumvents this problem by scaling F_{ST} to its maximum possible value [44]. Jost's D on the other hand [42] is based on the effective number of alleles not heterozygosity and is not sensitive to within population variation. In our study, the pattern of change in these statistics is generally consistent (Table 1), however they vary widely for their estimation of differentiation in P2. D_{est} shows a considerable reduction in P2 when compared to all other regions, F_{ST} shows a considerable increase compared to all other regions, and F'_{ST} indicates a modest reduction when compared to South Africa but a slight increase compared to P1. These differences reflect the different processes best described by these statistics, F_{ST} is a ratio of genetic variances [61] and a higher value indicates a within subpopulation reduction in expected heterozygosity relative to the total population. The differences observed between F_{ST} and F'_{ST} are explained by the scaling factor, making F'_{ST} more appropriate for comparing differentiation between regions. In contrast, D_{est} indicates the level of allelic differentiation between populations and the low result suggests that sites within P2 have very similar allelic composition. As expected heterozygosity is independent of the exact allelic

composition of populations, these differences can be simultaneously observed in the same population (here P2).

Our results indicated that there was significant inbreeding (as measured by F_{IS}) in all populations studied (Table 1). This result is surprising as *S. madagascariensis* is considered an obligate outcrosser [62,63]. The relatively high occurrence of potential null alleles in the data set may have artificially decreased observed heterozygosity (Document S1, Supporting Information). Breeding between close relatives (not strictly selfing) would also contribute to higher levels of F_{IS} within populations. Maintenance of self-incompatability but increased mate availability has been identified in the closely related *S. inaequidens* [64]. In their study, Lafuma and Maurice [64] postulate that an increase in the average level of dominance relationship between S-alleles that control self-incompatability, could have allowed *S. inaequidens* to retain selfing avoidance while reducing the disadvantage of limited mate availability. A similar scenario could have occurred in *S. madagascariensis* in Australia.

The single occurrence of two unique haplotypes in P2 is unusual and has several possible explanations. Firstly, these may be genuinely novel haplotypes introduced from the native range and occurring at such low frequencies in the environment that they have only been sampled once each in the Australian herbarium record. The absence of these haplotypes in the native range could be similarly due to their low occurrence in the environment leading to their omission in native range sampling. Another possible explanation is that these novel haplotypes are the result of mutations of existing haplotypes. Alternatively, the herbarium records for these two individual plants could have been misidentified as *S. madagascariensis*. There are other Australian native *Senecio* species which occur in these areas (e.g. *S. pinnatifolius*) and would likely have similar chloroplast haplotypes. Although we

are unable to conclusively explain the presence of these unique haplotypes, their low frequency means that their inclusion does not unduly affect study results.

Despite generally lower diversity in the invasive compared to the native range, both the haplotypic richness and genetic diversity (as measured by Simpsons index) in Australia increased over time. The appearance of new haplotypes in the herbarium record could be the result of homoplasic mutations (regeneration of identical native range haplotypes) within Australia, or very low initial haplotype frequencies leading to evasion of herbarium sampling. Our simulations indicate that the chances of the low frequency haplotypes (G and H) being missed in herbarium sampling is sufficiently small as to be unlikely, making a secondary introduction of material including these new haplotypes a more parsimonious explanation. However, our simulations presume that haplotypic proportions have remained steady over the course of the invasion, an assumption that may not hold true, particularly if significant genetic drift has occurred or if selection has acted to increase the frequency of particular haplotypes over time. The proximity of first occurrences of haplotypes G and H to the port of Sydney suggests arrival of additional material containing these haplotypes through Sydney. In support of this scenario, our simulations indicate that the chance of two new haplotypes occurring in the herbarium record within this range of a major port merely by chance is sufficiently low as to make it an unlikely explanation, supporting additional introduction(s) as a more parsimonious explanation.

Locations of the various haplotypes present in South Africa (Fig. 2) indicate that the two oldest and most common haplotypes in Australia (A and C) were also found in the majority of native sites, making identification of the initial source of introduction challenging. Both Tinley Manor and Durban have a very similar haplotypic composition to Australia in 1957, and Durban is the largest port in South Africa in

terms of shipping volume, providing a potential invasion pathway. Of the rarer haplotypes in Australia (G and H), which appear to have been introduced later through Sydney, H only occurs at two of the native sites sampled (Vryheid and Denny Dalton), which are within 80 km of each other in the Zululand District Municipality. The restriction of this haplotype to a specific native area suggests that the area may have been a source for *S. madagascariensis* invasion in Australia. It is also possible however that other native areas harbour this haplotype that were not sampled in this study. The similarity between Tinley Manor, Durban and Australia in 1957, with regards to haplotypic composition, as well as the significant difference between the haplotypic composition of Australia in 1957 and the Zululand sites, suggests that an initial introduction from the Durban area (of common haplotypes A and C) was followed by a later introduction from Zululand including haplotypes G and H. However, these results must be treated with caution as the haplotypic proportions in 1957 in Australia may not necessarily mirror those of the source area due to genetic drift and/or selection in South Africa and/or Australia since *S. madagascariensis* was introduced.

Previous efforts to manage *S. madagascariensis* in Australia led to an unsuccessful search for biological control agents in Madagascar [65]. Failure of the biocontrol program was likely due to poor adaptation of Madagascan agents to *S. madagascariensis* genotypes in Australia, as these most likely originated from South Africa [34,35]. Our work builds on these previous findings to suggest that the areas around Durban and Zululand in KwaZulu-Natal may be good prospects for future biological control search efforts

Using our nuclear microsatellite dataset we were unable to conclusively test for admixture between materials originating from disparate native sources. Clustering

analysis found majority support for two South African populations, with differentiation of the Eastern Cape but mixed population origins of all sites in KwaZulu Natal. This differentiation of the Eastern Cape site is corroborated by significant isolation by distance in the native range only when the Eastern Cape site is included. The virtual genetic homogeneity in the native range made source identification impossible from the nuclear microsatellite dataset, a common problem when native range F_{ST} is low [66]. The two Australian populations clustered separately from the single South African population when all samples were analysed together, possibly due to drift post introduction [67] or because we did not sample widely enough to include the true source population(s). Further sampling in the native range may therefore lead to identification of more likely sources

Conclusions

Our study successfully combines genetic analysis of contemporary field and historical herbarium collections to reconstruct the history of *S. madagascariensis* in Australia, from introduction, through lag phase and into the recent period of invasion. By utilising these different resources in combination, we highlight how a survey of contemporary genetic structure only (as undertaken for the majority of invasive species studies) would have failed to identify potential source populations, number of introductions, and changes in genetic diversity through time. Using this approach to reconstruct a more complete picture of the invasion history of introduced taxa will improve our understanding of invasion pathways and lag phase processes, shed further light on the role of multiple introductions and their effects on genetic diversity, and potentially pave the way for more effective control of invasive species.

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Tables and Figures

Table 1.

Location details and diversity metrics of all sites sampled for nuclear microsatellite analysis. Site locations: KZN, KwaZulu-Natal; EC, Eastern Cape; QLD, Queensland; NSW, New South Wales. Diversity metrics: *n*, number of samples per site; *A*, mean number of alleles per locus; A_r, allelic richness; A_{pr}, private allelic richness; H_o, observed heterozygosity; H_e unbiased expected heterozygosity; F_{IS}, inbreeding coefficient; D_{est}, Jost's estimator of actual differentiation; F_{ST}, Wright's fixation index; F'_{ST}, Wright's fixation index scaled to maximum possible value.

Population	Latitude	Longitude	n	A	A_r	A_{pr}	H_o	H_e	F_{IS}	D_{eq}	F_{ST} (±SE)*	F'_{ST} (±SE)*
South Africa (native)												
Vryheid, KZN	S 27° 47' 4"	E 30° 48' 25"	20	10.9	6.5	0.065	0.599	0.839	0.264			
Denny Dalton, KZN	S 28° 17' 3"	E 31° 12' 42"	20	10.9	6.3	0.234	0.606	0.826	0.263			
Ongoye, KZN	S 28° 54' 4"	E 31° 48' 38"	20	11.0	7.2	0.124	0.629	0.871	0.269			
Tinley Manor, KZN	S 29° 27' 2"	E 31° 16' 58"	20	12.1	7.6	0.052	0.513	0.860	0.380			
Pietermaritzburg, KZN	S 29° 38' 10"	E 30° 24' 8"	20	12.6	7.2	0.194	0.678	0.845	0.185			
Summerveld, KZN	S 29° 48' 6"	E 30° 42' 35"	20	13.7	7.3	0.671	0.628	0.870	0.261			
Durban, KZN	S 29° 51' 51"	E 30° 58' 56"	20	12.9	6.8	0.029	0.614	0.855	0.258			
Richmond, KZN	S 29° 52' 58"	E 30° 16' 48"	19	12.3	6.7	0.187	0.693	0.860	0.196			
Pennington, KZN	S 30° 22' 42"	E 30° 40' 34"	20	11.1	6.1	0.304	0.624	0.819	0.263			
Port Edward, KZN	S 31° 2' 5"	E 30° 10' 44"	19	12.0	7.9	0.203	0.585	0.865	0.305			
Boesmansriviermond, EC	S 33° 41' 4"	E 26° 39' 12"	20	8.4	6.2	0.530	0.493	0.711	0.237			
Mean (±SE) South Africa			19.8 (±0.1)	11.6 (±0.6)	6.9 (±0.2)	0.236 (±0.061)	0.606 (±0.028)	0.838 (±0.015)	0.262 (±0.030)			
Total South Africa			218	32.3	7.8	4.008	0.611	0.883	0.312	0.283	0.044 (±0.013)	0.289 (±0.085)
Australia (invasive)												
Far North Queensland												
Malanda, QLD†	S 17° 30' 30"	E 145° 31' 08"	16	6.1	4.9	<0.001	0.561	0.722	0.177			
Herberton, QLD‡	S 17° 25' 44"	E 145° 26' 15"	20	5.9	4.4	<0.001	0.382	0.687	0.473			
Mid-Eastern Australian population (P2)												
Mount Glorious, QLD	S 27° 22' 37"	E 152° 50' 31"	20	5.9	4.0	<0.001	0.552	0.670	0.182			
Mitchelton, QLD	S 27° 24' 44"	E 152° 57' 45"	19	5.9	4.3	0.001	0.458	0.681	0.340			
Oxenford, QLD	S 27° 53' 23"	E 153° 18' 43"	20	6.1	4.6	0.007	0.431	0.677	0.350			
Canungra, QLD	S 27° 58' 0"	E 153° 8' 36"	20	7.4	4.6	0.036	0.446	0.698	0.378			
Lamington, QLD	S 28° 11' 7"	E 153° 6' 56"	20	5.9	4.2	0.002	0.508	0.686	0.264			
Halfway Creek, NSW	S 29° 56' 6"	E 153° 5' 44"	20	7.9	5.0	<0.001	0.524	0.775	0.294			
Mean ±SE P2			19.8 (±0.2)	6.5 (±0.4)	4.5 (±0.1)	0.008 (±0.006)	0.486 (±0.044)	0.698 (±0.022)	0.301 (±0.057)			
Total P2			119	13.4	5.2	0.959	0.492	0.758	0.362	0.158	0.081 (±0.019)	0.276 (±0.065)
South-Eastern Australian population (P1)												
Crescent Head, NSW	S 31° 7' 45"	E 152° 52' 50"	20	9.9	6.2	0.042	0.584	0.812	0.272			
Elizabeth Beach, NSW	S 32° 19' 49"	E 152° 32' 9"	20	10.3	6.5	0.129	0.611	0.775	0.292			
Hunter Valley, NSW	S 32° 36' 55"	E 152° 4' 45"	20	10.1	5.9	0.001	0.645	0.812	0.195			
Black Hill, NSW	S 32° 50' 20"	E 151° 37' 48"	20	10.6	6.3	0.096	0.621	0.816	0.236			
Raymond Terrace, NSW	S 32° 45' 58"	E 151° 44' 55"	20	10.9	6.8	0.002	0.575	0.726	0.196			
Jilliby, NSW	S 33° 11' 58"	E 151° 27' 58"	20	7.1	6.6	<0.001	0.489	0.683	0.267			
Illawarra, NSW	S 34° 31' 5"	E 150° 45' 1"	20	8.4	5.6	0.160	0.552	0.740	0.247			
Gerroa, NSW	S 34° 46' 45"	E 150° 49' 19"	19	10.0	6.6	0.048	0.565	0.855	0.318			
Tabourie Lake, NSW	S 35° 23' 37"	E 150° 26' 18"	20	7.3	5.2	<0.001	0.543	0.604	0.128			
Cooma, NSW	S 32° 19' 49"	E 152° 32' 9"	19	7.3	6.6	0.005	0.577	0.780	0.121			
Tilba, NSW	S 36° 14' 59"	E 150° 6' 14"	20	8.0	5.5	0.068	0.606	0.750	0.191			
Eden, NSW	S 37° 2' 29"	E 149° 54' 30"	20	9.4	6.6	0.064	0.671	0.814	0.157			

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Tilba, NSW	S 36° 14' 59"	E 150° 6' 14"	20	8.0	5.5	0.068	0.606	0.750	0.191			
Eden, NSW	S 37° 2' 29"	E 149° 54' 30"	20	9.4	6.6	0.064	0.671	0.814	0.157			
Mean ±SE P1			19.8 (±0.1)	9.1 (±0.5)	6.2 (±0.2)	0.051 (±0.016)	0.587 (±0.028)	0.764 (±0.022)	0.210 (±0.031)			
Total P1			238	22.4	6.7	2.331	0.612	0.833	0.275	0.237§	0.049 (±0.010)	0.240 (±0.049)
Mean ±SE Australia			19.7 (±0.2)	8.0 (±1.3)	5.5 (±0.2)	0.038 (±0.011)	0.545 (±0.023)	0.738 (±0.015)	0.250 (±0.028)			
Total Australia			393	23.5	6.8	2.997	0.568	0.843	0.335	0.420§	0.100 (±0.012)	0.422 (±0.051)
Mean ±SE South Africa & Australia			19.8 (±0.1)	9.3 (±0.3)	6.0 (±0.2)	0.105 (±0.028)	0.567 (±0.018)	0.774 (±0.012)	0.254 (±0.021)			
Total South Africa & Australia			611	35.4	7.6		0.585	0.880	0.342	0.487§	0.100 (±0.013)	0.459 (±0.060)

*Jackknifed over all loci
†Site clusters with southern Australian population
‡Site clusters with mid Australian population
§Two Australian populations (Jilliby and Raymond Terrace) excluded from calculation due to missing data

Table 2.

Site details, diversity and haplotype frequencies of all individuals used for chloroplast microsatellite analysis at three loci. Population locations: KZN, KwaZulu-Natal; EC, Eastern Cape; Australian populations comprised of herbarium accessions falling into the geographic ranges of the mid-eastern population (P2) and south-eastern population (P1) , based on clustering of the nuclear data as defined by STRUCTURE [38]. Diversity measures: N_i, number of individuals; N_h, number of haplotypes (parentheses denote private haplotypes); R_h, haplotypic richness (rarefied to n = 11); H, Simpson's diversity index.

Population	N_i	N_h	R_h	H	Haplotypes														
					A	B	C	D	E	F	G	H	I	J	K	L	M	N	
South Africa (native)																			
Vryheid, KZN	18	5(0)	0.771	3.945	5	–	1	–	–	2	7	3	–	–	–	–	–	–	
Denny Dalton, KZN	20	7(1)	0.832	4.621	2	–	7	–	–	1	3	2	1	4	–	–	–	–	
Ongoye, KZN	19	4(0)	0.573	2.397	12	–	4	1	2	–	–	–	–	–	–	–	–	–	
Tinley Manor, KZN	14	2(0)	0.440	1.000	10	–	4	–	–	–	–	–	–	–	–	–	–	–	
Pietermaritzburg, KZN	11	4(0)	0.691	3.833	6	–	–	2	–	1	2	–	–	–	–	–	–	–	
Summerveld, KZN	19	6(0)	0.661	3.667	11	1	2	–	2	2	1	–	–	–	–	–	–	–	
Durban, KZN	19	3(0)	0.433	1.561	14	–	4	–	–	1	–	–	–	–	–	–	–	–	
Richmond, KZN	18	4(0)	0.471	2.179	13	1	3	–	–	–	1	–	–	–	–	–	–	–	
Pennington, KZN	20	4(1)	0.626	2.358	2	–	11	–	–	–	–	–	–	–	6	1	–	–	
Port Edward, KZN	17	5(0)	0.621	2.425	13	1	1	–	–	1	–	–	–	–	1	–	–	–	
Boesmansriviermond, EC	20	3(0)	0.195	1.100	18	–	1	–	–	–	1	–	–	–	–	–	–	–	
Mean ±SE South Africa	17.7 (±0.9)	4.3 (±0.4)	2.644 (±0.366)	0.574 (±0.054)															
Total South Africa	195	12(8)	3.331	0.659	106	3	38	3	4	8	15	5	1	4	7	1	–	–	
Australia (invasive)																			
Mid-Eastern Australian population (P2)	95	5(2*)	0.316	1.451	78	–	9	–	–	–	6	–	–	–	–	–	1	1	
South-Eastern Australian population (P1)	128	4(1†, 0*)	0.522	1.998	84	–	24	–	–	–	16	4	–	–	–	–	–	–	
Mean ±SE Australia	111.5 (±16.5)	3.5 (±0.5)	1.725 (±0.274)	0.419 (±0.103)															
Total Australia	223	4(0)	1.808	0.442	162	–	33	–	–	–	22	4	–	–	–	–	1	1	
Total South Africa & Australia	418	14	2.561	0.552	268	3	71	3	4	8	37	9	1	4	7	1	1	1	

* Australia and South Africa; † Australia only

Figure 1.

Maps illustrating the spread of *Senecio madagascariensis* in Australia through time. Panels (a)-(c) show density of herbarium records and location of chloroplast haplotypes. Panels (c) and (d) show location of P1 and P2 derived from nuclear microsatellite data from contemporary field collections (as defined by clusters in the program STRUCTURE [38]). Panel (d) shows clustering of sites in Far North Queensland with P1 and P2. Panel (e) shows extent of maps in relation to Australia as a whole.

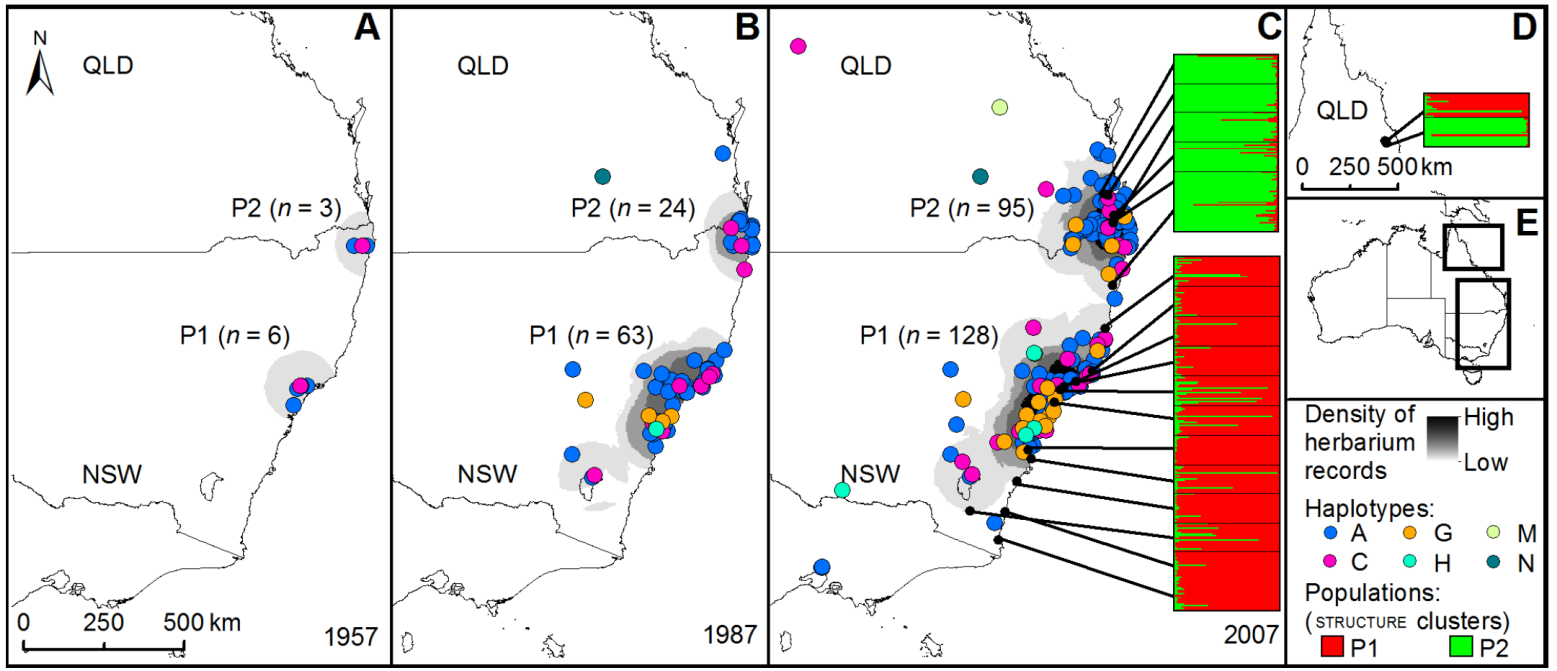

A
B
C
D
E
N
QLD
NSW
P2 (n = 3)
P1 (n = 6)
P2 (n = 24)
P1 (n = 63)
P2 (n = 95)
P1 (n = 128)
0 250 500 km
1957
1987
2007
0 250 500 km
Density of herbarium records
High
Low
Haplotypes:
A
G
M
C
H
N
Populations:
(STRUCTURE clusters)
P1
P2

Figure 2.

The location of *Senecio madagascariensis* haplotypes based on three chloroplast microsatellite loci. Panel (a) shows the proportion of haplotypes found at each sampled site in South Africa; panel (b) shows haplotypes from all herbarium records in Australia, according to their position in either the south-eastern Australian population (P1) or mid-eastern Australian population (P2). The size of the pie charts showing totals in both South Africa and Australia are proportional to the number of individuals sampled; panel (c) shows a median joining network of *S. madagascariensis*, where the smallest connector length represents one character change; panel (d) shows map extents. Colour codes for haplotypes are consistent throughout.

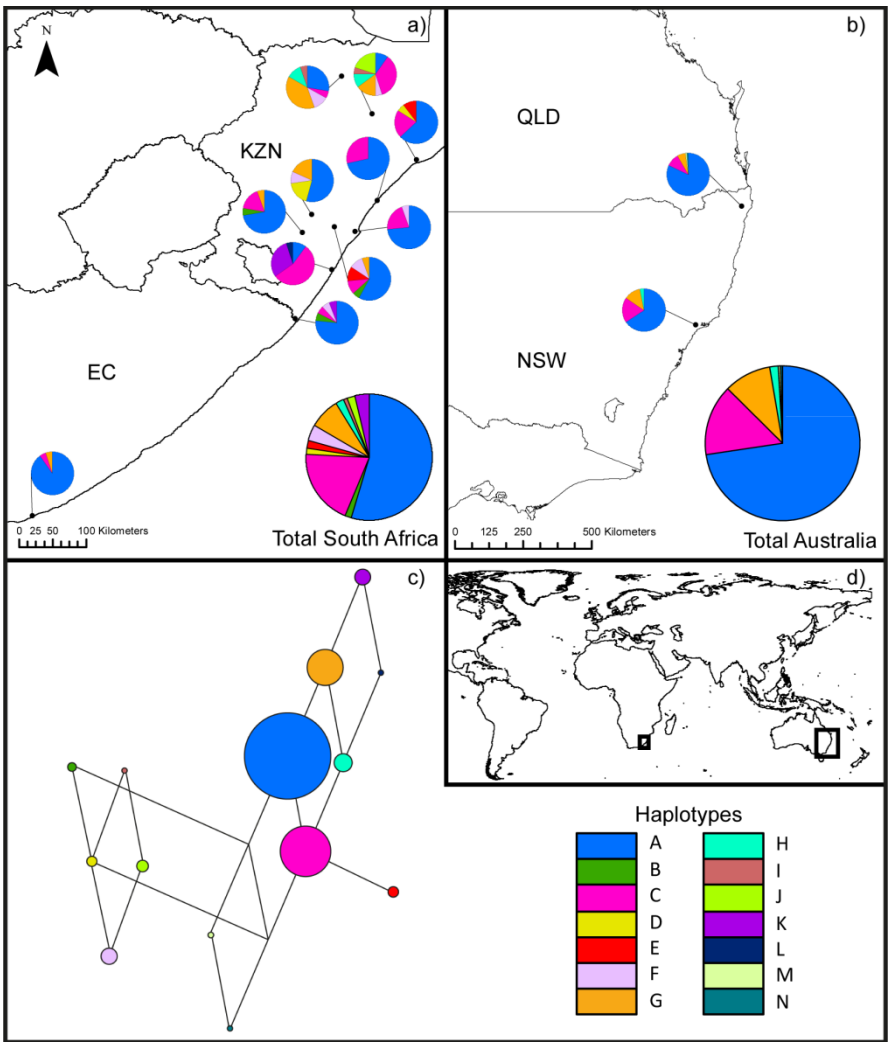
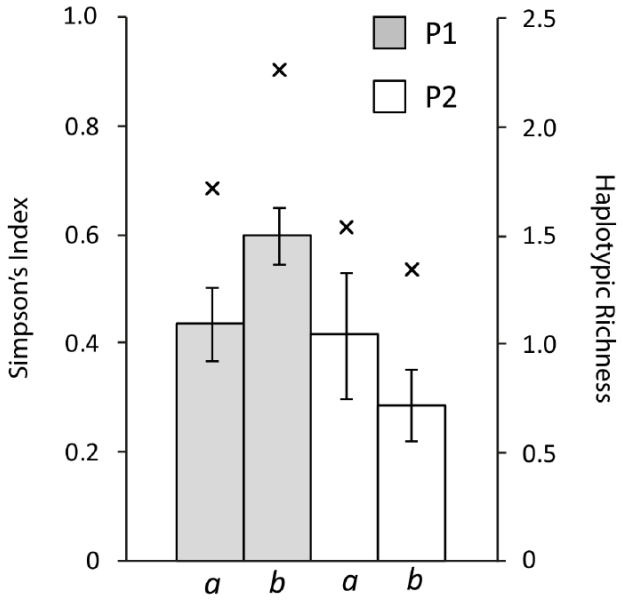


Figure 3.

Simpson's index of haplotype diversity (±SE) (primary y axis) and haplotypic richness (rarefied to *n* = 11) (cross symbols; secondary y axis) for populations P1 (grey) and P2 (white) during two different time periods, 1918 – 1987 (a) and 1988 – 2007 (b).



Supplementary Material

Document S1 – Microsatellite loci evaluation

Methods

Prior to scoring, all microsatellites were evaluated for consistency in banding pattern across individuals; any inconsistent loci were not scored and excluded from all subsequent analyses. For the nuclear microsatellite dataset, Linkage disequilibrium (LD) and deviations from Hardy-Weinberg (HW) equilibrium were investigated using GENEPOP on the web [1,2] and significance levels adjusted using sequential Bonferroni corrections [3]. The program MICRO-CHECKER [4] was used to examine large allele dropout, stuttering and null alleles as potential sources of error. Null allele frequencies were estimated using FREENA [5]. Genetic clusters in HW were determined using the program STRUCTURE v2.3.3 [6] using the admixture model. As the presence of null alleles introduces potential ambiguity around the true underlying genotype, we ran the program under two conditions; RECESSIVEALLELES set to 0 in which no ambiguity is assumed; and RECESSIVEALLELES set to1 where missing data is assigned as recessive to better account for null alleles [7]. To examine the effect of null alleles on F_{ST} estimates, a Mantel test with 9,999 permutations was carried out in GENALEX v6.4 [8] between the INA corrected and uncorrected F_{ST} estimates obtained from FREENA [5]. We used POWSIM v4.0 [9] to assess the power of our microsatellite dataset to detect genetic heterogeneity in Australia. Error rates were determined according to [10]; specifically error rates per allele, per locus and over all loci were calculated for both the nuclear and chloroplast microsatellite datasets.

Results

Two nuclear loci were excluded due to inconsistent banding patterns, the remaining seven were polymorphic and used in further analyses. There was no evidence of large allele dropout in any loci. Stuttering was identified as a potential issue at one

native and one invasive site for one locus. Significant LD was found for three different loci combinations, each at one of three different sites. All loci showing potential stuttering or LD were retained in the dataset as genuine stuttering and LD are expected to affect all sites equally. Significant deviations from HW equilibrium were found in 92 of 212 tests (43%), indicating heterozygote deficiency. Potential presence of null alleles was found in 80 of 182 tests (44%). Significant HW deviation corresponded with presence of null alleles in 90% of cases. The null allele frequency distribution was estimated to have a mean of 0.19, with 25% and 75% quartiles of 0.07 and 0.37 respectively. In STRUCTURE [6] there were no appreciable differences in optimum values of *K* or assignment of individuals to each cluster when RECESSIVEALLELES set to 0 or 1, therefore we only report results as per RECESSIVEALLELES set to 0. No loci were dropped due to presence of null alleles to minimise power loss, as when dealing with a low number of loci, it is generally preferable to account for null alleles rather than exclude loci [11]. Pairwise F_{ST} values corrected and uncorrected for null alleles were strongly and significantly correlated ($r = 0.97$, $P < 0.001$) suggesting that the effect of null alleles was similar across populations. Uncorrected F_{ST} values only are reported throughout the paper. Power analysis determined that our nuclear microsatellite dataset could accurately detect F_{ST} values > 0.01 ($P = 1$) indicating that our markers are adequate to detect the population structure in *S. madagascariensis*. Overall, the mean genotyping error rates per locus and per allele were 0.047 and 0.029 respectively. Errors per locus and per allele were less in Australia (0.023 and 0.035 respectively) than in South Africa (0.035 and 0.060 respectively). Error rates for individual loci are listed in Table S2, Supporting Information.

Figure S1.

Barplot output (where K = 2) from the program STRUCTURE [6] showing all South African sites in order of most northerly to most southerly (see Table 1 in main document for site names and locations).

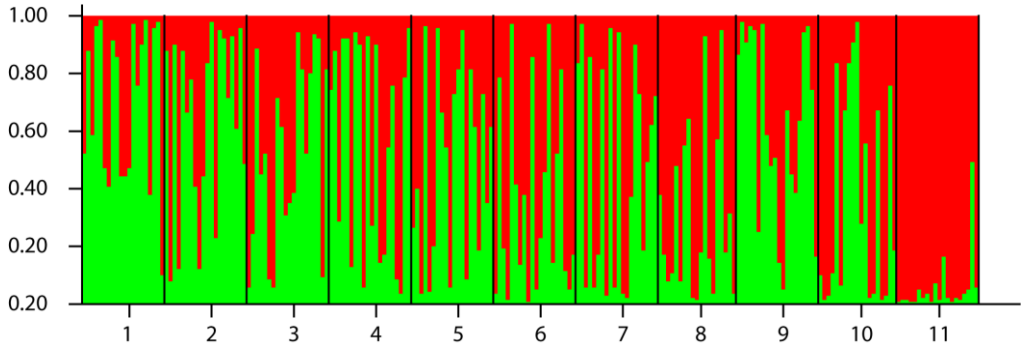


Table S1. *Senecio madagascariensis* herbarium voucher details

Herbarium	Site	Latitude	Longitude	Voucher number	Ploidy
South African National Herbarium	Vryheid, KZN	S 27° 47' 4"	E 30° 48' 25"	JGR 1101	diploid
	Hluhluwe, KZN	S 27° 48' 48"	E 32° 24' 5"	JGR 1103	polyploid
	Denny Dalton, KZN	S 28° 17' 3"	E 31° 12' 42"	JGR 1102	diploid
	Ongoye, KZN	S 28° 54' 4"	E 31° 48' 38"	JGR 1106	diploid
	Tinley Manor, KZN	S 29° 27' 2"	E 31° 16' 58"	JGR 1107	diploid
	Pietermaritzburg, KZN	S 29° 38' 10"	E 30° 24' 8"	JGR 1117	diploid
	Summerveld, KZN	S 29° 48' 6"	E 30° 42' 35"	JGR 1115	diploid
	Durban, KZN	S 29° 51' 51"	E 30° 58' 56"	JGR 1108	diploid
	Richmond, KZN	S 29° 52' 58"	E 30° 16' 48"	JGR 1116	diploid
	Pennington, KZN	S 30° 22' 42"	E 30° 40' 34"	JGR 1109	diploid
	Port Edward, KZN	S 31° 2' 5"	E 30° 10' 44"	JGR 1114	diploid
Stellenbosch University Herbarium	Boesmansriviermond, EC	S 33° 41' 4"	E 26° 39' 12"	SA/SMAD/A2/01	diploid
Queensland Herbarium	Halfway Creek, NSW	S 29° 56' 6"	E 153° 5' 44"	AQ741268	diploid

Table S2.

Polymorphic microsatellite primer pairs used in analysis, including fluorescent dye. Originally developed by Le Roux (nuclear) [12] and Weising and Gardner (chloroplast) [13]. Annealing temperatures used in PCR (T_a), number of alleles (A) and error rate per allele (E_a) and locus (E_l) are also shown.

Marker	Locus	Forward primer	Reverse primer	T_a (°C)	A	E_a	E_l
nSSR	Se-116	CCTTCTGGTTGATTTGGCTAAGC(FAM)	AGAACTGCACATTTGAAGCCTG	48	22	0.000	0.000
	Se-136	CAAAGGTAGGATGATGTGAAGCTC(FAM)	TCTTGTTGGGTCAATGCTCG	51	74	0.069	0.083
	Se-138	ACTTCGTGGGCCATTCCAG(VIC)	CTTCCTGCATAACATCCACCAC	58	30	0.044	0.086
	Se-176(a)	AGCATAGTGCAAGCATGTTCG(FAM)	CTTTGATGTTGGCTGCAATGC	60	29	0.007	0.014
	Se194	GTCGCAGTCACCGTCACTG(VIC)	GAGCAGCAGACAACGACAC	58	9	0.031	0.061
	Se-208	TTTTGGGCAGGCCATATCC(NED)	AGTGTCTCCACGGTTGTCG	55	61	0.020	0.040
	Se-220	AACTCGACCAGTCCTCAGC(NED)	GTCACACTGGAACCCAACTG	58	23	0.033	0.039
cpSSR	Ccmp1	CAGGTAAACTTCTCAACGGA(VIC)	CCGAAGTCAAAAGAGCGATT	50	3	0.053*	
	Ccmp2	GATCCCGGACGTAATCCTG(PET)	TCGTACCGAGGGTTCGAAT	50	3	0.043*	
	Ccmp5	TGTTCCAATATCTTCTTGTCATTT(FAM)	AGGTTCCATCGGAACAATTAT	50	2	0.000*	

*Error rate per locus and per allele are the same in haploid genomes

Table S3.

Hypotheses tested using Resampling Stats Add-In for Excel v4.0 (statistics.com) with 10000 repeat samples.

Null hypothesis	***P***
Haplotypic composition of Australia in 1957 is a random sample of the diversity present in the native range	0.633
Haplotypic composition of Australia in 1987 is a random sample of the diversity present in the native range	<0.01
Haplotypes G and H were not present in the first 32 herbarium records due to a sampling effect	0.019
Haplotypes G and H were both first found within 35 km of a major port by chance	0.012

Table S4.

Major ports in eastern Australia used in simulations. Name, location and size obtained from worldportsource.com

Port Name	**Size**	**Latitude**	**Longitude**
Port Botany	Medium	S 33° 58' 23"	E 151° 12' 54"
Port of Brisbane	Large	S 27° 22' 57"	E 153° 10' 10"
Port of Gladstone	Medium	S 23° 49' 48"	E 151° 15' 10"
Port Kembla	Medium	S 34° 27' 45"	E 150° 53' 58"
Port of Melbourne	Very Large	S 37° 49' 26"	E 144° 54' 40"
Port of Newcastle	Medium	S 32° 54' 27"	E 151° 46' 14"
Port of Sydney	Large	S 33° 51' 44"	E 151° 11' 33"

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CHAPTER 4

Australia First: Is Australia the bridgehead for intercontinental invasions of the global weed, *Senecio madagascariensis*?

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Statement of Authorship

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Contributed to initial manuscript conceptualisation, undertook laboratory work and completed all analyses acted as lead author in drafting manuscript and produced all figures and tables.

I hereby certify that the statement of contribution is accurate

Signature:..... Date: 28/03/2013

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Commented on and edited manuscript drafts.

I hereby certify that the statement of contribution is accurate and consent to the inclusion of this manuscript in Eleanor Dormontt's PhD thesis.

Signature:..... Date 28/03/2013

Prentis, P. J.
Commented on and edited manuscript drafts.

I hereby certify that the statement of contribution is accurate and consent to the inclusion of this manuscript in Eleanor Dormontt's PhD thesis.

Signature:..... Date 28/03/2013

Lowe, A. J.
Contributed to initial manuscript conceptualisation, and commented on and edited subsequent manuscript drafts.

I hereby certify that the statement of contribution is accurate and consent to the inclusion of this manuscript in Eleanor Dormontt's PhD thesis.

Signature:..... Date 28/03/2013

KEYWORDS: *Senecio madagascariensis*, biological invasions, chloroplast microsatellites, bridgehead, herbarium

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RUNNING TITLE: The genetics of an exotic plant's global invasions

Abstract

Understanding routes of invasions is crucial for our capacity to predict and manage the ongoing threat of invasive species. The globally invasive weed *Senecio madagascariensis* is native to South Africa and is presently known to have established in Japan, Hawaii, Australia, and parts of Africa and South America. Introduction to Australia is thought to have occurred from the native range (KwaZulu-Natal) in the early part of the 20th century. The history of introduction to other parts of the world has been less well characterised, although Australia has been suggested as a potential source for these secondary invasions. We used chloroplast microsatellites to look at the haplotypic variation present in the native range of *S. madagascariensis* and compared it to that found in Australia, Japan, Hawaii, Argentina and Brazil. We found greatly reduced numbers of haplotypes in all invasive ranges compared to the native range and all variation present in Japan, Hawaii, Argentina and Brazil could be found in Australia. However, a haplotype found commonly in Hawaii was only found in the Australian herbarium record, not contemporary populations, possibly as a result of founder effects in Hawaii increasing

the frequency of this otherwise rare haplotype. Our findings highlight the utility of herbarium records in assessing potential introduction sources and demonstrates how historical genetic signatures can be 'overwritten' by contemporary changes in haplotype frequencies. Our results support the role of Australia as a potential source population (i.e. bridgehead) for subsequent global invasions of *S. madagascariensis*. Similarities between invasive *S. madagascariensis* populations across the world emphasises the need for appropriate biosecurity measures to prevent further infestations from invasive as well as native source populations.

Introduction

Biological invasions are recognised as a significant threat to global biodiversity and food security, with many countries now employing sophisticated biosecurity protocols to limit further introductions and protect biological assets (Cook et al., 2011, Heikkilä, 2011, Perrings et al., 2005). Understanding invasions pathways is an important tool in the biosecurity tool belt, enabling prioritisation of resources both in terms of preventing entrance to a country (border control) and reducing the likelihood of arrival (improved transport practices) (Handley et al., 2011, Wilson et al., 2009). Introductions from the native range of invasive species are generally considered to pose the greatest risk for new invasions, or for further introduction of invasive stock that may exacerbate an existing problem.

Recently, the identification of invasive 'bridgehead' populations has highlighted the threat of secondary invasions originating not from native material but from invasive populations where a species is already well established (Lombaert et al., 2010). After initial introduction, successful establishment and spread of material originating from bridgehead populations may be the result of readily available invasion pathways,

rapid evolution of more invasive phenotypes in the bridgehead population, or a combination of both (Estoup and Guillemaud, 2010). Recognising bridgehead populations of invasive species will allow more cost effective management of invasion pathways and will impact on biosecurity risk-assessment criteria.

Reconstructing invasion pathways is often extremely complex (Boubou et al., 2012, Estoup and Guillemaud, 2010) but analysis of uniparentally inherited genomes (e.g. chloroplasts, mitochondria) can be useful due to their low mutation rate (Provan et al., 1999) and lack of recombination during meiosis. These characteristics make chloroplast microsatellites less variable but better equipped to identify broad scale patterns that might be obscured by gene flow in nuclear DNA (Agarwal et al., 2008), such as identification of putative source populations (e.g. Taylor and Keller, 2007, Prentis et al., 2009). The utility of chloroplast microsatellites in identifying exact source populations is limited however, as they provide no means of distinguishing between multiple sources that all share haplotypes with the invasive range.

In our previous work (Chapter 3) we reconstructed the invasion history of *Senecio madagascariensis* Poir. (Asteraceae) in Australia, using a combination of nuclear and chloroplast microsatellite loci in both native (South African) and invasive (Australian) populations. We also used chloroplast microsatellites to screen all specimens of *S. madagascariensis* kept in the Queensland Herbarium, National Herbarium of Victoria and National Herbarium of New South Wales, allowing analysis of temporal as well as spatial spread dynamics. *Senecio madagascariensis* is also invasive in other parts of the world; it is recorded as having been introduced to Japan, Hawaii, Australia, and parts of Africa and South America (Cruz et al., 2010, Kinoshita et al., 1999, Le Roux et al., 2006, Tracanna and Catullo, 1987, Invasive species compendium, www.cabi.org/isc). Australia has been suggested as the origin of

invasive material introduced to Hawaii in the 1980s (Le Roux et al., 2010), but this assertion has not been validated with genetic analyses to date. The origin of other global invasions of *S. madagascariensis* remains similarly unknown.

Here we extend our previous work (Chapter 3) by comparing variation at chloroplast microsatellite loci in contemporary *S. madagascariensis* samples collected from Australia, Hawaii, Japan, Brazil and Argentina, with the results obtained from Chapter 3 (in which chloroplast microsatellites were used to screen contemporary South African material and Australian herbarium samples). Specifically, we ask whether the haplotypic composition of global invasions is consistent with Australia acting as a bridgehead (source) population for secondary invasions; or conversely whether independent introductions from the native range provide a more parsimonious explanation of the observed diversity. Additionally, we explore the importance of historical herbarium data on *S. madagascariensis* in Australia and whether its inclusion impacts on inferences drawn from the results regarding likely source populations.

Methods

Senecio madagascariensis is diploid (2n = 20) and an obligate outcrosser. Inflorescences are bright yellow and insect pollinated. Seeds are predominantly wind dispersed but their small size means they are often found as contaminants in other products (e.g. hay, turf, crop seed) and they can also attach easily to animals, clothes and machinery. Leaf samples and/or seeds from individual plants in Australia, Hawaii, Japan, Brazil, Argentina and South Africa were collected and either stored in silica gel for DNA extraction (in the case of leaf material) or germinated and grown to maturity in the glass house to facilitate DNA extraction from fresh leaf material (in the

case of seeds). Details of all sampling locations can be found in Table 1. In addition to the contemporary collections, all herbarium specimens from Australia were also sampled as described in Chapter 3.

DNA extraction was undertaken according to the protocol described in Chapter 3, or (Comes et al., 1997) with modifications as described in (Brennan et al., 2009). PCR reactions were carried out using the methodology explained in Chapter 3 (at Universtiy of Adelaide) or in 15 µL reactions prepared with ~8 ng of template DNA, 1 x reaction buffer, 0.2 x PolyMate, 0.25 mM each dNTP, 0.04 mM MgCl2, 6 ng each of label primer (Sigma-Aldrich) and reverse primer (VHBio), 1.5 ng of forward primer (VHBio) and 0.6 U Taq DNA polymerase (Bioline) (at St Andrews University). Fluorescent dye labels (D2, D3 and D4 (Sigma-Aldrich, UK)) were used to distinguish products with universal 18 bp M13 5′-labelled oligonucleotides, 5′ M13-tagged forward primers and standard reverse primers (Schuelke, 2000). Reactions were carried out with an initial denaturation step of 95 °C for 2 minute, then 10 cycles of 95 °C for 20 seconds, 58-49 °C for 1 minute (reduce by 1°C per cycle), 72 °C for 2 minutes, then 25 cycles of 95 °C for 20 s, 48 °C for 1 minute, 72 °C for 2 minutes, and a final extension at 72 °C for 10 minutes. DNA was amplified using primers for the three chloroplast microsatellite identified as polymorphic in Chapter 3. Genotyping and scoring were carried out as described in Chapter 3 or on a Beckman Coulter CEQ 8000 sequencer and scored using ceq v9.0 (Beckman Coulter Inc). Approximately 10% of samples were repeated to allow error rate calculation according to the recommendations of DeWoody et al. (2006).

Each unique combination of alleles was defined as a separate haplotype. Counts were made of total number of haplotypes and private haplotypes in a particular location. Shannon's diversity index (*H*) was determined using the following equation:

$H = -\sum p_i \ln (p_i)$ where p_i is the proportion of the *i*th haplotype in the population (Shannon, 1948). For the herbarium data, number of haplotypes and Shannon's index of diversity (*H*) were determined for the genetic clusters identified in Australia (i.e. populations) (Chapter 3). A median-joining network (Bandelt et al., 1999) was created in network v4.6.0.0 (Fluxus Technology Ltd. 2010). Detailed maps of haplotype distribution were created in ArcGIS v10 (ESRI).

Results

The chloroplast microsatellite haplotypes of *Senecio madagascariensis* found in Hawaii, Japan, Brazil and Argentina were all also found in Australia as well as the native South African range. Fourteen different chloroplast haplotypes were identified worldwide (Figures 1, 2, Tables 1, 2). Six haplotypes were found in invasive ranges; four of these haplotypes were found in the majority of samples. The additional two invasive haplotypes were found in remote Australia only. All invasive haplotypes (except the additional two from remote Australia) were also found in the South African native range. In all ranges studied, haplotype A was the most frequent, making up 54 % in the native range, 77 % in Australia, 90 % in Hawaii, 91 % in Argentina and 100 % in Brazil and Japan of the total number of samples taken. Less frequent haplotypes found in invasive populations were C, G, H, M and N. In contemporary invasive populations only haplotypes A, C and G were present (Table 1), haplotypes H, M and N were only found in the Australian herbarium record (Table 2) between 1967 and 2005. In Hawaii, haplotype G was found in 10 % of samples. This haplotype was also found in the native range in 8 % of individuals across six sites (out of a possible 11 sites sampled), and in the Australian herbarium record.

Diversity as measured by Shannon's Index (Table 1) was highest in the native range, followed by Australia (when herbarium and contemporary samples were considered together, *H* = 0.745), then Hawaii, Argentina, Brazil and Japan. The overall error rate was 0.04.

Discussion

The *Senecio madagascariensis* haplotypes found in Hawaii, Japan, Brazil and Argentina were also found in Australia and the native South African range. This finding is consistent with Australia acting as a source for secondary invasions of *S. madagascariensis* across the world but does not rule out separate introductions from the native range of haplotypes identical to those found in Australia. The diversity of haplotypes found in all invaded ranges studied was less than that found in the native range, indicating genetic bottleneck events occurred during introduction. Diversity in Australia was intermediate to the native range and other global invasions; the higher diversity present in Australia could reflect a larger, more diverse immigrant pool and/or successive introductions of additional diversity over time (as supported by the results of Chapter 3). Alternatively it could be a sampling effect; many more samples were analysed in Australia than in any other region (including the native range) and the inclusion of herbarium records may also have captured haplotypes that are no longer present in contemporary populations.

Founding of secondary invasions from successful invasive rather than native populations, has recently been identified in the Harlequin ladybird *Harmonia axyridis*, and termed the 'bridgehead effect' (Estoup and Guillemaud, 2010, Lombaert et al., 2010). Other examples of this phenomenon include the invasion of South America, then subsequently North America by *Drosophila subobscura*, (Pascual et al., 2007),

and the wild olive *Olea europaea* subsp. *cuspidata*, where an initial introduction to Australia from South Africa subsequently founded the invasion of Hawaii and was accompanied by a significant reduction in diversity (Besnard et al., 2007). Our study confirms that Australia could have acted as a bridgehead population for *S. madagascariensis* invasions worldwide.

The utility of the herbarium record for studying biological invasions is also highlighted by the results of this work (for a more in-depth treatment of this issue, see Chapter 3). In Hawaii, four individuals displayed a rare haplotype found only in native contemporary populations. From these contemporary samples alone, it is tempting to conclude that the Hawaiian invasion was likely the result of a separate introduction from the native range, or a subsequent introduction that augmented the diversity introduced initially from Australia. However, when the Australian herbarium record is considered, the same haplotype is found at the same frequency as that found in Hawaii (10 %) meaning that an introduction from Australia (as postulated by Le Roux et al., 2010) is sufficient to explain the observed diversity in Hawaii. This haplotype also first appears in the Australian herbarium record in 1975, just prior to the reported introduction to Hawaii in the 1980s. In this case Australia might have acted as a 'cryptic' bridgehead population, since surveying only contemporary populations, rather than those present during the period of invasion into Hawaii, would have pointed to South Africa as the more likely direct source. Another possible explanation may be the introduction of the same material to Australia and Hawaii from South Africa. Chapter 3 pointed towards an additional introduction of *S. madagascariensis* to Australia in the 1970s; although we consider it to be unlikely, it's possible that both invasions were founded from material present in the same introduction vector (most likely as dry ballast).

Sample numbers in this study were low for all invasions outside of Australia, which will have impacted our ability to correctly identify all haplotypes present in a given area; and it is likely that some diversity has been underestimated. Larger sample sizes and the inclusion of herbarium records from other *S. madagascariensis* invasions would improve the resolution of this work and potentially better distinguished between the various possible invasion pathway scenarios.

Although the results obtained here do not preclude separate introductions from the native range or other invasions (all ranges were dominated by one or both of two haplotypes), exportation of *S. madagascariensis* from Australia should still be considered as a significant threat for future invasions. International biosecurity protocols should include focus on potential invasion pathways from Australia (Wilson et al., 2009) and seek to prevent the accidental importation of *S. madagascariensis* propagules. Being wind dispersed, *S. madagascariensis* has very small mobile seeds which can easily contaminate agricultural products and machinery, and establishment of new invasion foci in Australia has likely occurred as a result of these processes (Tablelands Regional Council, personal communication, Sindel, 1986). The seeds can also remain viable for many years (Radford and Cousens, 2000) making effective quarantine procedures particularly important.

From an Australian perspective, introduction of novel *S. madagascariensis* genotypes is most likely to occur from South Africa, as opposed to the other global invasions studied here, as the genetic diversity present in other invasions (as captured by chloroplast microsatellite analysis) is already present in Australia. Introduction of novel genotypes can increase genetic diversity which is often associated with evolutionary potential and can lead to more aggressive invasive populations (Prentis et al., 2008, Facon et al., 2008, Lavergne and Molofsky, 2007).

Even in the absence of adaptive evolution, introduction of genetic novelty would increase the chances of a superior pre-adapted genotype establishing and spreading through an existing invasion. However, propagules from other successful global invasions (with haplotypes that are already present in Australia), would still present an unacceptable risk, as even populations that have experienced strong bottlenecks during colonisation can undergo rapid evolution (Dlugosch and Parker, 2008) and a reduction in diversity at neutral loci does not necessarily reflect a diversity reduction in quantitative traits (Knopp et al., 2007). Other global invasions of *S. madagascariensis* may still comprise individuals with invasive traits not currently found in Australia. Australia's biosecurity policies are considered some of the best in the world (Thompson et al., 2003) and should continue to guard against future introductions of *S. madagascariensis* from all potential sources.

Conclusions

Here we present the first genetic study of global invasions of *Senecio madagascariensis* and find that the diversity present across multiple invasions is consistent with Australia acting as a bridgehead for subsequent global introductions. However, results cannot rule out the possibility of independent introductions from the native range of material with identical haplotypes. Diversity in all global introductions was reduced compared to the native range, indicating a genetic bottleneck at some point along the invasion pathway. Inclusion of the Australian herbarium record allowed identification of historic diversity not found in the contemporary Australian populations sampled. One of these haplotypes was present in contemporary Hawaiian collections, highlighting the utility of genetic analysis of herbarium records in preventing the inference of more complex invasion pathways than is necessary to explain observed patterns. Biosecurity measures should consider Australia as a

significant threat for exportation of invasive *S. madagascariensis* to new suitable areas worldwide.

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Tables and Figures

Table 1.

Site details, diversity and haplotype frequencies of all contemporary individuals used for chloroplast microsatellite analysis at three loci. Population locations: KZN, KwaZulu-Natal; EC, Eastern Cape; QLD, Queensland; NSW, New South Wales; MAU, Maui;. Diversity measures: N_i, number of individuals; N_h, number of haplotypes (parentheses denote private haplotypes); H, Shannon's index of diversity

Population	Latitude	Longitude	N_i	N_h	H	Haplotypes											
						A	B	C	D	E	F	G	H	I	J	K	L
South Africa (native)																	
Vryheid, KZN*	S 27° 47' 4"	E 30° 48' 25"	18	5(0)	1.426	5	-	1	-	-	2	7	3	-	-	-	-
Denny Dalton, KZN*	S 28° 17' 3"	E 31° 12' 42"	20	7(1)	1.734	2	-	7	-	-	1	3	2	1	4	-	-
Ongoye, KZN†	S 28° 54' 4"	E 31° 48' 38"	19	4(0)	1.010	12	-	4	1	2	-	-	-	-	-	-	-
Tinley Manor, KZN*	S 29° 27' 2"	E 31° 16' 58"	14	2(0)	0.598	10	-	4	-	-	-	-	-	-	-	-	-
Pietermaritzburg, KZN*	S 29° 38' 10"	E 30° 24' 8"	11	4(0)	1.119	6	-	-	2	-	1	2	-	-	-	-	-
Summerveld, KZN*	S 29° 48' 6"	E 30° 42' 35"	19	6(0)	1.337	11	1	2	-	2	2	1	-	-	-	-	-
Durban, KZN*	S 29° 51' 51"	E 30° 58' 56"	19	3(0)	0.708	14	-	4	-	-	1	-	-	-	-	-	-
Richmond, KZN*	S 29° 52' 58"	E 30° 16' 48"	18	4(0)	0.855	13	1	3	-	-	-	1	-	-	-	-	-
Pennington, KZN*	S 30° 22' 42"	E 30° 40' 34"	20	4(1)	1.070	2	-	11	-	-	-	-	-	-	-	6	1
Port Edward, KZN*	S 31° 2' 5"	E 30° 10' 44"	17	5(0)	0.872	13	1	1	-	-	1	-	-	-	-	1	-
Boesmansriviermond, EC*	S 33° 41' 4"	E 26° 39' 12"	20	3(0)	0.394	18	-	1	-	-	-	1	-	-	-	-	-
Mean ±SE South Africa			17.7 (±0.9)	4.4 (±0.4)	1.011 (±0.117)												
Total South Africa			195	12(8)	1.534	106	3	38	3	4	8	15	5	1	4	7	1
Argentina (invasive)																	
Buenos Aires (i) ‡	S 34° 36' 12"¶	W 58° 22' 54"¶	4	1(0)	0.000	4	-	-	-	-	-	-	-	-	-	-	-
Buenos Aires (ii) ‡	S 34° 36' 12"¶	W 58° 22' 54"¶	3	2(0)	0.562	2	-	1	-	-	-	-	-	-	-	-	-
Buenos Aires (iii) ‡	S 34° 36' 12"¶	W 58° 22' 54"¶	4	1(0)	0.000	4	-	-	-	-	-	-	-	-	-	-	-
Buenos Aires (iv) ‡	S 34° 36' 12"¶	W 58° 22' 54"¶	4	2(0)	0.562	3	-	1	-	-	-	-	-	-	-	-	-
Buenos Aires (v) ‡	S 34° 36' 12"¶	W 58° 22' 54"¶	4	1(0)	0.000	4	-	-	-	-	-	-	-	-	-	-	-
Buenos Aires (vi) ‡	S 34° 36' 12"¶	W 58° 22' 54"¶	3	1(0)	0.000	3	-	-	-	-	-	-	-	-	-	-	-
Mean ±SE Argentina			3.7 (±0.2)	1.3 (±0.2)	0.187 (±0.118)												
Total Argentina			22	2(0)	0.305	20	-	2	-	-	-	-	-	-	-	-	-
Australia (invasive)																	
Herberton, QLD‡	S 17° 25' 44"	E 145° 26' 15"	13	1(0)	0.000	13	-	-	-	-	-	-	-	-	-	-	-
Oxenford, QLD‡	S 27° 53' 23"	E 153° 18' 43"	14	1(0)	0.000	14	-	-	-	-	-	-	-	-	-	-	-
Halfway Creek, NSW‡	S 29° 56' 6"	E 153° 5' 44"	19	1(0)	0.000	19	-	-	-	-	-	-	-	-	-	-	-
Crescent Head, NSW‡	S 31° 7' 45"	E 152° 52' 50"	18	2(0)	0.687	10	-	8	-	-	-	-	-	-	-	-	-
Raymond Terrace, NSW‡	S 32° 45' 58"	E 151° 44' 55"	18	1(0)	0.000	18	-	-	-	-	-	-	-	-	-	-	-
Illawarra, NSW‡	S 34° 31' 5"	E 150° 45' 1"	17	2(0)	0.362	15	-	2	-	-	-	-	-	-	-	-	-
Cooma, NSW‡	S 32° 19' 49"	E 152° 32' 9"	17	2(0)	0.677	10	-	7	-	-	-	-	-	-	-	-	-
Eden, NSW‡	S 37° 2' 29"	E 149° 54' 30"	10	2(0)	0.611	7	-	3	-	-	-	-	-	-	-	-	-
Mean ±SE Australia			15.8 (±1.0)	1.5 (±0.2)	0.292 (±0.108)												
Total Australia			126	2(0)	0.438	106	-	20	-	-	-	-	-	-	-	-	-

Brazil (invasive)																		
Charqueadas*	S 30° 00' 30"	W 51° 31' 26"	10	1(0)	0.000	10	-	-	-	-	-	-	-	-	-	-	-	
Viamão*	S 30° 06' 47"	W 50° 58' 55"	10	1(0)	0.000	10	-	-	-	-	-	-	-	-	-	-	-	
Mean ±SE Brazil			10 (±0.0)	1.0 (±0.0)	0.000 (±0.000)													
Total Brazil			20	1(0)	0.000	20	-	-	-	-	-	-	-	-	-	-	-	
Hawaii (invasive)																		
Makawao, MAU§	N 20° 50' 40"	W 156° 17' 38"	21	2(1\\, 0**)	0.487	17	-	-	-	-	-	4	-	-	-	-	-	
Ulupaleka, MAU§	N 20° 37' 55"	W 156° 22' 46"	19	1(0)	0.000	19	-	-	-	-	-	-	-	-	-	-	-	
Mean ±SE Hawaii			20	1.5 (±0.5)	0.239 (±0.169)													
Total Hawaii			40	2(0)	0.325	36	-	-	-	-	-	4	-	-	-	-	-	
Japan (invasive)																		
Shikoku§	N 34° 12' 30"	W 134° 35' 40"	21	1(0)	0.000	21	-	-	-	-	-	-	-	-	-	-	-	
Mean ±SE invasive			12.1 (±1.5)	1.4 (±0.1)	0.208 (±0.066)													
Total invasive			229	3(0)	0.403	203	-	22	-	-	-	4	-	-	-	-	-	
Mean ±SE global			14.1 (±1.1)	2.4 (±0.3)	0.502 (±0.093)													
Total global			424	12	1.033	309	3	60	3	4	8	19	5	1	4	7	1	

* DNA extraction, PCR and genotyping undertaken in Adelaide
† DNA extraction, PCR and genotyping undertaken in both Adelaide and St Andrews to calibrate results
‡ DNA extraction undertaken in Adelaide, PCR and genotyping undertaken in St Andrews
§ DNA extraction, PCR and genotyping undertaken in St Andrews
¶ Exact coordinates unavailable, nearest major city coordinates provided.
\\ Nationally
** Globally

Table 2.

Site details, diversity and haplotype frequencies of all herbarium individuals used for chloroplast microsatellite analysis at three loci, data taken from Chapter 3. Population comprised of herbarium accessions falling into the geographic

Population	N_i	N_h	H	Haplotypes													
				A	B	C	D	E	F	G	H	I	J	K	L	M	N
Australia (herbarium) (invasive)																	
Mid-Eastern Australian population (P2)	95	4(2)	0.665	78	-	9	-	-	-	6	-	-	-	-	-	1	1
South-Eastern Australian population (P1)	128	4(1*, 0†)	0.959	84	-	24	-	-	-	16	4	-	-	-	-	-	-
Mean ±SE Australia (herbarium)	111.5 (±16.5)	4 (±0.0)	0.812 (±0.147)														
Total Australia (herbarium)	223	4(0)	0.859	164	-	33	-	-	-	22	4	-	-	-	-	1	1

*Nationally
†Globally

Figure 1.

Map showing the global distribution of *Senecio madagascariensis* chloroplast haplotypes. Pie charts show the proportion of each haplotype found at each location and are proportional in size to the number of individuals sampled.

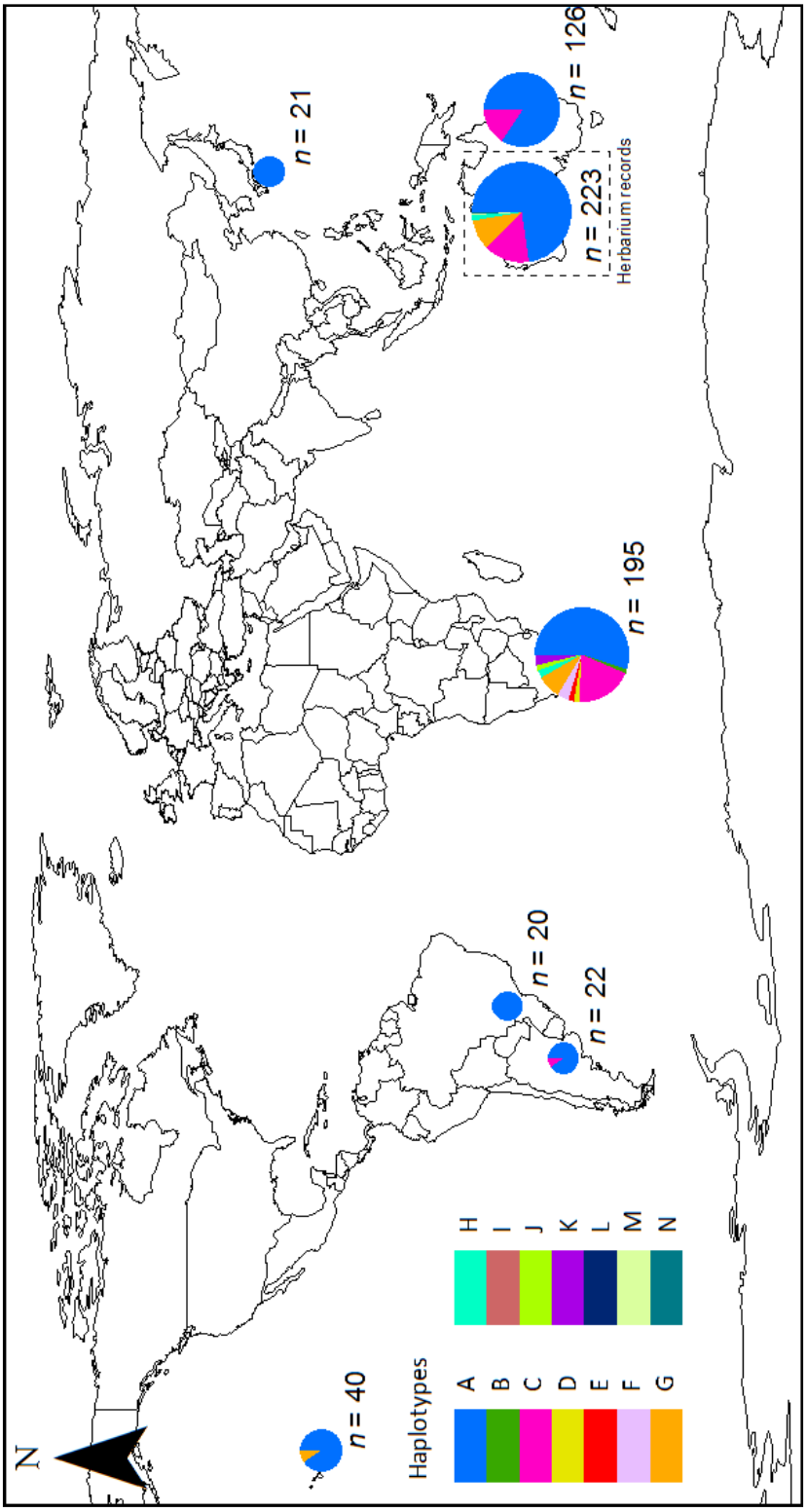
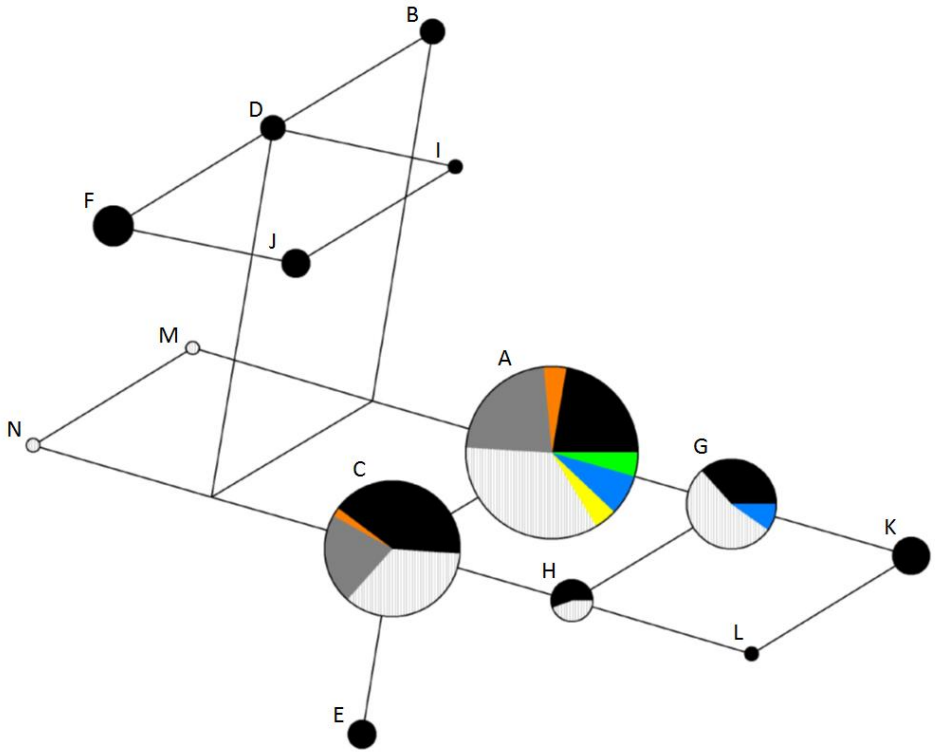


Figure 2.

Median joining network of *Senecio madagascariensis*, where the smallest connector length represents one character change. Circle area is proportional to haplotype frequency with the smallest circle representing one sample. Ranges are colour coded; South Africa, black; Australia (contemporary), grey; Australia (herbarium), grey hatched; Hawaii, blue; Japan, green; Brazil, yellow; Argentina, orange.



CHAPTER 5

Adaptive evolution in invasive species

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CHAPTER 6

Occasional hybridisation between a native and invasive species pair unlikely to contribute to invasive success

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ORIGINAL ARTICLE

Occasional hybridisation between a native and invasive species pair unlikely to contribute to invasive success

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RUNNING TITLE: Hybridisation between a native and invasive species pair

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ABSTRACT

Background and Aims Hybridisation between native and invasive species can facilitate introgression of native genes that increase invasive potential by providing exotic species with pre-adapted genes suitable for new environments. In this study assessed the outcome of hybridisation between native *Senecio pinnatifolius* 'dune variant' and invasive *S. madagascariensis* to investigate the potential for introgression of adaptive genes to have facilitated *S. madagascariensis* spread in Australia.

Methods We used amplified fragment length polymorphisms (141 loci) and nuclear microsatellites (2 loci) to genotype a total of 118 adults and 223 seeds from *S. pinnatifolius* and *S. madagascariensis* at one allopatric and two shared sites. We used model based clustering and assignment methods to establish whether hybrid seed set and mature hybrids occur in the field.

Key Results We detected no adult hybrids in any population. Low incidence of hybrid seed set was found at Lennox Head (6% and 22% of total seeds sampled for *S. pinnatifolius* and *S. madagascariensis* respectively), where the contact zone overlapped for 20 m. One hybrid seed was detected at Ballina (2% of total seeds sampled for *S. madagascariensis*) where a gap of approximately 150 m was present between species.

Conclusions We found no evidence of adult hybrid plants at two shared sites. Hybrid seed set from both species was identified at low levels. Based on these findings we conclude that introgression of adaptive genes from *S. pinnatifolius* 'dune variant' is unlikely to have facilitated *S. madagascariensis* invasions in Australia. Revisitation of one site after two years could find no remaining *S. pinnatifolius* 'dune variant', suggesting that contact zones between these species are dynamic and that *S. pinnatifolius* may be at risk of displacement by *S. madagascariensis* in coastal areas.

KEY WORDS

Senecio madagascariensis, *Senecio pinnatifolius*, hybridisation, biological invasions, introgression, AFLP, microsatellites.

INTRODUCTION

The study of hybridisation between related species has continued to fascinate biologists since the early 19th century (Stebbins 1959) with the potential role of hybridisation in evolutionary diversification of particular interest (Abbott, Albach *et al.* 2013; Anderson and Stebbins 1954; Arnold 2004; Seehausen 2004; Stebbins 1959). Hybridisation can have diverse outcomes including the formation or extinction of species (Abbott, Hegarty *et al.* 2010; Rhymer and Simberloff 1996), introgression of genes from one parental taxa to another (e.g. Whitney, Randell *et al.* 2010), and demographic swamping (e.g. Field, Ayre *et al.* 2008; Prentis, White *et al.* 2007). Alternatively, successful hybridisation between co-occurring species may be rare enough to have little long term impact on either parental taxa.

Hybridisation between native and invasive species is of particular interest, indeed in their seminal review, Ellstrand and Schierenbeck (2000) argue that hybridisation (inter- and intra-specific) can act as a stimulus for the evolution of invasiveness. One mechanism by which this can occur is through introgression of adaptive genes resulting from hybridisation followed by repeated backcrossing with parental taxa. Introgression of native genes can increase invasive potential by providing exotic species with pre-adapted genes suitable for new environments (e.g. Whitney,

Randell *et al.* 2010), conversely introgression of exotic genes can facilitate the transfer of weedy traits to native species, jeopardising genetic integrity (e.g. Fitzpatrick, Johnson *et al.* 2010). Simulation studies on neutral genes have revealed that the very nature of the invasive process is likely to promote almost exclusively unidirectional introgression, from the native species into the invader (Currat, Ruedi *et al.* 2008) increasing the likelihood of locally adapted genes facilitating invasive species spread. Aside from introgression, hybrid progeny can go on to become invasive species in their own right, such as *Senecio squalidus* which evolved via homoploid hybrid speciation from the parental species *S. aethnensis* and *S. chrysanthemifolius* (Abbott, Hegarty *et al.* 2010). In extreme cases, hybrid progeny can be so successful that they completely displace their parental species in the field, such as the Californian wild radish, an invasive hybrid lineage derived from introduced *Raphanus sativus* and *R. raphanistrum* (Hegde, Nason *et al.* 2006).

In the current study, we focus on a native and invasive species pair, *Senecio pinnatifolius* 'dune variant' and *S. madagascariensis*, which co-occur along ~2000 km of coast line in New South Wales, Australia. *Senecio madagascariensis* is a successful invasive plant in Australia and typically a weed of agricultural pastures, however it can also be found growing alongside the native *S. pinnatifolius* in natural systems, raising the possibility that introgression of adaptive genes from the native has facilitated its spread into these areas. Previous work on *S. pinnatifolius* 'tableland variant' (Prentis, White *et al.* 2007) found hybrid seed set but no adult hybrids in the field. Prentis, White *et al.* (2007) also modelled loss of viable seeds to hybridisation and predicting the eventual displacement of *S. pinnatifolius* 'tableland variant' by the invasive *S. madagascariensis* at their study sites.

Whether Prentis, White *et al.*'s (2007) conclusions are more broadly applicable to other *S. pinnatifolius* variants is not clear. Reports of potential hybrids between *S. madagascariensis* and *S. pinnatifolius* 'dune variant' (Scott 1994; EM White, Queensland University of Technology, Australia, 'pers. comm.') have served as a stimulus for the current study which sought to assess the incidence of hybridisation between *S. madagascariensis* and *S. pinnatifolius* 'dune variant' at two sites where the species co-occur. The species have overlapping flower times (Radford 1997; Radford and Cousens 2000) and share pollinators (White 2008) making hybridisation in the field possible. The two species do possess different ploidy however (*S. pinnatifolius* tetraploid, *S. madagascariensis* diploid) with the triploid hybrid offspring that would most often result, typically having low fertility due to meiotic pairing problems between homeologous chromosomes. Despite the low fertility typical of triploid hybrids, they have been shown to act as a bridge between taxa facilitating introgression (e.g. Koutecký, Baďurová *et al.* 2011; Lowe and Abbott 2000) and it may be possible for *S. madagascariensis* to produce unreduced gametes (Brownfield and Köhler 2010; Koutecký, Baďurová *et al.* 2011; Ramsey and Schemske 1998) that could fuse with normal *S. pinnatifolius* gametes to produce tetraploid hybrid offspring.

As a successful invader, *S. madagascariensis* is spreading through south-eastern Australia into new habitats, possibly due in part to introgression of adaptive genes from *S. pinnatifolius*' dune variant'. As a first step to examining this possibility, we assessed the extent of hybrid seed set and the incidence of adult hybrids at our field sites. We hypothesised that if introgression was occurring between *S. pinnatifolius* 'dune variant' and *S. madagascariensis*, then evidence of mature hybrids should exists at sites where the two species co-occur.

We use amplified fragment length polymorphisms (AFLPs) and microsatellites from mature individuals of both species at two sites where the species co-occur and one site for each species that was at least 2 km away from any other known populations of the congener ('allopatric'). We also sampled open pollinated progeny arrays in areas of co-occurence. We asked whether hybrid seed set occurs in the field and whether adult hybrids are present that could backcross with either species to facilitate introgression.

MATERIALS AND METHODS

Study species

Senecio madagascariensis is a diploid plant, initially introduced to south-eastern Australia from the KwaZulu-Natal province of South Africa in the early part of the 20th century (Radford, Muller *et al.* 2000). Molecular analysis of contemporary and historical field collections has pointed to at least two separate introductions (Chapter 3). *Senecio pinnatifolius* (previously *S. lautus*) is a tetraploid plant native to Australia. There have been multiple morphological treatments of the species complex (Ali 1969; Radford 1997; Thompson 2005), with each agreeing on distinction of 'dune', 'headland' and 'tableland variants. *Senecio pinnatifolius* 'dune variant' occurs on coastal sands along the east coast of Australia and is the only *S. pinnatifolius* variant analysed in the current study. Previous work (Prentis, White *et al.* 2007) has assessed the occurrence of hybridisation between *S. madagascariensis* and *S. pinnatifolius* 'tableland variant' but whether their findings similarly apply to other variants is currently unknown.

Initially included in the *S. pinnatifolius* complex, *S. madagascariensis* was recognised as a separate species after Hilliard's (1977) treatment of Asteraceae in Natal (Sindel,

Radford *et al.* 1998). This separation has been supported by morphological comparisons (Thompson 2005) and cytological studies (Radford, King *et al.* 1995) finding 2*n* = 20 for *S. madagascariensis* and 2*n* = 40 for *S. pinnatifolius*.

Both species look superficially identical, growing to approximately 0.6 m and with bright green leaves and yellow inflorescences that are heterogamous and radiate. The species can be reliably distinguished by the number of involucral bracts present, 18-21 in *S. madagascariensis*, 11-14 in *S. pinnatifolius*. Both species are outcrossing, self-incompatible (Ali 1966) and insect pollinated, predominantly by the introduced European honey bee *Apis mellifera* and various species of Syrphidae (White 2008); seeds are wind dispersed. In comparisons of life history traits between the species, *S. madagascariensis* was found to perform better than *S. pinnatifolius* with respect to seedling, growth and fecundity measures but *S. pinnatifolius* maintained a stronger soil seed bank (Radford and Cousens 2000).

Sample collection and seed germination

One allopatric population of each species was sampled along with two sites where the two species occurred together (Table 1). At each site, twenty individuals of each species were sampled with fresh leaf material preserved in silica beads for DNA extraction. Additionally, at shared sites, mature seed heads were collected from sampled plants where available and stored for later germination. Plants were sampled in a systematic fashion (across the contact zone at shared sites) and the location of all plants recorded with GPS (with the exception of the allopatric *S. madagascariensis* population where coordinates were not recorded). Plants that were not sampled for DNA were identified in the field and GPS coordinates recorded. The GPS recorded relative position to within 1 m accuracy. In the laboratory, mature achenes were detached from their pappus and the seed coat nicked with a scalpel.

Seeds were grown on moist filter paper with gibberellic acid (GA_3) in a 12 hour photoperiod at 25 °C to stimulate germination. Up to ten seedlings per parent plant were frozen at -80 °C prior to DNA extraction (see Table 2 for exact numbers). Two years after initial sampling, we revisited one of the shared sites to survey the changes in abundance of *S. pinnatifolius* and *S. madagascariensis*.

Genetic analysis

DNA extractions were carried out using the Machery-Nagel Nucleospin Plant II Kit with the PL2/PL3 buffer system. Two published microsatellite loci (Le Roux and Wieczorek 2007) (Table 2) originally developed for *S. madagascariensis* and found to be cross compatible with *S. pinnatifolius*, were used to screen all adults and seedlings from both species. PCR reactions were prepared with ~20 ng of template DNA, 1x reaction buffer, 0.2 mM of each dNTP, 2.5 mM $MgCl_2$, 0.4 μM of each primer, and 0.02 U Amplitaq Gold® (Applied Biosystems) to give a final PCR reaction volume of 10 μL. Reactions involved an initial denaturation step of 94 ºC for 2 minutes, followed by 35 cycles at 94 ºC for 1 minute, the loci specific annealing temperature for 1 minute (Table 2), 72 ºC for 1 minute and 30 seconds, and a final extension at 72 ºC for 30 minutes. One published chloroplast microsatellite locus (Weising and Gardner 1999) (Table 2) was found to produce bands mutually exclusive to *S. pinnatifolius* and *S. madagascariensis* and so was included to allow identification of the maternal parent of any hybrid adults detected in the field. Reactions were prepared with ~20 ng of template DNA, 1x reaction buffer, 0.2 mM of each dNTP, 2.5 mM $MgCl_2$, 0.5 μM of each primer, and 1 U IMMOLASE™ DNA polymerase (Bioline) to give a final PCR reaction volume of 10 μL. Reactions involved an initial denaturation step of 94 ºC for 5 minutes, 30 cycles of 94 ºC for 20 seconds, 50 ºC for 20 seconds, 72 ºC for 20 seconds, and a final extension at 72ºC for 30 minutes. Products were separated using the ABI 3730 DNA analyzer (Applied

Biosystems) with the GeneScan™ –500 LIZ® size standard. Genemapper® Software v4.0 (Applied Biosystems) was used to score fragments. Scoring was recorded in a binary matrix with presence or absence of particular alleles indicated by a 1 or 0. This method allowed for polyploidy and diploid data to be directly compared and analysed together. DNA from thirty one individuals (9% of samples) were amplified twice for microsatellite analysis to enable estimation of error rates, calculated according to DeWoody, Nason *et al.* (2006).

Amplified fragment length polymorphisms (AFLPs) were assessed according to the method of Vos, Hogers *et al.* (1995) (with modifications). Restriction digests were performed in 20 µl reactions with ~200 ng of DNA, 1 x restriction digest buffer 2, 10 U MseI (New England Biolabs), 10 U EcoRI (New England Biolabs), and 1 x BSA. Reactions were incubated for 3 hours at 37 °C, followed by 20 minutes at 65 °C to denature the enzymes. Adapters were ligated to the digested fragments in reactions containing 20 µl of digested DNA, 1 x T4 ligase buffer, 2.5 µM EcoRI adapter, 0.25 µM MseI adapter and 3 U of T4 DNA ligase (New England Biolabs). Reactions were incubated overnight at 16 °C.

Pre-selective amplifications contained 2 µl of digested and ligated DNA, 1 x Optimised DyNAzyme™ EXT buffer (including 1.5 mM Mg^{2+}), 0.2 mM of each dNTP, 0.5µM MseI (+C), 0.5µM EcoRI (+A) primers and 0.25 U DyNAzyme™ EXT DNA polymerase to give a final PCR reaction volume of 25 µL. Reactions involved an initial denaturation step of 75 °C for 2 minutes, then 20 cycles of 94 °C for 30 seconds, 56 °C for 30 seconds, 75 °C for 2 minutes, and a final extension at 60ºC for 30 minutes. PCR products were run on agarose gel to check for successful amplification.

Selective amplifications contained 1 µl of 1 in 30 diluted pre-selective PCR product, 1 x TaqGold buffer (Applied Biosystems), 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.3 µM MseI + 3bp primers, 0.3 µM EcoRI +3 bp primers and 0.75 U TaqGold (Applied Biosystems) in a final PCR reaction volume of 15 µL. Reactions involved an initial denaturation step of 94 °C for 2 minutes, then 10 cycles of 94 °C for 30 seconds, 65-56 °C for 30 seconds (reduce by 1°C per cycle), 72 °C for 2 minutes, then 26 cycles of 94 °C for 30 seconds, 56 °C for 30 seconds, 72 °C for 2 minutes and a final extension at 60ºC for 5 minutes. Twelve selective amplifications were trialled using a range of + 3bp primer combinations on four individuals of each species. Products were run on 5% acrylamide gels using a Gelscan GS2000 (Corbet Research) and the three most suitable combinations (based on appropriate number and strength of bands, polymorphisms and ease of scoring) were chosen for selective amplification of all samples (Table 2). Products were separated using the ABI 3730 DNA analyzer (Applied Biosystems) with the GeneScan™ –500 LIZ® size standard. Forty one adult individuals (12% of total individuals) were re-extracted for DNA and the AFLP process repeated to allow loci validation and error rate calculations. Vegetative material from seedlings was too small to allow for repeated extractions, so only adults were used. A negative control was included throughout the extraction/AFLP process to enable exclusion of non-specific bands.

Genemapper® Software v4.0 (Applied Biosystems) was used to manually allocate bins to appropriate loci, all duplicated samples were visualised and where consistent banding was apparent between samples, this was assigned as a specific locus. Once manual binning was complete, the full dataset was automatically scored using Genemapper® Software v4.0 (Applied Biosystems) and raw peak height data obtained. The raw peak height data were then used with AFLPScore v1.4 (Whitlock, Hipperson *et al.* 2008) to minimise error whilst maximising number of retained loci.

AFLPScore allows the user to select a range of loci selection thresholds (the average intensity of bands at a specific locus, above which a locus is retained in the dataset) and phenotype calling thresholds (the intensity of a given band, either in absolute terms, or as a percentage of the average for that locus, above which band presence will be called). By comparing combinations of different locus selection and phenotype calling thresholds, the user can select thresholds which result in reduced error and maximised retained loci. After error reduction via AFLPscore, a phenotype matrix was exported and loci with the highest error rates systematically removed to create 11 seperate datasets with error rates of 1%, 2%, 3% etc, up to 10% and additionally one with 17% error rate (the output from AFLPscore with no loci removed). To assess the effects of each error rate on overall information content, the data from the allopatric populations of each species were analysed using the program STRUCTURE (Pritchard, Stephens *et al.* 2000) with RECESSIVEALLELES set to 1 to account for dominant data (Falush, Stephens *et al.* 2007). Number of predefined populations (*K*) was set from 1 to 5. Each run consisted of a burn-in period of 100,000 Markov Chain Monte Carlo (MCMC) repetitions, followed by 1,000,000 MCMC repetitions, the program was run five times to allow averaging of results in CLUMPP (Jakobsson and Rosenberg 2007). Plots were displayed in DISTRUCT (Rosenberg 2004). The final dataset was chosen based on how well it could detect the expected structure (designation of *K*=2, highest probability of individuals belonging to the appropriate species cluster) and how robust it was to the negative impacts of higher error (such as the signal from plate effects) see Zhang and Hare (2012) for an in-depth discussion and analysis of this approach.

Data analysis

To assess hybridisation, the AFLP and microsatellite data were combined into one data matrix, in the case of the microsatellites, each allele was either designated as

present or absent. Assignment of an individual as either a pure parental species or a hybrid was based on a consensus between two different analysis methods, with the most conservative (i.e. non-hybrid) designation accepted if results were inconsistent between methods. The first method used the allocation procedure in the program AFLPOP (Duchesne and Bernatchez 2002). The allopatric populations of each species were set as sources, and the remaining samples allocated to either one of the pure species or hybrid origin by the program. Zero frequencies were corrected as 1/*n* +1, where *n* is the sample size. The allocation minimal log-likelihood difference (MLD) was initially set to 1 (meaning allocation only occurred when designation was 10 times more likely than any other possible origin). Samples that could not be allocated in this way were re-run with MLD set to 0 (allocating to highest likelihood source regardless of the magnitude of difference between alternate likelihoods).

The second method used the program STRUCTURE (Pritchard, Stephens *et al.* 2000) with extensions implemented by Falush, Stephens *et al.* (2007) to account for genotypic ambiguity that is inherent in dominant markers; RECESSIVEALLELES was set to 1. STRUCTURE has been used successfully to assess datasets comprised of individuals with different ploidy levels (e.g. De Hert, Jacquemyn *et al.* 2012; Pinheiro, de Barros *et al.* 2010; Zalapa, Price *et al.* 2011). Number of predefined populations (*K*) was set to 2. Each run consisted of a burn-in period of 100,000 Markov Chain Monte Carlo (MCMC) repetitions, followed by 1,000,000 MCMC repetitions, the program was run five times to allow averaging of results in CLUMPP (Jakobsson and Rosenberg 2007). Plots were displayed in DISTRUCT (Rosenberg 2004). Clustering of adult and seedling genotypes of each species at both allopatric and shared sites were visualised with a principal coordinate analysis (PCoA) in GENALEX v6.4 (Peakall and Smouse 2006). Hybrid zone mapping was completed using ArcGIS v9.2 (ESRI 2009).

RESULTS

Loci selection

Both nuclear microsatellite loci were polymorphic in both species and retained for further analysis (Table 2). The single chloroplast microsatellite locus was polymorphic in *S. pinnatifolius* (2 alleles) and monomorphic in *S. madagascariensis* but alleles were not shared between species. All adults and seedlings genotyped conformed to their expected species specific chloroplast haplotypes. The error rate per allele and per locus for the nuclear microsatellites was zero. Of the 12 AFLP primer combinations trialled, three were chosen for screening all samples (Table 2).

In AFLPScore v1.4 (Whitlock, Hipperson *et al.* 2008), mismatch error rates were used to optimise scoring parameters using both absolute and relative phenotype calling thresholds on an initial dataset containing 247 loci. The error rate of the exported data set was 0.17 with 233 retained loci, achieved by filtering data using an absolute phenotype-calling threshold of 250 relative fluorescence units (RFU), prior to application of a 50 RFU locus-selection threshold. After STRUCTURE analysis, the data set equating to an average error rate of 6% was chosen, as it correctly identified K=2, indicated high assignment rates of individuals to their correct species, did not display any significant plate effects at K=3 (the number of plates) and contained a reasonable number of loci (142) (Fig. 1). An overall error rate of 6% (Fig 2) is high compared to the 2-5% reported for most AFLP studies (Bonin, Bellemain *et al.* 2004) but under the maximum threshold of 10% recommended by Bonin, Ehrich *et al.* (2007). Systematically evaluating the effects of different error rates on result and selecting that which is most informative and least confounding allows the information content of the dataset to be maximised without limiting the included loci in order to conform to an arbitrary cut off point (Zhang and Hare 2012).

Hybridisation

No adult hybrids were detected in the field. In total, 17 hybrids were observed from 223 seeds (8% of seeds and 5% of all individuals genotyped including adults). Fourteen of these hybrid seeds were from *S. madagascariensis* mothers and three from *S. pinnatifolius* mothers. Hybrid seed set was observed at Lennox Head where 6% and 22% of the total seeds sampled for each species at that site were hybrid for *S. pinnatifolius* and *S. madagascariensis* respectively. All hybrid seeds were observed from one *S. madagascariensis* mother and two *S. pinnatifolius* mothers. (Fig. 3). For each adult with hybrid seed set, the distance to the nearest congeneric was <15 m (Fig. 3). There was uncertainty in the field about seeds collected from what appeared to be a single plant but may have been two adjacent plants. Twenty seeds were germinated from this sample with the hope that separation of individuals could be made in the lab from the results of the genetic analysis. The microsatellite data confirmed that these seeds did indeed come from the same individual, and so one adult has 20 genotyped offspring instead of the usual 10. A single hybrid seed was detected at Ballina from a *S. madagascariensis* mother (Fig 3), as designated by agreement between AFLPop and STRUCTURE, however this individual does closely cluster with other pure *S. madagascariensis* seedlings in the PCoA analysis (Fig. 4), which may indicate a false positive result. AFLPOP (Duchesne and Bernatchez 2002) allocated 90% of adults and 68% of seedlings with a minimal log-likelihood difference (MLD) of 1 (indicating that the allocation was at least 10 times more likely than any other). The remaining samples allocated with MLD set to 0. One *S. madagascariensis* seedling was allocated to *S. pinnatifolius* 'dune variant' with MLD set to 0. The chloroplast haplotype of this individual was consistent with *S. madagascariensis* maternity and it clustered with the hybrid seedlings in the PCoA (Fig. 4), so has been designated as a hybrid. Hybrid origin was more conservatively allocated in the program STRUCTURE (Pritchard, Stephens *et al.* 2000) (Fig. 5) with

92% consensus between the two methods. Final designation used the most conservative (non-hybrid) allocation.

Site revisit

The Lennox Head site was revisited in 2009, two years after initial sampling and a visual survey of plant species identity undertaken. All plants observed were identified as *S. madagascariensis*.

DISCUSSION

Hybridisation between native and exotic species can affect biological invasions in several ways, including via introgression (Currat, Ruedi *et al.* 2008; Prentis, Wilson *et al.* 2008; Whitney, Randell *et al.* 2006; Whitney, Randell *et al.* 2010) and pollen swamping (Buggs and Pannell 2006; Petit, Bodenes *et al.* 2004; Prentis, White *et al.* 2007). Despite occasional hybrid seed set between native *Senecio pinnatifolius* 'dune variant' and invasive *S. madagascariensis*, we found no evidence to support the role of introgression in this system. We found low levels of hybrid seed formation in both *S. pinnatifolius* 'dune variant' and *S. madagascariensis* mothers at one site (Lennox Head, NSW) where the two species occur together with a minimum distance of approximately 15 m. At another site (Ballina, NSW) a single hybrid seed was detected in a *S. madagascariensis* mother, with a distance of approximately 155 m to the closest *S. pinnatifolius* 'dune variant' plant. No adult hybrids were identified at either site.

The absence of adult hybrids in the field may be the result of reduced hybrid fitness acting as a post-zygotic mating barrier between *S. pinnatifolius* and

S. madagascariensis. To further explore this possibility it will be necessary to compare the performance of hybrid and pure seedlings in common garden conditions. As the levels of overall hybrid seed set were low, it may be that hybrid seedlings were not present simply by chance in study year, and that their overall fitness does not necessarily result in mortality prior to reproductive maturity. Occasional adult hybrid occurrence may explain the findings of (Scott 1994; EM White, Queensland University of Technology, Australia, 'pers. comm.').

The present study did not identify the ploidy level of the hybrid seedlings identified from our open pollinated progeny arrays which would be an interesting topic for further research. Koutecký, Baďurová *et al.* (2011) found that hybrids formed from reduced gametes between diploid *Centaurea pseudophrygia* and tetraploid *C. jacea* were less common in the seed set of maternal plants but more common in the adult hybrid plants found in the field, suggestive of increased fitness of the tetrapliod hybrids. These tetraploids were also able to backcross with *C. Jacea*, facilitating introgression of *C. pseudophrygia* genes into *C. jacea*. In *S. pinnatifolius* 'dune variant' x *S. madagascariensis* hybrids there may be similar fitness asymmetries associated with ploidy level.

Selection against hybrids in the field would constitute a post-zygotic mating barrier, yet the prevailing view is that pre-zygotic mating barriers are stronger in flowering plants (Dell'Olivo, Hoballah *et al.* 2011; Rieseberg and Willis 2007; Widmer, Lexer *et al.* 2009). Evidence for pre-zygotic isolation barriers between *S. pinnatifolius* 'dune variant' and *S. madagascariensis* are sparse at present. The two species can be found occurring in shared sites (Prentis, White *et al.* 2007; Radford 1997; White 2008), their flowering times overlap (Radford 1997; Radford and Cousens 2000) and a similar suit of pollinators visit both species (White 2008). However, it should be

noted that reproductive isolation can still be favoured even when flowering times overlap but not completely, as is the case with *S. pinnatifolius* and *S. madagascariensis* (Radford 1997). And even when pollinators are shared, they may prefer conspecific over heterospecific visitation (White 2008). The relative contribution of these potentially reproductively isolating barriers remains to be tested in this system with more extensive field and laboratory studies, incorporating greater geographical and temporal breadth.

We set out to explore whether hybridisation between *S. pinnatifolius* 'dune variant' and *S. madagascariensis* was likely to have facilitated the spread of *S. madagascariensis* by way of introgression of adaptive genes. Due to the very low level of hybrid seed set and the absence of adult hybrids, we must conclude that introgression via fertile hybrids in the field is probably rare, at least at the sites we studied. As only two field sites were included it is difficult to generalise across the entire ~2000 km range in which the two species overlap, however we can tentatively support the findings of Prentis, White *et al.* (2007) who found similar results in their study of hybridisation between *S. pinnatifolius* 'tableland variant' and *S. madagascariensis*. It may be the case that all *S. pinnatifolius* variants exhibit the same patterns when in sympatry with *S. madagascariensis*.

In their modelling of these hybrid zones Prentis, White *et al.* (2007) also predicted a demographic swamping of *S. pinnatifolius* 'tableland variant' by *S. madagascariensis* assuming that hybridisation is plant density dependent. In the present study, overall levels of hybridisation were too low to assess density dependence but we did find the greatest proportion of hybrid seed set in an area of high congeneric plant density (Fig. 3). To adequately assess the density dependent nature of hybridisation, artificial manipulation of plant densities in open pollinated conditions would be required. Our

subsequent revisitation of the Lennox Head site, two years after initial sampling, offers tentative support to the predictions of Prentis, White *et al.* (2007) as we were unable to find any *S. pinnatifolius* individuals. It is possible that demographic swamping of *S. pinnatifolius* by *S. madagascariensis* has occurred at this site, or that *S. madagascariensis* has achieved dominance via other competitive advantages (Radford and Cousens 2000).

CONCLUSIONS

Despite limited obvious pre-zygotic isolating barriers restricting hybridisation between the native *Senecio pinnatifolius* 'dune variant' and invasive *S. madagascariensis* in coastal areas of eastern Australia, we did not find any evidence of adult hybrid plants at two shared sites surveyed in 2007 and analysed with a combination of AFLPs and microsatellites. Hybrid seeds from both *S. pinnatifolius* 'dune variant' and *S. madagascariensis* were identified at low levels from open pollinated progeny arrays in the field. Based on these preliminary investigations we conclude that introgression of adaptive genes from *S. pinnatifolius* 'dune variant' is unlikely to have played a significant role in the success of *S. madagascariensis* invasions in Australia.

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TABLES AND FIGURES

Table 1

Information on sites and samples included in the study. Number of adults sampled (n_a), number of adults sampled for seed (n_s), number of seedlings analysed (s) and range of seedlings per mother plant.

Species	**Population**	**Latitude**	**Longitude**	n_a	n_s	*s (range)*
S. pinnatifolius	Southport	S 27° 56' 15"	E 153° 25' 35"	20	0	–
	Lennox Head	S 28° 47' 9"	E 153° 35' 38"	20	7	52 (2-10)
	Ballina	S 28° 52' 25"	E 153° 35' 21"	20	7	61 (4-10)
S. madagascariensis	Oxenford	S 27° 53' 23"	E 153° 18' 43"	18	0	–
	Lennox Head	S 28° 47' 9"	E 153° 35' 38"	20	6	59 (10-19)
	Ballina	S 28° 52' 25"	E 153° 35' 21"	20	6	51 (4-10)
			Total	118	26	223 (2-19)

Table 2

Details of final round PCR primers used in study. Markers used were amplified fragment length polymorphisms (AFLP), nuclear microsatellites (nSSR), and one chloroplast microsatellite (cpSSR). Primer information includes primer type (EcoRI or Mse origin for AFLPs, locus name and primer direction for microsatellites); primer sequence including fluorescent dye (PET, FAM, NED or VIC); annealing temperature (T_a); and number of loci (for AFLPs) or alleles (for microsatellites) scored for each pair (n). Error rates per loci are also given.

Marker	Primer #1		Primer #2		T_a (°C)	n	Error Rate
AFLP	EcoRI	TACTGCGTACCAATTCAGC(PET)	Mse	GACGATGAGTCCTGAGTAACAA	65-56	48	5.23
	EcoRI	TACTGCGTACCAATTCAGC(FAM)	Mse	GACGATGAGTCCTGAGTAACAG	65-56	57	5.39
	EcoRI	TACTGCGTACCAATTCAGC(NED)	Mse	GACGATGAGTCCTGAGTAACCG	65-56	37	8.11
nSSR	Se-116F	CCTTCTGGTTGATTTGGCTAAGC(FAM)	Se-116R	AGAACTGCACATTTGAAGCCTG	48	15	0
	Se-138F	ACTTCGTGGGCCATTCCAG(VIC)	Se-138R	CTTCCTGCATAACATCCACCAC	58	24	0
cpSSR	Ccmp3F	CAGACCAAAAGCTGACATAG(PET)	Ccmp3R	GTTTCATTCGGCTCCTTTAT	50	3	0

Figure 1

Panels (a)-(g) show STRUCTURE runs for the different AFLP datasets produced with varying error rates. Allopatric populations of the two species were used only, to avoid the confounding effect that detection of hybrids might have on the output. *Senecio madagascariensis* is shown on the left side of the plots, *S. pinnatifolius* 'dune variant' on the right. Results are shown for $K = 2$ (equating to two species) and $K = 3$ (which is the number of different plates the samples were run on). The dotted lines represent the plate boundaries. The final dataset chosen is shown in (g) where both species are clearly define and at $K = 3$ there are no obvious plate effects. The number of loci included in each run are as follows, a) 33; b) 56; c) 79; d) 96; e) 112; f) 128; g) 141; h) 154; i) 165; j) 175; k) 184; l) 233.

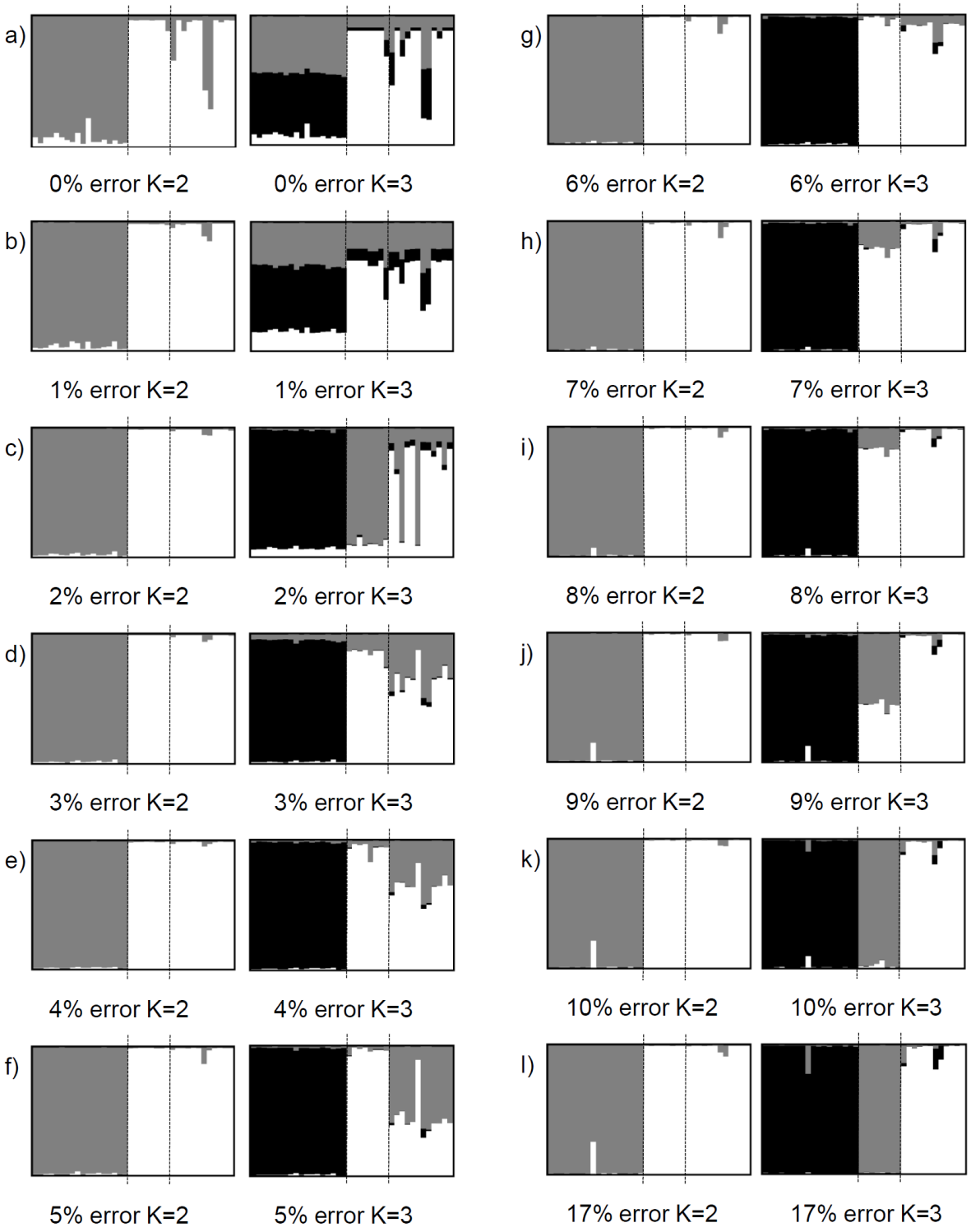

a)
0% error K=2
0% error K=3
b)
1% error K=2
1% error K=3
c)
2% error K=2
2% error K=3
d)
3% error K=2
3% error K=3
e)
4% error K=2
4% error K=3
f)
5% error K=2
5% error K=3
g)
6% error K=2
6% error K=3
h)
7% error K=2
7% error K=3
i)
8% error K=2
8% error K=3
j)
9% error K=2
9% error K=3
k)
10% error K=2
10% error K=3
l)
17% error K=2
17% error K=3

Figure 2

Frequency histogram of locus specific error rates in the final AFLP dataset with an overall mean error rate of 6% across 141 loci.

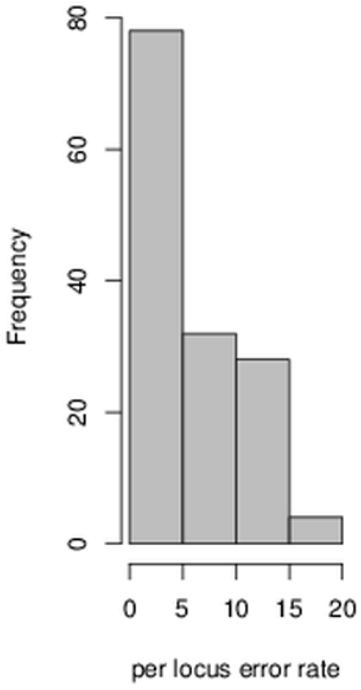


Figure 3

Location of samples at shared field sites. Panel (a) shows location of sites in New South Wales; panel (b) shows the Ballina site; panel (c) shows the Lennox Head site. *Senecio pinnatifolius* is depicted with white symbols, *S. madagascariensis* with black symbols. The position of un-sampled plants is shown by crosses, small circles are genotyped adult plants, large circles are genotyped plants with genotyped seed. The proportion of seeds with pure or hybrid origin is shown in the large circles, grey indicating hybrid. Number of seeds sampled per adult (*n*) is indicated.

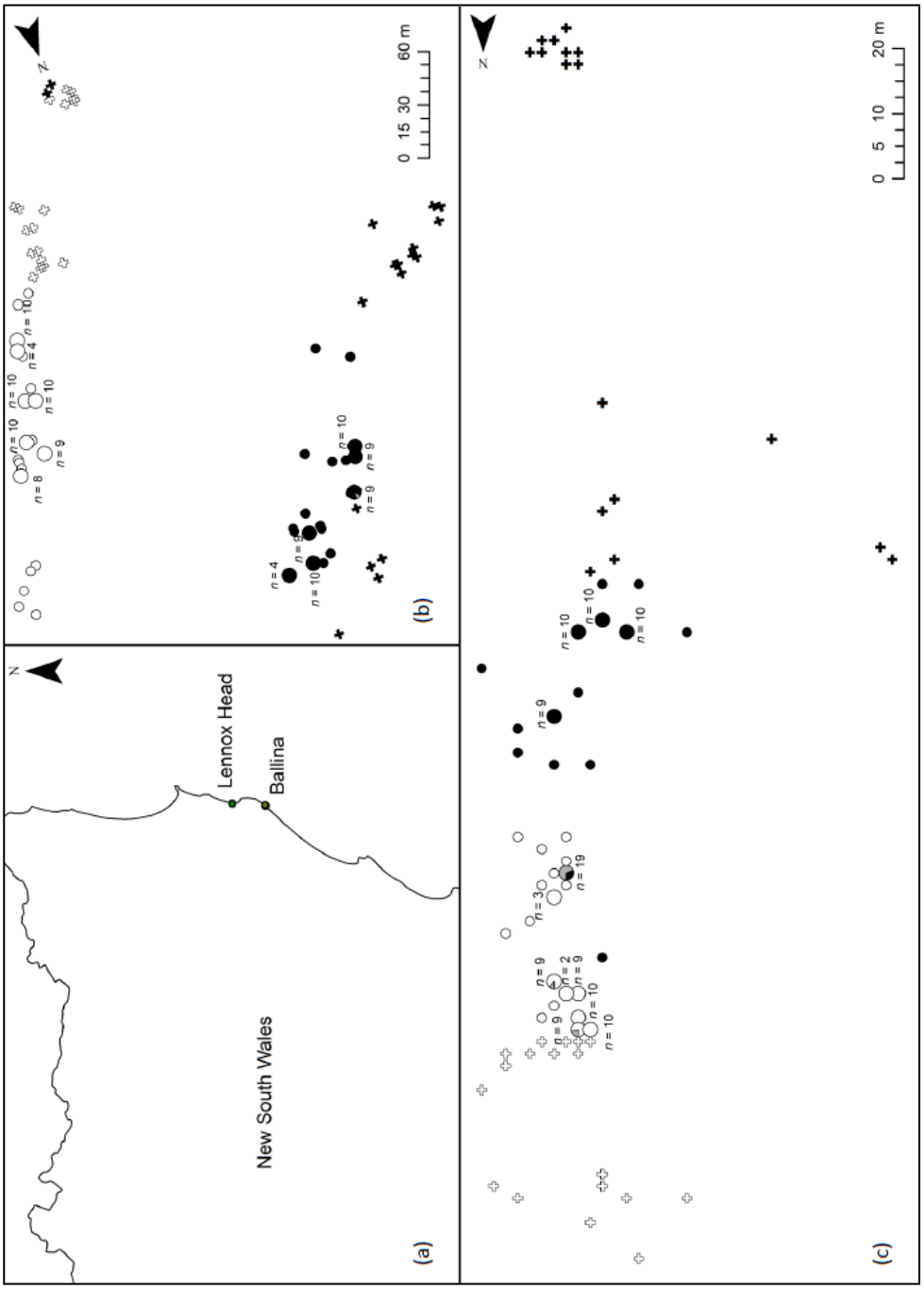
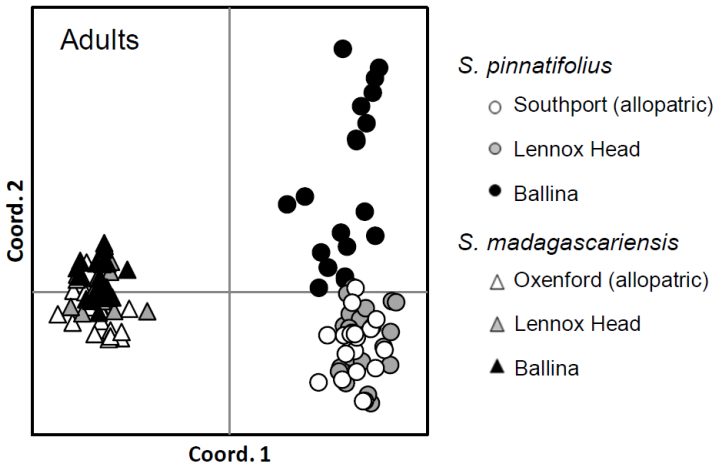

N
Lennox Head
Ballina
New South Wales
(a)
N
0 15 30 60 m
(b)
N
0 5 10 20 m
(c)

Figure 4

Principal coordinates analysis illustrating clustering of adults (a) and seeds (b) of *S. pinnatifolius* and *S. madagascariensis* at allopatric and shared sites. Hybrid designation is based on the combined results from STRUCTURE and AFLPop.



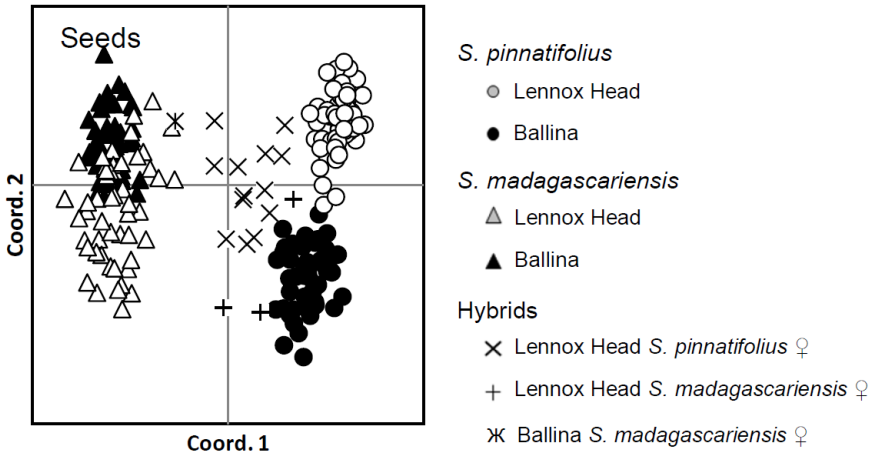
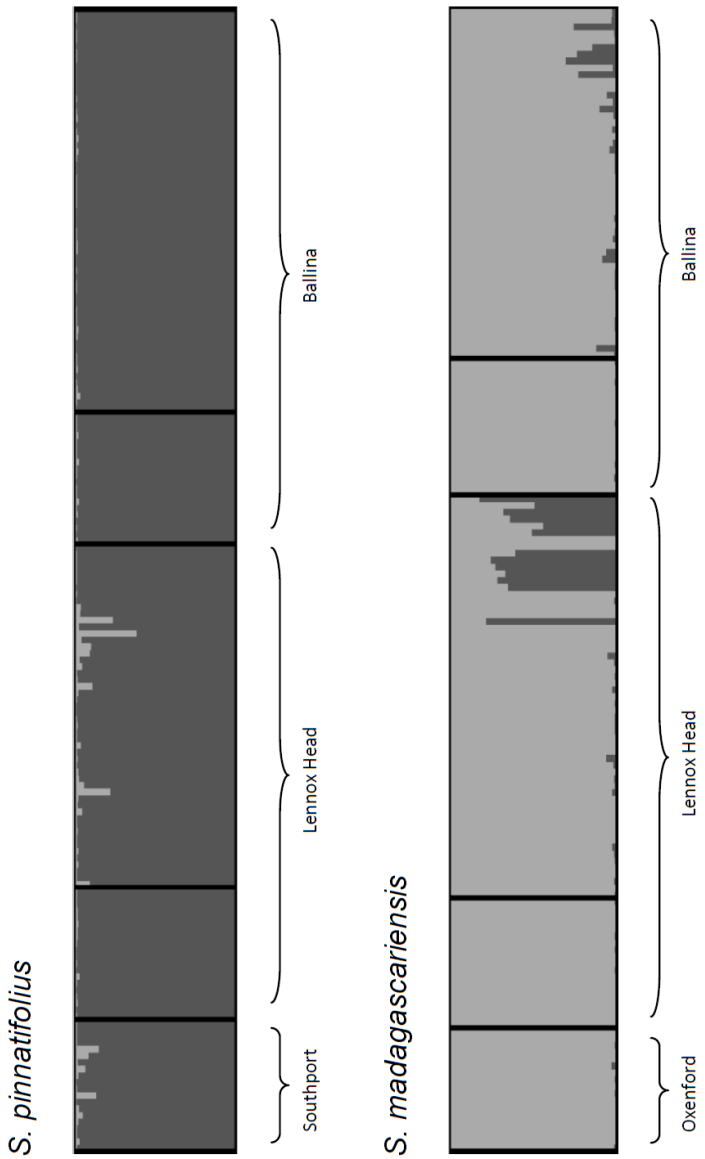


Figure 5

Output from STRUCTURE using all individuals and the 6% error dataset.



CHAPTER 7

Detecting outlier loci and putative selective agents in a rapid plant invader

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RUNNING TITLE: detecting selection in invasions

Abstract

Identifying contemporary evolution is a major goal in invasion biology. A first step in this endeavour is to identify loci which may have been subject to recent selection and demonstrate correlations with environmental parameters with which they may be associated. Here we used a genome scan of 164 loci on a total of 316 *Senecio madagascariensis* plants collected from across its invasive range in Australia. Half of these (*n* = 158) were collected from eight discreet demographic populations >100 km apart and the remainder collected as individual plants >2 km apart. The demographic population and individual based samples were analysed separately using complementary approaches for identifying putative loci under selection. Demographic populations were analysed using outlier approaches in two programs, MCHEZA and BAYESCAN; individuals were analysed using a spatial analysis method in SAM to look for associations between particular loci and environmental variables. Two loci were identified in both datasets as potentially under selection and were associated with monthly environmental variables describing light and water availability between May and August which correspond with flowering time. Overall 6% of loci were identified as candidates for selection in one or more of the analysis methods, all three software programs only agreed on a single locus (0.6% of all loci) and this was associated with eight environmental parameters describing rainfall. Results are discussed with reference to selection and alternative explanations for the observed patterns. The

two identified loci present promising targets for future investigations into contemporary selection in invasive *S. madagascariensis* in Australia.

Introduction

Rapid adaptation in exotic species has become a critical area of investigation over the last decade with a swathe of theoretical and empirical studies supporting the role of contemporary evolution in the success of many introduced species (Barrett et al., 2008, Chun et al., 2011, Keller and Taylor, 2008, Lee, 2002, Prentis et al., 2008, Urbanski et al., 2012, Whitney and Gabler, 2008). Whether or not a species is responding to selective pressures in a novel environment has important implications for management and risk assessment, particularly with regards to response of species to future climate change (Boman et al., 2008, Hoffmann and Sgro, 2011, Neve et al., 2009). Similarly, predictions made about species based on knowledge derived from their native range, could have limited application if invasive individuals are diverging from source populations (e.g. Chun et al., 2011, Xu et al., 2010 but see Alexander et al., 2012). Identification of the selective processes shaping contemporary invasive populations facilitates better understanding of current and future invasion dynamics and improves our capacity to respond with appropriately targeted control measures (Neve et al., 2009).

Methods for detecting signatures of selection make use of the expected differences in differentiation between loci under selection and neutral loci. Under positive selection, a locus is expected to show greater divergence between populations than neutral loci; under balancing selection, the divergence is expected to be lower (Lewontin and Krakauer, 1973). Several analysis programs have been developed to

distinguish between neutral loci and those associated with selection by identifying higher or lower F_{ST} than expected under neutrality e.g. FDIST (Beaumont and Nichols, 1996) (which also underpins the program LOTISAN (Antao et al., 2008)); DFDIST (a modification of FDIST for dominant markers, which also underpins MCHEZA (Antao and Beaumont, 2011)); DETSEL (Vitalis et al., 2003) and DETSELD for dominant markers (cited in Perez-Figueroa et al., 2010); and BAYESCAN (Foll and Gaggiotti, 2008). These programs differ in how they estimate allelic frequencies and often produce different results when used on the same dataset (Perez-Figueroa et al., 2010). In their simulation study of three commonly used programs for dominant markers (DFDIST, DETSELD and BAYESCAN), Perez-Figueroa et al. (2010) found BAYESCAN to perform most efficiently but also advocate using one of the alternative programs with a multi-test correction. MCHEZA allows for application of this correction within the program itself using false discovery rates (FDR) (Antao and Beaumont, 2011).

Another method for identifying potential loci under selection is the spatial analysis method (SAM) implemented in SAM (Joost et al., 2007, Joost et al., 2008). The program tests for association between environmental variables and allelic frequencies at marker loci using multiple univariate logistic regression models and accounts for multiple testing with a Bonferroni correction (Shaffer, 1995). Using a SAM in addition to more traditional F_{ST} outlier approaches is particularly advantageous as the analyses are not reliant on any notion of population, which can be difficult to define correctly (Joost et al., 2007). SAM also provides an indication of the possible selective force(s) acting on populations which has led to the apparent differences in F_{ST} revealed by outlier methods. A drawback of SAM however, is the potential impact of spatial autocorrelation where individuals within the same area share the same alleles due to relatedness (identical by descent), and are found to occur within the same environmental conditions by virtue of their spatial proximity. In

this situation loci may be erroneously designated as associated with selection (Manel et al., 2010).

Detecting outlier loci and those associated with particular environmental variables must be interpreted with caution. Demographic factors can affect outcomes, and outputs should always be considered in light of species' demographic histories (Thornton et al., 2007). More fundamentally, a locus' designation as 'under selection' can be viewed as evidence that selection occurs somewhere within the genome but not necessarily directly on the particular locus in question (or surrounding genomic region) due to coupling of endogenous and exogenous barriers to gene flow (Bierne et al., 2011). Given these limitations, the combination of genome scans and spatial analysis methods should be considered a valuable first step in identifying putative contemporary selection.

In this study we investigate the potential for contemporary selection in the invasive plant *Senecio madagascariensis* in Australia. *Senecio madagascariensis* was originally introduced accidentally from South Africa and now has a wide distribution across the eastern coast of Australia (Chapter 3). Understanding how *S. madagascariensis* may be responding to Australian conditions is particularly relevant to on-going control efforts, and can assist in predictions of future distribution and therefore identification of at-risk areas under various climate change scenarios (Hoffmann and Sgro, 2011, Neve et al., 2009).

As each method for detecting selection works optimally with different sampling designs, we have followed two different collection protocols (one population based, one individual based) and undertaken a genome scan for all samples using amplified fragment length polymorphisms (AFLPs). AFLPs were chosen as an appropriate

marker due to their relatively low cost and spread across the genome (Caballero et al., 2008). Each set of samples were analysed separately by either SAM (in the case of the individual based collections) or MCHEZA and BAYESCAN (in the case of the population based collections). We sought to identify loci associated with selection and specific environmental variables that may be acting as selective agents. By utilising a range of available methods with two independent data sets we aim to provide robust identification of potential selection in the Australian invasion of *S. madagascariensis*.

Methods

Leaf samples of invasive *Senecio madagascariensis* were collected from across the known range in Australia. Collections were undertaken in two parts, eight geographically separate demographic populations (*n* = 20) and 158 individuals (>2 km apart) were sampled (Fig. 1, Table 1, Table S1). Between five and 10 young leaves were collected from each plant and placed in silica gel to preserve for DNA extraction. Exact GPS coordinates were recorded for each sample. One herbarium specimen was collected from Halfway Creek, NSW to confirm correct identification in the field (submitted to Queensland Herbarium, record number AQ741268).

DNA extraction, genetic analysis using amplified fragment length polymorphisms (AFLPs) were undertaken according to the procedures described in Chapter 6. AFLP loci were scored first manually by comparing the AFLP fingerprints of repeated samples and binning consistent peaks. The resulting bin set was then used to score the entire data set and the raw peak height data imported into AFLPSCORE v1.4 (Whitlock et al., 2008) where scoring parameters such as the minimum average peak

height used to include a locus ('locus selection threshold') and minimum peak height used to call a the presence of a band a particular locus ('phenotype calling threshold') are manipulated. The effects of these changing parameters on mismatch error rates can then be evaluated and minimized for optimum scoring.

The effect of the eventual error rate on our ability to identify genuine population signal was assessed with the program STRUCTURE v2.3.3 (Pritchard et al., 2000). The theory behind taking this approach has been explored by Zhang and Hare (2012). AFLP datasets vary with respect to the error rates of individual loci. By testing whether a dataset is able to detect expected populations structure, and avoid artifactual structure (such as that produced by plate effects), use of a particular error rate can be justified without simply seeking the lowest rate possible (in which genuine signal can be lost). In the present study, expected population structure was taken from Chapter 3, in which two distinct populations were identified from microsatellite data and were consistent with that expected from two sequential founder events in Australia. Results from the demographic populations assessed here were examined to see if the dominant population structure identified was consistent with the genuine signal expected from the data, or the signal expected if plate effects were dominating the results (as is expected when error rate is too high, Zhang and Hare, 2012).

Each STRUCTURE run used the admixture model with RECESSIVEALLELES set to 1 to account for dominant data (Falush et al., 2007). and consisted of a burn-in period of 100,000 Markov Chain Monte Carlo (MCMC) repetitions, followed by 1,000,000 MCMC repetitions. Data from the eight demographic populations (n = 158) were analysed only as expected population structure obtained in Chapter 3 pertained specifically to these samples. Possible numbers of discrete populations (K) were set from one to eight (the total number of sites sampled). Each value of K had five

separate runs to allow averaging of results in CLUMPP (Jakobsson and Rosenberg, 2007). The most likely value of *K* was determined using STRUCTURE HARVESTER v0.6.92 (Earl and Vonholdt, 2012) and taken as the value of *K* at which Δ*K* is greatest (Evanno et al., 2005). Graphic representations of population clustering were created using DISTRUCT (Rosenberg, 2004).

Measures of population level diversity were obtained using GENALEX v6.4 (Peakall and Smouse, 2006), specifically expected heterozygosity (H_e) and Shannon's Diversity Index (*H*) were calculated.

Outlier loci in the eight demographic populations were analysed using two different and complementary software packages designed to identify candidate loci under selection using allele frequency data from populations. The first package, BAYESCAN v2.01 (Foll and Gaggiotti, 2008) estimates directly the posterior probability that loci are under selection using a Bayesian methodology and is an extension of the method put forward by Beaumont and Balding (2004). The second package, MCHEZA (Antao and Beaumont, 2011) is based on the program DFDIST (a modification of FDIST (Beaumont and Nichols, 1996) developed to analyse dominant markers) and again uses Bayesian methods but in this case uses the proportion of recessive phenotypes to estimate allelic frequencies. F_{ST} between subpopulations (in this case, our demographic populations) is calculated and compared to a null sampling distribution of F_{ST} estimates generated using coalescent simulations based on neutral expectations. Loci which appear to have unusually high or low F_{ST} values are identified as 'outliers' and considered to be influenced by some form of selection, either directly or indirectly.

In BAYESCAN (Foll and Gaggiotti, 2008) the following parameters were set: burn in period of 50000 iterations; thinning interval of 10 iterations (total number of iterations = 100000, sample size = 5000); 20 pilot runs of 5000 iterations; prior odds for the neutral model = 10; F_{IS} beta priors were set to a mean of 0.303 with a standard deviation of 0.089 (as obtained from nuclear microsatellite data of the same populations in Chapter 3).

In MCHEZA (Antao and Beaumont, 2011), neutral mean F_{ST} was calculated by excluding putative loci under selection and the simulated F_{ST} distribution approximated using a bisection algorithm over repeated simulations. The confidence interval was set to 95% and the false discovery rate (FDR) set to 0.1.Theta was set to 0.1 and the parameters of the beta distribution set to 0.25. The critical frequency was set to 0.99 (applicable only in the simulated data) which is the frequency threshold of the most common allele, above which a locus is ignored.

The dataset of individuals was analysed using SAM v1, to test for association between environmental variables and allelic frequencies at marker loci using multiple univariate logistic regression models (Joost et al., 2008, Joost et al., 2007). Models are considered significant when both a likelihood ratio G and a Wald statistical test reject the null hypothesis of the observed distribution being better explained by a constant only, rather than the environmental variable considered. SAM implements a Bonferroni correction (Shaffer, 1995) to account for multiple testing. Climate variables were acquired from the Australian Government's Bureau of Meteorology (BOM) as interpolated gridded datasets with variable spatial and temporal resolution, and specific point values obtained using the SAMPLE tool in ARCGIS v10 (ESRI). Other variables included were latitude, longitude and various parameters derived from the AUSLIG 9 Second Digital Elevation Model of Australia. The full list of variables used

in the analysis can be found in Table 2. All maps were created in ARCGIS v10 (ESRI) with the probability of allelic presence interpolated via Kriging.

Results

In total, 316 *Senecio madagascariensis* individuals were successfully genotyped at 164 AFLP loci (Table 3). Population outlier analyses were conducted on 158 individuals from eight distinct demographic populations (Fig. 1, Table 2). A spatial analysis method (SAM) to detect putative loci under selection and associated environmental variables was undertaken with a separate set of 158 individuals collected from across the known region of *S. madagascariensis* infestation in Australia, plus one individual randomly selected from each of the populations included in the outlier analysis, bringing the total to 166 used for the SAM (Fig. 1, Table S1).

From the analysis of demographic populations samples, one locus ('y23') was found to be an outlier by both BAYESCAN (Foll and Gaggiotti, 2008) (Fig. 2) and MCHEZA (Antao and Beaumont, 2011) (Fig. 3). This same locus ('y23') also had a significant association with environmental variables in the analysis of individuals in SAM (Joost et al., 2008) (Table 4, 5, Fig. 4). One other locus ('y59') was designated as an outlier by BAYESCAN but not MCHEZA, and associated with an environmental variable in SAM. Six loci were considered outliers by BAYESCAN only ('b2', 'b25', 'b48' 'r24', 'r30' and 'y30'). Two loci ('r11 and 'r12') were found to significantly associate with one or more environmental variable in the individual analysis in SAM but were not recognised as outliers by BAYESCAN or MCHEZA. Overall, ten different loci were identified as outliers by one or more methods, constituting approximately 6% of loci. MCHEZA identified

0.6% of loci as outliers, BAYESCAN identified 4.9% AND SAM 2.4%. The distribution of F_{ST} across loci was 'L' shaped (Fig. 5), as expected where only a few genes are the subject of divergent selection and the remainder of the genome is more homogeneous due to gene flow.

The final mismatch error rate obtained in AFLPSCORE v1.4 (Whitlock et al., 2008) was 0.095. Data was filtered using an absolute phenotype-calling threshold of 1000 relative fluorescence units (RFU), prior to application of a 200 RFU locus-selection threshold. Using the demographic population based samples, STRUCTURE identified two distinct genetic clusters corresponding to the south-eastern (P1) and mid-eastern (P2) populations identified in Chapter 3 and no appreciable population structure that could be attributed to plate effects (Figure S1). As found in Chapter 6, the error rate was higher than often reported in AFLP studies (2-5%) (Bonin et al., 2004), but less than 0.1 as recommended by Bonin et al. (2007). The detection of expected population structure and no plate effects supports the acceptance of this relatively high error rate. Levels of genetic diversity were similar across sites (Table 1).

Discussion

In this study we have used AFLP genome scans analysed with two independent detection methods to identify putative loci under selection in the invasive plant *Senecio madagascariensis* in Australia. As each method is optimised by a different sampling regime, we have undertaken these two types of analyses on separate data sets and found two loci ('y23' and 'y59') detected by both methods and associated with environmental parameters describing water and light availability over the flowering period of *S. madagascariensis*.

Overall, 6% of our loci were identified as potentially associated with selection by one or more of our analysis methods which is in keeping with the 5-10% reported by Nosil et al. (2009). One locus ('y23') was identified by all three software packages used to identify putative selection and significantly associated with eight (presumably highly correlated) environmental parameters describing rainfall over the months of May-August. Another locus ('y59') was identified as an outlier by BAYESCAN but not MCHEZA and was found to correlate with daily sunshine hours in May by SAM (Table 5). The corroboration between results from population based outlier methodologies and the individual based spatial analysis method is reassuring and provides confidence that a genuine signal has been detected in the data.

The environmental variables identified as significantly associated with the outlier loci are specific to the months of flowering of *S. madagascariensis* (May-September) (Radford, 1997), an intuitive result given the importance of flowering in the life cycle, particularly of annual plants. Using monthly environmental data for highly variable parameters such as rainfall has distinct advantages when using the spatial analysis method as it allows detection of finer scale patterns which might otherwise be hidden by datasets with less temporal granularity such as annual averages (Cox et al., 2011). Short-lived organisms such as *S. madagascariensis* (an annual) might also be more sensitive to finer temporal scale environmental fluctuations due to their single reproductive season. The quality of the spatial data will also impact results; in particular, gridded climate datasets interpolated from weather station information may not be accurate enough to allow detection of significant associations with particular loci (Parisod and Holderegger, 2012). The precision of environmental datasets will also affect our ability to correctly identify associations. In our study we identified sunshine hours as being significantly correlated with the presence of allele 'y59' in

our individual samples. A variable such as sunshine hours will be continuous in reality; however the measurements recorded are discreet, rounded to the nearest hour. Access to more precise datasets would improve our ability to detect correlations, and may strengthen or weaken the associations uncovered here.

It is tempting to conclude that the loci identified as outliers are the targets of selection, or at least linked closely to these chromosomal regions. Although this scenario is plausible, there are other possibilities that deserve attention. Bierne et al. (2011) postulate that the observed pattern of outlier loci associated with environmental variables (or genetic-environment association, GEA) is often, if not predominantly, the result of coupling of endogenous and exogenous barriers to gene flow. The authors argue that environment-independent genetic incompatibilities can create endogenous reproductive barriers which fluctuate in space but can become trapped by exogenous reproductive barriers as a result of ecological selection. In light of this possibility, our results could be cautiously interpreted as evidence for selection occurring somewhere in the genome of *S. madagascariensis*, but not necessarily directly in the genomic areas surrounding the identified loci.

Perhaps more fundamentally, selection is not the only process capable of producing outlier loci in the genome; demographic factors can also mimic signatures of selection and disentangling the two processes is not a trivial exercise (Li et al., 2012, Thornton et al., 2007). For example, recent range expansion can result in ‘surfing’ of non-adaptive mutations (Excoffier and Ray, 2008) and hierarchical population structure can generate an excess of false positives because neutral variance is higher than expected by the null model (Excoffier et al., 2009, Bonhomme et al., 2010).

With these potential scenarios in mind, it is prudent to interpret the results of our study as a first step in identifying potential contemporary selection in *S. madagascariensis* in Australia but this must be rigorously tested experimentally before more confident conclusions can be drawn. There are two methods for further identifying genes under selection that could be appropriate future prospects for *S. madagascariensis* research. The first is quantitative trait loci (QTL) mapping, which when used in association with genome scans can provide experimental confirmation of selection on ecologically relevant traits (Stinchcombe and Hoekstra, 2008). Traits that differ between populations subjected to different light and water regimes (determined either by common garden or reciprocal transplant experiments) could be focal to incorporate the results of the current study (reviewed in Dormontt et al. 2011). The second method is to directly sequence AFLP fragments that are detected as outlier loci and search for closely mapped genes of interest which may be the targets of selection (e.g. Paris and Despres, 2012). Recent work has identified that molecular transducer genes (involved in response to biotic stimuli) may be under-expressed in Australian *S. madagascariensis* populations relative to the native range (Prentis et al., 2010). Mapping of candidate loci under selection in relation to molecular tranducer genes provides promising potential avenues for future research. Each method for candidate gene identification has its drawbacks; a recent simulation study looked at the success of the outlier methods used in the present research in identifying loci linked to QTL and found a false positive rate of ~30% (Vilas et al., 2012). Similarly in their AFLP sequencing study, Paris and Despres (2012) found only one candidate gene from 14 outliers that warranted further investigation. The authors also faced significant technical challenges such as the impact of long homonucleotides on correct assignment of fragment length (Paris and Despres, 2012) and the presence of homoplasy (Caballero et al., 2008). Each of the above methods can provide useful insights but must be analysed and interpreted cautiously,

particularly in light of the potential for coupling of exogenous and endogenous reproductive barriers (Bierne et al., 2011), which can lead to the erroneous assignment of loci differentiation to selection.

Conclusions

In this study we have indentified two genomic loci in the invasive plant *Senecio madagascariensis* which are considered 'outliers' and significantly associated with environmental variables describing water and light availability. The use of two separate datasets and independent analysis methodologies gives us confidence that we have detected a genuine signal in the data. These loci constitute good candidates for further research into the possible role of contemporary selection in this significant weed. If *S. madagascariensis* is indeed responding to selection in Australia, current predictions of its potential distribution (e.g. Sindel and Michael, 1992) may be incorrect and will likely be confounded further by future climate change (Hoffmann and Sgro, 2011). By integrating knowledge about potential evolutionary adaptations into our distributional models and invasive control strategies (Neve et al., 2009), we may be able to better predict and manage future *S. madagascariensis* spread in Australia.

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Tables and Figures

Table 1.

Locations and genetic diversity parameters of populations used in outlier analyses; *n*, number of *Senecio madagascariensis* individuals; *H*, Shannon's diversity index; H_e, expected heterozygosity.

Population	Latitude	Longitude	*n*	*H*	H_e
Herberton, QLD‡	S 17° 25' 44"	E 145° 26' 15"	19	0.207	0.132
Oxenford, QLD	S 27° 53' 23"	E 153° 18' 43"	20	0.209	0.133
Halfway Creek, NSW	S 29° 56' 6"	E 153° 5' 44"	19	0.228	0.146
Crescent Head, NSW	S 31° 7' 45"	E 152° 52' 50"	20	0.230	0.150
Raymond Terrace, NSW	S 32° 45' 58"	E 151° 44' 55"	20	0.227	0.144
Illawarra, NSW	S 34° 31' 5"	E 150° 45' 1"	20	0.211	0.136
Cooma, NSW	S 32° 19' 49"	E 152° 32' 9"	20	0.223	0.146
Eden, NSW	S 37° 2' 29"	E 149° 54' 30"	20	0.240	0.155
Mean ±SE			19.8 (±0.2)	0.222 (±0.007)	0.143 (±0.005)
Total			158	0.260	0.158

Table 2.

Environmental datasets used in spatial analysis method.

Variable	Number of datasets	Date	Spatial resolution ~degrees/km
Mean monthly and mean annual rainfall (mm)	13	1961-1990	0.025/2.5
Mean monthly and mean annual maximum and minimum temperature (°C)	25	1961-1990	0.025/2.5
Mean monthly and mean annual days with minimum temperature less than -5, -2, 0, 2 °C (frost potential)	52	1976-2005	0.05/5
Mean monthly and mean annual sunshine (hours)	13	At least 15 years of records	0.25/25
Clear sky UV (index)	13	1979-2007	1.5/150
Monthly and annual solar exposure (MJ/m^2)	13	1998-2007	0.05/5
Mean monthly and mean annual relative humidity (9 am, 3 pm)	26	1976-2005	0.05/5
Mean monthly and three-monthly rainfall percentage (%)	24	1961-1990	0.025/2.5
Mean days of rain - monthly and annual	78	1961-1990	0.05/5
Average rainfall percentiles (1, 3, 6, 9 & 12 months)	143	1900-2005	0.25/25
Three-monthly and annual rainfall variability (index)	13	1900-2003	0.25/25
Latitude	1	2007-2008	0.000001/0.1
Longitude	1	2007-2008	0.000001/0.1
Aspect	1	2001	0.0025/0.25
Elevation	1	2001	0.0025/0.25
Slope	1	2001	0.0025/0.25

Table 3.

Primer sequences and number of amplified fragment length polymorphism (AFLP) loci scored from each primer pair.

EcoRI primer	Mse I primer	*n*
TACTGCGTACCAATTCAGC(PET)	GACGATGAGTCCTGAGTAACAA	66
TACTGCGTACCAATTCAGC(FAM)	GACGATGAGTCCTGAGTAACAG	46
TACTGCGTACCAATTCAGC(NED)	GACGATGAGTCCTGAGTAACCG	52

Table 4.

Loci designated as under selection by three different outlier detection programs: BAYESCAN (Foll and Gaggiotti, 2008); MCHEZA (Antao and Beaumont, 2011); SAM (Joost et al., 2008).

Locus	BAYESCAN	MCHEZA	SAM
b2	✓		
b25	✓		
b48	✓		
r11			✓
r12			✓
r24	✓		
r30	✓		
y23	✓	✓	✓
y30	✓		
y59	✓		✓

Table 5.

Environmental variables significantly associated with specific loci in the program SAM (Joost et al., 2008)

Environmental variable	**Locus**			
	r11	**r12**	**y23**	**y59**
Average rainfall variability May-July	✓*	✓**	✓**	
Average rainfall variability June-August			✓*	
Mean days of rain over 2 mm in June			✓*	
10^{th} percentile monthly rainfall in July			✓*	
10^{th} percentile monthly rainfall in June			✓**	
20^{th} percentile monthly rainfall in August			✓**	
30^{th} percentile monthly rainfall in August			✓*	
40^{th} percentile monthly rainfall in August			✓**	
Mean daily sunshine hours in May				✓*

*P < 0.05
**P < 0.01

Figure 1.

Locations of all *Senecio madagascariensis* samples included in study. Panel (a) shows all individuals (crosses) and demographic populations (circles) in south eastern Queensland (QLD) and New South Wales (NSW). Panel (b) shows samples from far north Queensland and panel (c) shows collection site in relation to Australia as a whole.

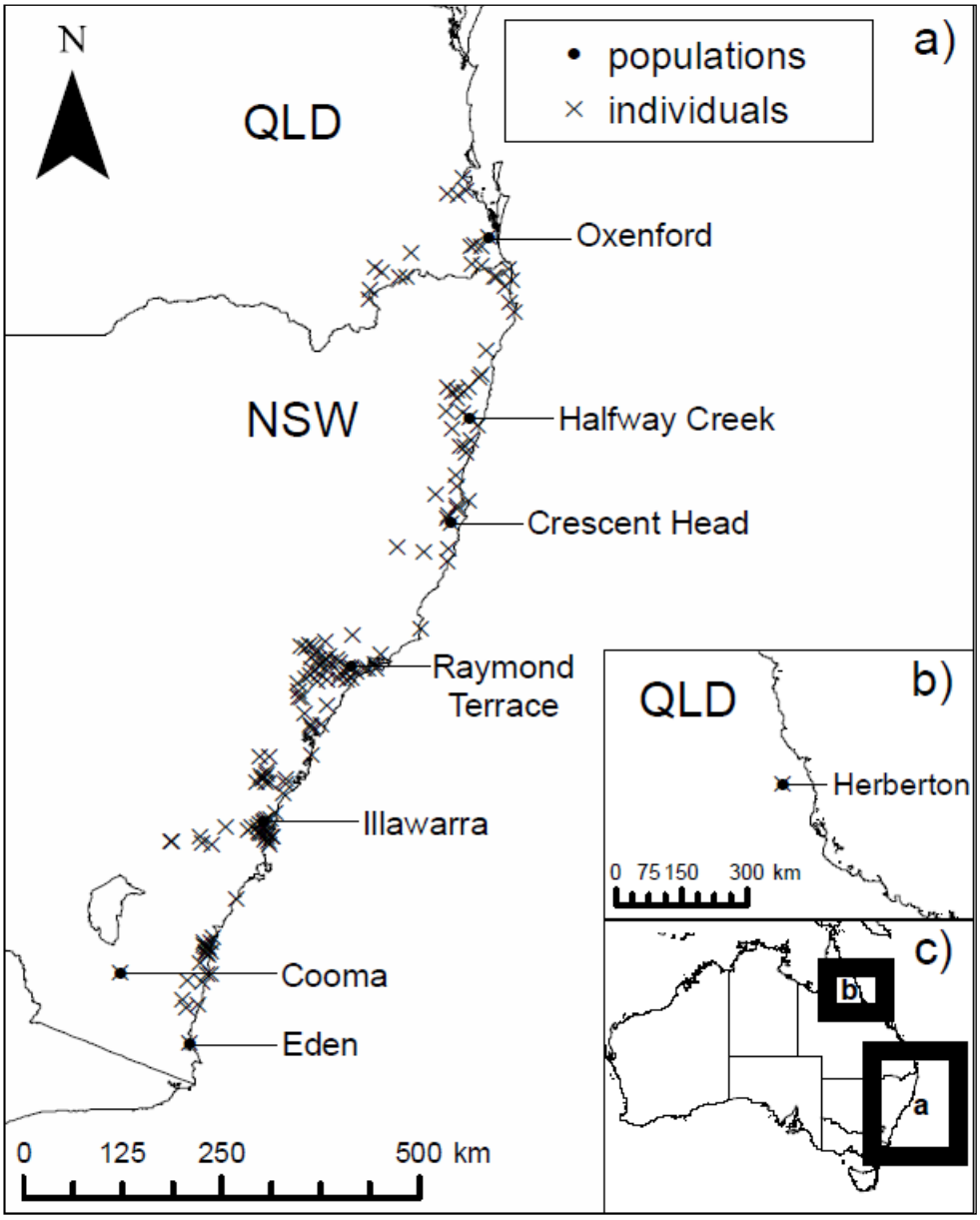


Figure 2.

Output from the BAYESCAN analysis of 164 amplified fragment length polymorphisms (AFLP) loci in eight demographic populations. F_{ST} is shown against the log of the posterior odds (PO). Dashed lines represent different false discovery rates (FDR). Loci designated as putatively under selection with an FDR of 0.05 are labelled.

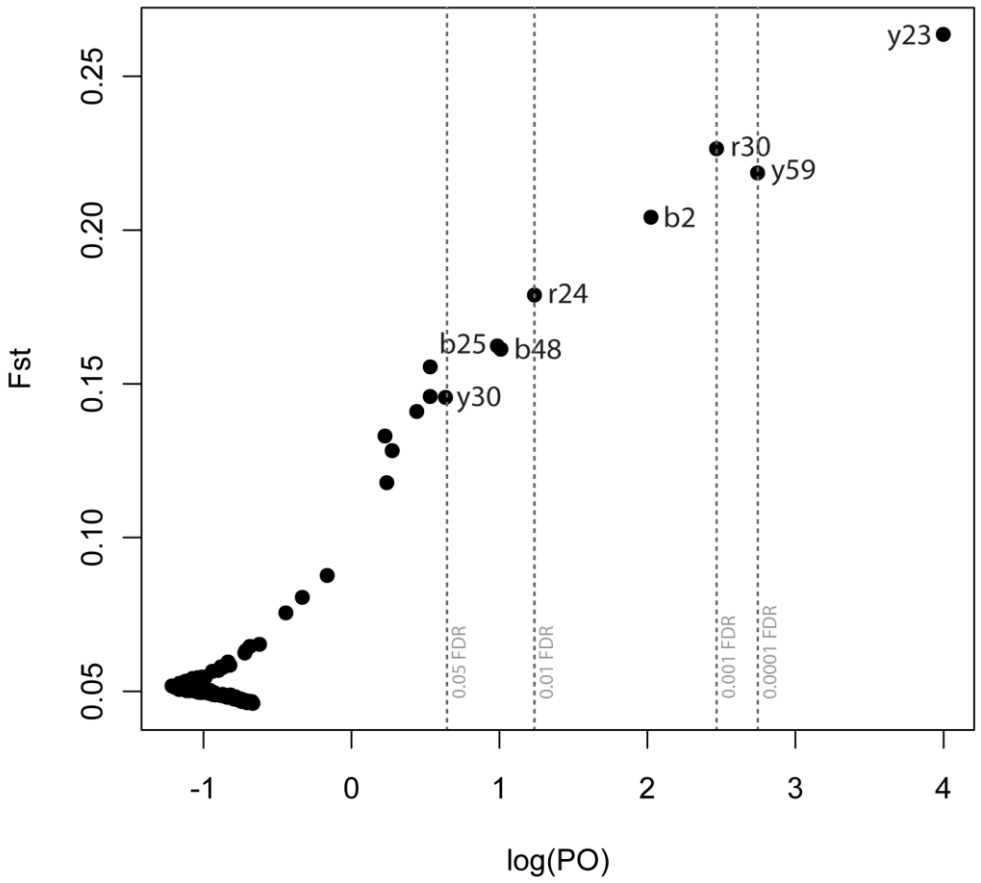


Figure 3.

Output from MCHEZA analysis. The empirical distributions of F_{ST} values for amplified fragment length polymorphisms (AFLP) loci. The *x* axis is expected heterozygosity and the *y* axis is F_{ST}. The solid line represents the 95th quantile. Dots exceeding the 95th quantile represent potential outlier loci, however, after correction for the false discovery rate, only locus 'y23' (labelled) was designated as an outlier

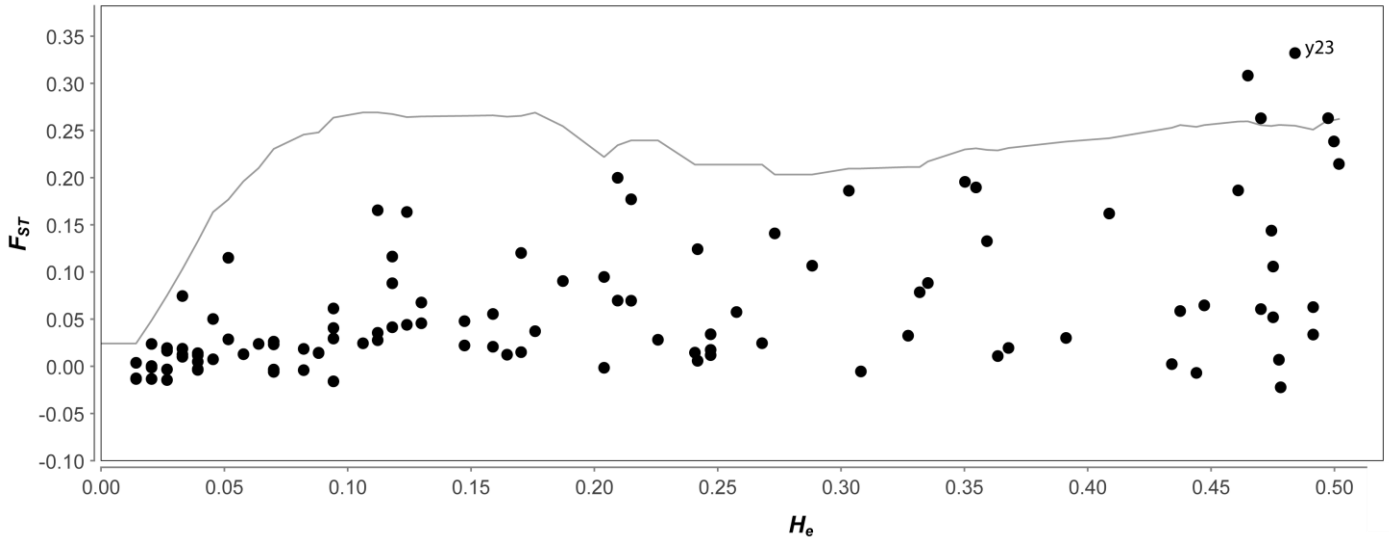


Figure 4.

Probability of allele presence at putatively selected loci across the study area along with associated environmental variable. Panel (a) shows the probability of allele ‘y59’ being present in the study area; panel (b) shows mean daily sunshine hours which significantly associated with the distribution of ‘y59’; panel (c) shows the probability of allele ‘y23’ being present in the study area; panel (d) shows average rainfall variability between May and July which significantly associated with the distribution of ‘y23’ (other variables correlated with ‘y23’ are not shown (see Table 5).

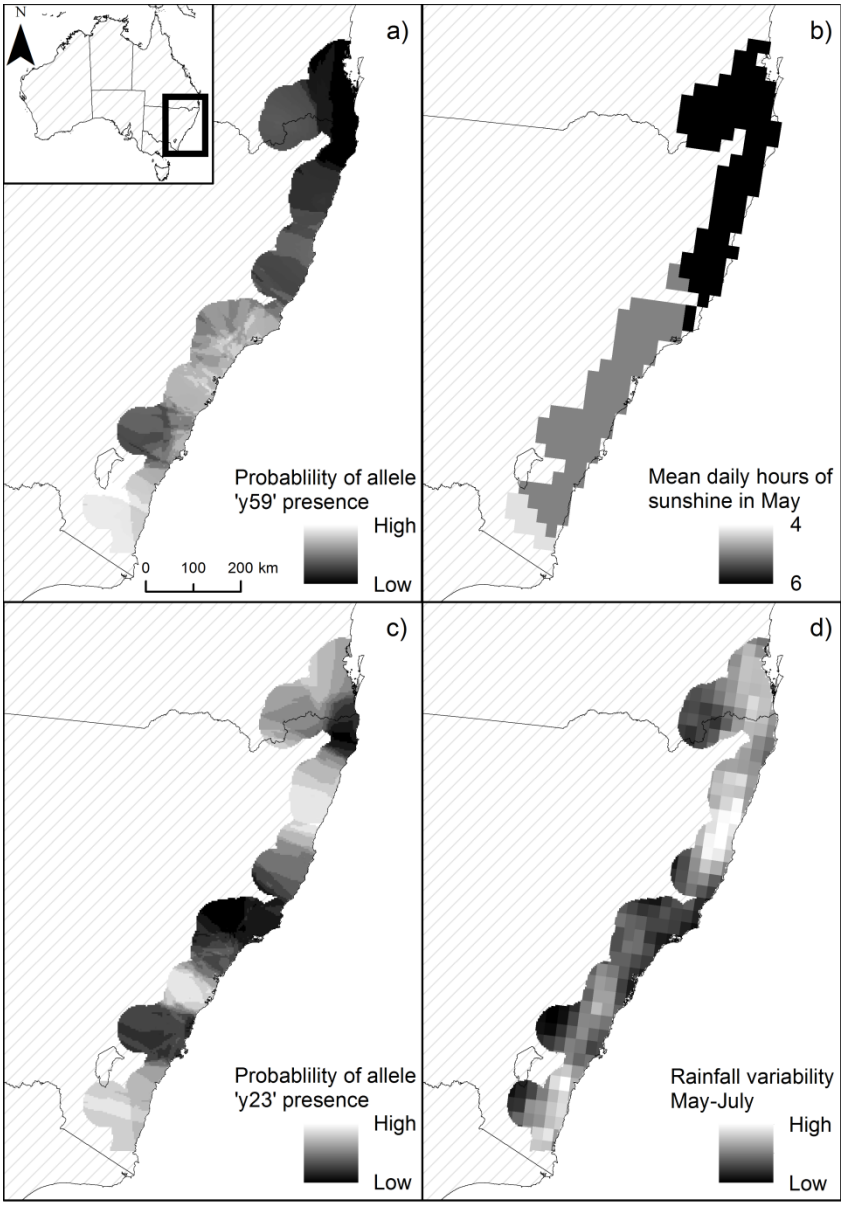
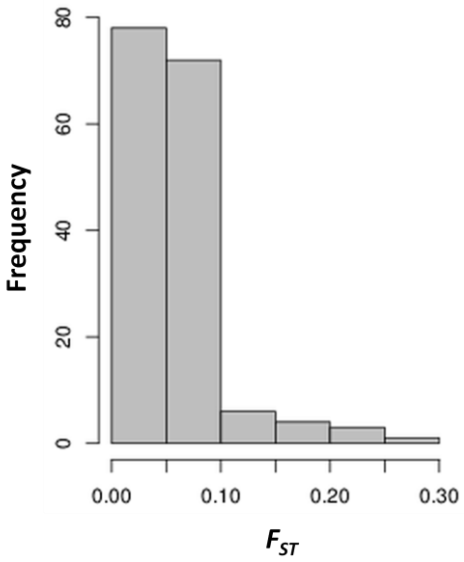


Figure 5.

Histogram of F_{ST} values (as calculated in BAYESCAN) across all loci showing an 'L' shaped distribution, as expected where a few genes are under divergent selection and the remainder of the genome is more homogeneous due to gene flow.



CHAPTER 8

Conclusions, including excerpt from:

Is rapid adaptive evolution important in successful invasions?

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Statement of Authorship

Is rapid adaptive evolution important in successful invasions?

***Fifty Years of Invasion Ecology: The Legacy of Charles Elton*, 1st edition. Edited by David M. Richardson, Chapter 14, pp 175-193.**

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Contributed to initial manuscript conceptualisation, acted as lead author in drafting initial manuscript, coordinated addressing reviewers' comments, prepared figures and tables.

I hereby certify that the statement of contribution is accurate

Signature:..... Date: 28/03/2013

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Contributed to initial manuscript conceptualisation, and commented on and edited subsequent manuscript drafts.

I hereby certify that the statement of contribution is accurate and consent to the inclusion of this manuscript in Eleanor Dormontt's PhD thesis.

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Contributed to initial manuscript conceptualisation and initial drafting, commented on and edited subsequent manuscript drafts.

I hereby certify that the statement of contribution is accurate and consent to the inclusion of this manuscript in Eleanor Dormontt's PhD thesis.

Signature:..... Date: 28/03/2013

Conclusions

This thesis has been written as a selection of manuscripts, either published, or in preparation for submission for publication. Each chapter has its own discussion where results are considered in light of the chapter aims and associated literature. Here, in this final chapter, I present a more cohesive synthesis of the overall contribution of this body of work to our understanding of *Senecio madagascariensis* invasions.

Invasion pathways unique to human mediated introductions, *Senecio madagascariensis* and mass transit

Chapter 2 presented a literature review on invasion pathways which recognised that invasion parameters such as propagule pressure and enemy release can vary in a predictable way depending on the mode of introduction of an exotic species. The review identified two categories of invasion pathway unique to human-mediated introductions, which increase the likelihood of establishment and the potential for exotic species to adapt rapidly to their new environments. These new categories are cultivation and mass transit. In cultivation, propagules are selected for particular traits favoured humans and given resources to aid in their establishment. The ubiquity of this invasion pathway can be seen by the vast number of horticultural escapes that have become invasive, and this trend seems to be growing (Bradley, Blumenthal *et al.* 2012). The second category, mass transit, is where propagules are able to co-opt the human transport network, resulting in the mass movement of multiple propagules to multiple locations on multiple occasions. This pathway can be exemplified by the multitude of hull fouling organisms that move between ports, attached to the outside of ships or taken in as ballast water (Reusch, Bolte *et al.* 2010; Roman and Darling

2007). Each of these invasion routes increases the likelihood of higher propagule pressure, enemy release and multiple introductions from multiple sources, all of which have been associated with increased invasive potential (Dlugosch and Parker 2008a; Orians and Ward 2010; Simberloff 2009).

Chapter 3 supports the notion that *S. madagascariensis* was introduced to Australia via mass transit. The chapter examines the invasion history of *S. madagascariensis* in Australia using a combination of contemporary and historical collections analysed with nuclear and chloroplast microsatellites. Anecdotal evidence suggests that *S madagascariensis* was most likely introduced initially to Australia via contaminated dry ballast in ships trading between South Africa and Europe via Australia (Sindel, Radford *et al.* 1998). Although genetic diversity had been reduced in Australia compared to the native range, *S. madagascariensis* still exhibit high diversity in absolute terms, and analysis of the herbarium record points to at least two separate introduction events. By combining analyses of contemporary and historical samples, Chapter 3 demonstrates how population genetics can be used in conjunction with traditional mapping of invasion spread, to reconstruct a rich history of invasion pathways and dynamics.

Chapter 4 looked at *S. madagascariensis* invasions on a global scale, and found that the diversity present in Hawaii, Japan, Brazil and Argentina could all be found in individuals from Australia, as well as the native range. This result suggests that Australia may have been the source of secondary invasions of *S. madagascariensis* around the world, possibly again through a mass-transit invasion pathway. There has been a growing body of evidence that secondary invasions from existing invasive populations are often the source of subsequent invasions, not the native range (Boubou, Migeon *et al.* 2012; Hirsch, Zimmermann *et al.* 2011; Lombaert, Guillemaud

et al. 2010; Yang, Sun *et al.* 2012). The work presented here is consistent with this scenario for *S. madagascariensis* invasions worldwide but will need more thorough treatment before alternative explanations (i.e. introduction of genetically similar material from the native range) can be confidently ruled out.

The potential for adaptive evolution in *Senecio madagascariensis*

Chapter 5 presented another review paper, this time looking at the potential mechanisms behind adaptive evolution in invasive species. Chapters 6 and 7 have looked at two of these mechanisms in the study species *S. madagascariensis*. Adaptive evolution can be considered to require two things – genetic variation and selection. Chapter 6 looks at hybridisation with *S. pinnatifolius* 'dune variant' as a possible source of adaptive variation in *S. madagascariensis*. The very low incidence of hybridisation in the field and the lack of mature hybrids suggest that hybridisation is probably not playing a large role in providing *S. madagascariensis* with adaptive genetic variation. Chapter 7 looks at the second part of the adaptive evolution question, selection. Here I asked whether there is evidence of selection acting on the genome of *S. madagascariensis* and whether I could identify and environmental parameters that may be exerting a selective pressure. The results indicate that there is such evidence and that certain environmental variables associated with water and light availability may well be exerting a measurable selective pressure on *S. madagascariensis* in Australia. Chapter 3 has already identified large amounts of genetic diversity in *S. madagascariensis* populations in Australia, probably due to its outcrossing habit and likely multiple introductions. It is perfectly plausible that *S. madagascariensis* has sufficient standing genetic variation on which selection can act.

Limitations of the current work

The data presented here are of course far from perfect; as in any research project, more questions are generated than answered. In Chapter 3 the sampling in the native range could have been more extensive to include populations further afield from KZN and improve the likelihood that we sampled representative diversity in the area. There was no obvious population structure evident in the populations we did sample from South Africa and it would have been enlightening to sample widely enough to capture some native range structure. It would also have been preferable to use all the markers available on all the specimens (contemporary and herbarium specimens for both nuclear and chloroplast markers). This regime was not possible however due to problems with the DNA obtained from herbarium specimens. With more time and money, it may have been possible to more closely compare the genetic diversity of the contemporary and historical collections. Similarly, use of herbarium specimens from the native range could have allowed a direct comparison between materials that existed at the time of introduction, not a century later. We cannot be 100% sure whether the current native range distribution of genotypes actually mirrors that of the time of introduction.

In Chapter 4, logistic constraints made wide sampling from the worldwide invasions of *S. madagascariensis* very difficult, I relied on good will from international collaborators to provide samples and so had little opportunity to affect the sampling regime. The work does serve to highlight *S. madagascariensis* as a potential bridgehead species and I hope this will stimulate further work that seeks to explore this issue with more rigorous sampling and analytical methods.

In Chapter 6 there were significant problems in the laboratory. Initially microsatellites only were going to be used instead of AFLPs but unfortunately, despite good results

on pilot runs, when the entire sample set was run the results were very poor. The majority of microsatellite loci did not amplify properly, hence only two (out of an initial seven) were kept for the final dataset and used in the chapter. AFLPs were chosen as an appropriate alternative to the microsatellite dataset but due to financial constraints, the sample sizes had to be reduced to 20 adults per population instead of 40 as were initially planned. This has impacted on the overall power of the analyses to detect hybrids. Furthermore, as the study was limited to only two shared sites, I cannot be certain of the broader generality of the results obtained. Future work looking at more sites with more powerful markers and combining a purely genetic study with one that includes morphological comparisons would be particularly useful.

In Chapter 7, the comparative analyses might have been improved with less plants per population and more populations in the outlier analyses, as has been recently advised by (De Mita, Thuillet *et al.* 2013) who used simulated populations to compare the effectiveness of different methods of detecting selection. The use of AFLP loci is also limiting as methods for moving beyond the 'genome scan' stage and on to identifying specific genes that might be under selection, is particularly challenging from AFLP datasets.

The remainder of this concluding chapter is taken from:

Dormontt EE, Lowe AJ, Prentis PJ. 2011. Is rapid adaptive evolution important in successful invasions? In: Richardson DM ed. *Fifty Years of Invasion Ecology: The Legacy of Charles Elton.* 1st edition, Blackwell Publishing Ltd.

Full text of this chapter in its original form can be found in Appendix 1.

Future directions in studying adaptive evolution in invasive species: a genomic approach

Biological invasions provide a unique opportunity to examine how adaptive and neutral processes influence phenotypic evolution over ecological timescales. Although evolutionary change was hypothesized as a process that could increase invasion success over 35 years ago (Baker 1974), most research in this area has been conducted in the past decade. This research has largely concentrated on comparisons of genetic diversity between the native and introduced range of invasive species (reviewed in Dlugosch and Parker 2008a). Recently, there has been a surge of interest in investigating adaptive evolution in invasive species (Keller and Taylor 2008; Maron, Vila *et al.* 2004; Prentis, Wilson *et al.* 2008), yet many studies have not adequately controlled for neutral evolutionary processes (but see Colautti, Eckert *et al.* 2010; Keller, Sowell *et al.* 2009; Maron, Vila *et al.* 2004; Xu, Julien *et al.* 2010). Several methods exist to determine whether adaptive evolution is responsible for rapid phenotypic change in biological invasions, while controlling for neutral evolution, phenotypic plasticity and pre-adaptation. These approaches include phenotypic ancestor–descendent comparisons between native and introduced populations when the source populations are known (Dlugosch and Parker 2008b); comparisons of genetic differentiation at neutral genetic markers (F_{ST}) versus phenotypic traits (Q_{ST}) (Xu, Julien *et al.* 2010); and finally reciprocal transplant experiments between source populations from the native range and introduced populations, using selection gradient analysis (Rundle & Whitlock 2001). Although these three approaches can demonstrate that phenotypic change in introduced populations is adaptive, they will not elucidate the genetic variation underlying this evolution.

Understanding the gene or genes underlying ecologically relevant traits that are involved in adaptive evolution to novel environments is a major goal in evolutionary biology and should be equally important to scientists studying biological invasions. Through the application of such methods the invasion biology community will be able to determine whether rapid adaptive evolution in the invasive range results from standing genetic variation rather than new mutation. Further, we can begin to understand whether the same genes are the targets of selection across multiple independent invasions of the same species; or if similar genes are the target of selection in different species invading the same habitat. Of course to get the most out of these experiments we first need to know which traits have been the target of selection-driven adaptive change in the introduced range.

Once adaptive phenotypic evolution has been established, such as in the case of *Phyla canescens* (Xu, Julien *et al.* 2010) we advocate the approach put forward by Stinchcombe and Hoekstra (2008) of a genome scan in combination with quantitative trait loci (QTL) mapping. These methods will allow identification of chromosomal regions and genes within these regions that underlie ecologically relevant traits important to adaptive evolution in invasive species.

Genome scans involve genotyping numerous loci throughout the genome of many individuals in several populations (Beaumont and Balding 2004) to detect 'outlier' loci that show unusually high levels of differentiation. This technique allows a distinction to be made between evolutionary forces that affect the whole genome (e.g. bottlenecks and drift) and those that affect particular loci (i.e. selection) (Stinchcombe and Hoekstra 2008). Historically, genome scans have used amplified fragment length polymorphism (AFLP) markers or microsatellites (SSRs) obtained from the expressed portion of the genome to detect outlier loci. Although this approach has had some

success in finding candidate genes underlying adaptive phenotypic evolution (Stinchcombe and Hoekstra 2008), it can lack power to detect outliers using these marker systems. By applying this analysis technique to RAD markers (Miller, Dunham *et al.* 2007), generated with high-throughput next generation sequencing, the power of genome scans can be substantially improved. Hohenlohe, Bassham *et al.* (2010) conducted a genome scan using Illumina-sequenced RAD tags in two marine and three freshwater populations of threespine stickleback and were able to identify and genotype over 45,000 single nucleotide polymorphisms (SNPs). Using the statistical power of population genomics, this study identified several genomic regions indicative of divergent selection after the colonization of freshwater. This approach in isolation, however, has a major limitation as it is often unclear which 'outlier' loci underlie phenotypic traits of interest. To overcome this problem, genome scans should be used in combination with QTL mapping.

QTL mapping uses statistical analyses of molecular markers distributed throughout the genome and traits measured in the progeny of controlled crosses to identify stretches of DNA that are closely linked to genes that underlie the trait in question (Stinchcombe and Hoekstra 2008). Consequently, if it is possible to make controlled crosses between native and introduced individuals of the same species differing in ecologically relevant traits of interest, QTL mapping can be used to identify the genomic regions associated with these traits. This approach requires a large amount of genetic markers to saturate the genome, and population genomic markers such as sequenced RAD tags make an ideal marker for QTL mapping. QTL mapping with RAD tags in controlled crosses of freshwater and marine sticklebacks (see Baird, Etter *et al.* 2008) fine mapped the genetic bases conferring reduction of lateral plate armour in freshwater populations of the three-spine stickleback by identifying recombinant breakpoints in F2 individuals. Theoretically, by adding the outlier loci

detected by Hohenlohe, Bassham *et al.* (2010) to this QTL map, it would be possible to determine if the outlier loci identified in genome scans are found in the chromosomal regions underlying the reduction of lateral plate armour. Experimental evolution research has determined that a reduction in lateral plate number is under selection in freshwater populations of three-spine stickleback (Barrett, Colautti *et al.* 2008). Therefore, a population genomics approach using sequenced RAD tags has identified the genetic bases of adaptive evolution in this case.

A combined genome scan and QTL mapping approach can help to identify genes or small chromosomal regions underlying adaptive phenotypic change. A well-illustrated example of this approach comes from maize, where outlier loci identified from genome scans were integrated onto linkage maps to determine if they map to chromosomal regions underlying domestication traits that have a history of artificial selection (Vigouroux, McMullen *et al.* 2002) *Zea mays* ssp. *mays* (maize) was crossed to its wild progenitor *Z. mays* ssp. *parviglumis* (Teosinte) and two outlier loci detected from genome scans were found to map close to a chromosomal region controlling two traits that differ significantly between maize and teosinte (Vigouroux, McMullen *et al.* 2002). Although this example is not of an invasive species, it does highlight how a combined approach can be applied to study adaptive phenotypic change in any organism including invasive species.

Overall these examples demonstrate that genome scans and QTL mapping, using RAD markers generated by next generation sequencing, can be used to determine the genetic bases of adaptive phenotypic evolution of species. Using these new technological advances has provided unprecedented insights into the genetics underlying adaptive trait evolution and validated previous research with much less effort. It is now time to apply these new technologies to biological invasions. We

believe that using this combined population genomics approach can move the field of invasion biology forward by allowing researchers to determine the genetic basis of ecologically relevant traits involved in successful invasions, and potentially to identify candidate genes or mutations involved in rapid adaptive evolution of invasive species.

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Appendices

Appendix 1: Full version as published of Dormontt EE, Lowe AJ, Prentis PJ. 2011. Is rapid adaptive evolution important in successful invasions? In: Richardson DM ed. *Fifty Years of Invasion Ecology: The Legacy of Charles Elton.* 1st edition, Blackwell Publishing Ltd.

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