Can hybridization cause local extinction: a case for demographic swamping of the Australian native *Senecio pinnatifolius* by the invasive *Senecio madagascariensis*?

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Summary

• Hybridization between native and invasive species can have several outcomes, including enhanced weediness in hybrid progeny, evolution of new hybrid lineages and decline of hybridizing species. Whether there is a decline of hybridizing species largely depends on the relative frequencies of parental taxa and the viability of hybrid progeny.

• Here, the individual- and population-level consequences of hybridization between the Australian native *Senecio pinnatifolius* and the exotic *Senecio madagascariensis* were investigated with amplified fragment length polymorphism (AFLP) markers, and this information was used to estimate the annual loss of viable seeds to hybridization.

• A high frequency (range 8.3–75.6%) of hybrids was detected in open pollinated seeds of both species, but mature hybrids were absent from sympatric populations. A hybridization advantage was observed for *S. madagascariensis*, where significantly more progeny than expected were sired based on proportional representation of the two species in sympatric populations. Calculations indicated that *S. pinnatifolius* would produce less viable seed than *S. madagascariensis*, if hybridization was frequency dependent and *S. madagascariensis* reached a frequency of between 10 and 60%.

• For this native–exotic species pair, prezygotic isolating barriers are weak, but low hybrid viability maintains a strong postzygotic barrier to introgression. As a result of asymmetric hybridization, *S. pinnatifolius* would appear to be under threat if *S. madagascariensis* increases numerically in areas of contact.

Key words: amplified fragment length polymorphism (AFLP), demographic swamping, hybrid viability, invasive species, *Senecio*, triploid.

Introduction

The importance of hybridization in the evolution and speciation of plants has long been recognized (Rieseberg *et al.*, 1995; Arnold, 1997; Rieseberg *et al.*, 2003; Abbott & Lowe, 2004; Hegarty & Hiscock, 2005; Buggs & Pannell, 2006). Hybridization can result when divergent lineages, or species formed in allopatry, change ranges and come into reproductive contact, potentially forming a zone of secondary contact (Anderson, 1949; Lagercrantz & Ryman, 1990; Cruzan, 2005; Hoskin *et al.*, 2005). The formation of hybrid zones can be promoted by biological invasions, if introduced species are sufficiently closely related to native species. As global trade and passenger travel continue to accelerate (Hanfling & Kollmann, 2002), it seems probable that alien plant invasions will continue at an alarming rate, leading to increasing contact

and hybridization between previously allopatric species (Abbott, 1992; Abbott & Lowe, 2004). In contrast to natural range changes, biological invasions are more likely to form extensive zones of contact, potentially accelerating the eventual outcome of hybridization (Wolf *et al.*, 2001).

Hybridization between natives and exotics can have several outcomes, including enhanced weediness in hybrid offspring (Ellstrand & Schierenbeck, 2000; Morrell *et al.*, 2005; Whitney *et al.*, 2006), evolution of new hybrid lineages (Lowe & Abbott, 2004) and decline or even extinction of hybridizing species (Levin *et al.*, 1996). The last is the most potentially destructive outcome of interspecific hybridization, and can occur via two main potential mechanisms (Wolf *et al.*, 2001). First, introgressive hybridization, the transfer of genes between species via fertile or semifertile hybrids, may produce hybrid derivatives of superior fitness that displace one or both pure conspecifics; this process is defined as genetic assimilation (Wolf *et al.*, 2001). Secondly, if hybrids are sterile or display reduced fitness, the population growth rate of the hybridizing taxa may decrease below that required for replacement of one or both parental species; this process is termed demographic swamping (Wolf *et al.*, 2001).

The potential for introgression is regulated in part by the strength of chromosomal or genic sterility barriers that prevent the formation of fertile interspecific offspring (Arnold, 1997; Lowe & Abbott, 2004; Erickson & Fenster, 2006). These barriers can be particularly strong for triploid hybrids resulting from crosses between diploid and tetraploid species (Lowe & Abbott, 2000; Husband, 2004). A combination of both genetic assimilation and demographic swamping may also result in the decline of hybridizing taxa, making it difficult to discern the true causative process. In many cases molecular methods can be applied to demonstrate the potential for introgression and to distinguish between processes.

Senecio, one of the largest genera of flowering plants, is known world-wide for its globally important weed species (Holm *et al.*, 1997) and the widespread occurrence of interspecific hybridization between native and introduced taxa (Abbott, 1992; Lowe & Abbott, 2004; Kadereit *et al.*, 2006). *Senecio madagascariensis* (fireweed), a native of southern Africa and Madagascar, was introduced to Australia > 80 yr ago and is now an aggressive weed in its invasive range (Radford, 1997; Radford *et al.*, 1995a; Sindel *et al.*, 1998). In Australia, molecular genetic and morphological studies have demonstrated a close affinity between fireweed and Australian native *Senecio* species, including *Senecio pinnatifolius* (formerly *Senecio lautus*) (Scott *et al.*, 1998). Although *S. madagascariensis* ($2n = 2x = 20$) and *S. pinnatifolius* ($2n = 4x = 40$) differ in ploidy (Radford *et al.*, 1995b), empirical and experimental crossing studies have established that both species can serve as paternal and maternal parents of synthetic hybrids (Radford, 1997). Under glasshouse conditions, synthetic triploid hybrids between the two species exhibit low viability and are highly sterile (sterile pollen, low pollen production and no stigmatic viability;

Radford, 1997). Despite low fertility, triploid hybrids can still act as a genetic bridge between diploid and tetraploid taxa, as demonstrated by Lowe & Abbott (2000). Thus, despite low fertility, F1 triploid hybrids could enable introgression of *S. madagascariensis* genes into *S. pinnatifolius* (or vice versa), but this remains untested in the field.

Populations of *S. madagascariensis* exist in sympatry with populations of the native *S. pinnatifolius* across many regions of Australia's east coast. Within this area, *S. madagascariensis* and *S. pinnatifolius* grow in close physical proximity, have flowering periods that overlap and are pollinated by the same insect species (Radford, 1997; Radford & Cousens, 2000; White, 2007). Hybrid formation has also been observed in sympatric populations of the two species in the field (Scott, 1994; Radford, 1997), but may be restricted to certain variants of *S. pinnatifolius*, such as the varieties Tableland, Headland and Dune (Radford, 1997).

To examine in greater detail the outcome of hybridization between the native *S. pinnatifolius* and the invasive *S. madagascariensis*, comparisons were made at population (in sympatric vs allopatric populations) and individual (in sympatric populations) levels to investigate contemporary and long-term outcomes of hybridization. Amplified fragment length polymorphisms (AFLPs) were used as molecular markers in this analysis. Three primary questions are addressed in this paper.

1 What is the viability of hybrids in the field? We compared the frequency of hybrids in open pollinated seed of both species and the incidence of adult-stage hybrids in sympatric populations in an attempt to answer this question.

2 Does hybridization influence the degree of genetic diversity or differentiation within sympatric compared with allopatric populations of these hybridizing species?

3 Can we estimate the likely outcome of hybridization within this native–invasive species pair under a number of hybridization scenarios, and is *S. pinnatifolius* at risk of genetic assimilation and/or demographic swamping in sympatric populations?

Materials and Methods

Study species

Senecio madagascariensis Poir. (fireweed), a diploid annual weed from South Africa, has invaded large areas of farmland and grassland in south-eastern Australia (Radford *et al.*, 1995a; Radford, 1997). *Senecio pinnatifolius* A. Rich is an herbaceous perennial tetraploid (Orduff, 1964; Ali, 1966; Radford *et al.*, 1995b, 2004), and has a similar geographic range to *S. madagascariensis* in the eastern states, but generally occurs in smaller, more scattered populations than the exotic (Radford, 1997; Radford & Cousens, 2000). *Senecio pinnatifolius* (var. Tableland, formerly known as *Senecio lautus* ssp. *lanceolatus*), the focus of this study, inhabits disturbed areas and pasture usually close to the edge of rainforest or

moist eucalypt forest and flowers between February and June in south-eastern Australia (Radford & Cousens, 2000; Supplementary material Fig. S1). There is a 4-month period of overlap between the flowering periods of the native and exotic *Senecio* species; the latter flowers between the months of March and December in Australia (Radford & Cousens, 2000). Previous studies have indicated that both species are self-incompatible and insect-pollinated (Ali, 1966; Lawrence, 1985; Radford, 1997). The two species are superficially morphologically similar (differing in plant size, bract number and time to senescence), both producing similar-sized yellow capitula which occur in clusters on the plant: floral visitors move freely between the two species when they grow together in the field (White, 2007).

Study sites and sample collections

To assess genetic diversity in allopatric populations of each *Senecio* species, leaf material was collected from approx. 45 (minimum 42) flowering plants from each of three allopatric populations of *S. pinnatifolius* (var. Tableland) and three allopatric populations of *S. madagascariensis*.

To determine the number of mature hybrid plants as well as genetic diversity for each species when they grow in sympatry, leaf material was collected from approx. 45 plants (minimum 43, maximum 47) from two sympatric sites across the morphological range of flowering plants of each species. Plants from which leaf material was collected were identified as either *S. pinnatifolius* or *S. madagascariensis* using morphological

features, including bract number and leaf morphology, following Ali (1969) and Nelson (1980). Despite repeated searches over two consecutive flowering seasons, no obvious hybrids (i.e. plants with intermediate morphology) were observed in the field. In addition, ~20 seeds per plant were collected from a random selection of 10 plants of each species from which leaf material had been collected (a total of ~200 seeds per species for each of the two populations).

All allopatric and sympatric populations sampled occurred within the Border Ranges, a group of linked mountain ranges running along the eastern portion of the Queensland/New South Wales state border (population locations are indicated in Table 1). All sites, regardless of location, occurred within a similar altitudinal range (between 550 m and 700 m above sea level), had similar types of neighbouring vegetation (pasture and moist eucalypt forest or rainforest), and were surveyed during May when both species were flowering. Allopatric populations were separated by at least 5 km from the nearest known population of the other species. In sympatric populations, *S. pinnatifolius* grew along the rainforest edges, and in nearby creek beds, while *S. madagascariensis* inhabited adjacent pasture, with considerable mixing of the species at the interface.

All leaf samples from allopatric and sympatric populations were transported on ice, then frozen and stored at –80°C until DNA extractions were performed. Seeds were germinated on moist filter paper until they reached approx. 20 mm in height, at which point they were removed, frozen and stored at –80°C. Germination percentages for *S. pinnatifolius* and

Table 1 Population locations and relative frequencies of plants and flowers of native *Senecio pinnatifolius* (*Sp*) and exotic *Senecio madagascariensis* (*Sm*) used in the current study

| Population | Location | Relative densities (*Sp:Sm*) | | Sample size | |
		Plants	Capitula	Parents	Progeny
Allopatric					
Hampton	East of Hampton, Northern Darling Downs (27°22′S, 152°10′E)	100 : 0	100 : 0	45	
Swanfels 1	North of Killarney, Southern Darling Downs (28°07′S, 152°23′E)			42	
Swanfels 2	North of Killarney, Southern Darling Downs (28°08′S, 152°23′E)			45	
Beechmont	Near Beechmont, Gold Coast Hinterland (28°07′S, 153°10′E)	0 : 100	0 : 100	45	
Tamborine	Mt Tamborine, Gold Coast Hinterland (27°58′S, 153°12′E)			45	
Springbrook	Springbrook Plateau, Gold Coast Hinterland (28°11′S, 153°16′E)			45	
Sympatric					
Queen Mary Falls	Near Queen Mary Falls section of Main Range National Park, Southern Darling Downs (28°20′S, 152°21′E)	0.77 : 0.23	0.96 : 0.04	*Sp* 45; *Sm* 45	*Sp* 109; *Sm* 49
O'Reillys'	Near Lamington National Park, Gold Coast Hinterland (28°13′S, 153°07′E)	0.84 : 0.16	0.97 : 0.03	*Sp* 43; *Sm* 47	*Sp* 72; *Sm* 41

S. madagascariensis from both sympatric sites were generally quite low, particularly for *S. madagascariensis* (< 35% at both sites); numbers of resulting progeny for each species for each site are shown in Table 1. Low seed germination was not the result of seed dormancy, as neither species exhibits dormancy when grown on filter paper (Radford, 1997), but rather a result of the collection relatively immature fruiting capitula.

Reciprocal crossing experiments

A reciprocal crossing experiment was undertaken to examine the viability and number of seed produced from interspecific and intraspecific crosses. Plants were germinated and grown using the methodology described in Radford & Cousens (2000). Once plants reached reproductive maturity, inflorescences to be used in the reciprocal crossing experiment were bagged before flowers opened. Once flowers opened, bags were removed and crosses performed. Hand pollinations were performed by applying mature anthers from pollen donors to the stigmatic surface of pollen receivers with forceps. This procedure was repeated for all florets on an inflorescence. Inflorescences were rebagged until maturation of capitula as indicated by the exposure of mature pappus. Bags were then removed, and the number of seed produced for both interspecific and intraspecific crosses was recorded. To assess the viability of seed produced from crosses, seeds were germinated according to the protocol of Radford & Cousens (2000). ANOVA was used to determine if differences existed in the number and viability of seeds produced from interspecific and intraspecific crosses.

Relative densities of plants and flowers

Relative plant and capitulum densities of each species at each site were determined using the point centred quarter (PCQ) method (Krebs, 1989), using 30 random plants of each species at each site as 'centre points'. A χ^2 test was used to determine whether the proportion of hybrids produced in the progeny of each species was concordant with capitulum densities of each species at each site.

AFLP profiling

Total cellular DNA was extracted from 0.1 g of plant material per sample according to the protocol of Doyle & Doyle (1987) with slight modifications. DNA was quantified visually on ethidium bromide-stained agarose gels and samples were diluted with 0.5 Tris-Edta buffer to obtain concentrations between 100 and 200 ng μl^{-1}.

AFLP restriction/ligation was performed following the protocol of Prentis *et al.* (2004). AFLP polymerase chain reaction (PCR) was performed following the method of Zawko *et al.* (2001), using two primer pairs: E-AAG/M-AG and E-AAG/M-GA, where the selective *Eco*RI primer was

Hex labelled (Geneworks, Adelaide, Australia). The fluorescently labelled amplified products were analysed by gel electrophoresis (5% acrylamide gels), using a Gelscan GS2000 (Corbet Research, Sydney, Australia) with a TAMRA 500 size standard (Applied Biosystems, Warrington, UK). To confirm reproducibility, five adult samples of each *Senecio* species were run blindly six times from different extractions for both primer combinations and loci that were ambiguous were not scored in the full analysis. This information was also used to produce an error rate of fragment misscoring for both primer combinations. At an individual locus, bands of similar size and intensity were considered to be homologous, following previous studies of closely related species (Rieseberg, 1996; O'Hanlon & Peakall, 2000). AFLP profiles were scored for the presence and absence of bands between 50 and 500 bp in size.

Data analysis

Population level Genetic diversity within each population was quantified by calculating Shannon's index of diversity (Shannon, 1948), as this diversity measure has been used previously to obtain accurate estimates of genetic diversity in polyploid plants with AFLP markers (Abbott *et al.*, 2007). Shannon's index was calculated using the following equation: $H = -\Sigma(p_i \ln p_i)$, where p_i is the frequency of a band at a particular locus. This value was then averaged over all polymorphic loci. A *t*-test was used to compare whether degrees of genetic diversity were similar in sympatric and allopatric populations of both species.

Global F_{ST} (*F* statistic) and pairwise F_{ST}, used to characterize the extent of population differentiation among all population pairs within each species separately, were estimated in SPAGEDI (Hardy & Vekemans, 2002). This program was chosen as it can estimate *F* statistics in both diploids and polyploids with dominant marker data. PHYLIP (Felsenstein, 2005) was used to construct a neighbour-joining (NJ) phenogram in TREEVIEW (Page, 1996) from the pairwise F_{ST} matrix.

Individual level Principal co-ordinates analysis (PCOA) was used to examine clustering of individual *S. pinnatifolius* and *S. madagascariensis* genotypes from both sympatric and allopatric sites using GENALEX (Peakall & Smouse, 2006). To assign individuals to their most likely species of origin, or hybrid status, the assignment method of Duchesne & Bernatchez (2002) in AFLPOP was used. The assignment method utilizes multilocus AFLP data to test the likelihood that an individual genotype (G) is a pure species or interspecific hybrid based on population-level allele frequencies. If the frequency of an AFLP fragment was 0, log(0) was replaced by log(ε), where ε was chosen as 0.001. Individuals were assigned to species or hybrid populations displaying the highest log-likelihood for G; however, allocation of genotypes was only made if the minimal log-likelihood difference (MLD) was ≥ 1 for mature individuals. This means that a genotype is 10 times more

likely to originate from a particular population than any other candidate population. A MLD of 0 was used to allocate progeny genotypes to parental species or hybrid swarms, as many individuals were unassigned at higher MLD stringency levels. The MLDs chosen here are similar to those in most previous studies (Potvin & Bernatchez, 2001; Campbell *et al.*, 2003; He *et al.*, 2004).

To determine the probability of incorrect assignment the AFLPOP simulator was used. The simulation technique produces 1000 random samples from the source population file and calculates the proportion of allocations (P) to the second population. When P is small the incorrect assignment of individuals is highly unlikely. If P values for an individual were < 0.001 for both species and all possible hybrid populations, then the individual could not be assigned.

First generation, F1 parental backcrosses and F2 hybrid populations were simulated in AFLPOP for all pairs of allopatric populations of *S. pinnatifolius* and *S. madagascariensis*. Mature sympatric individuals of the two species were then assigned to either allopatric populations or simulated hybrid swarms. Progeny raised from seed collected from sympatric sites were also allocated to their species of origin or simulated interspecific hybrid status using the same assignment method as already described.

Risk posed by hybridization

The number of nonhybrid adults of each species that would be produced from seeds in a single year under various rates of F1 seed production was estimated. Data from other studies were incorporated for the following parameters: monthly capitulum production for both species in allopatric sites (Supplementary material Fig. S1) to estimate the proportion of total capitula produced per year during synchronous (S) and nonsynchronous (N) flowering, annual seed production (A), percentage germination under field conditions (G), survival transition to maturity of both species in *S. pinnatifolius* (var. Tableland) habitat (E), and hybridization rate (H) (see Supplementary material Table S1 for values). Annual viable seed production (AVSP) was then calculated for both species using the following equation:

$$AVSP = ((S \times A) \times (1 - H) \times G \times E) + ((N \times A) \times G \times E)$$

Hybridization scenarios examined using this equation were: no hybridization, maximum hybridization (all seeds produced during synchronous flowering were hybrids), fixed level hybridization (based on actual levels of hybridization observed in field-collected progeny in this study), and linear frequency-dependent hybridization. The hybridization rate (H) was calculated for each month, based on flowering synchrony data from field observations (Radford & Cousens, 2000; Supplementary material Fig. S1). The proportion of *S. madagascariensis* (P_m) in a population was used to estimate the proportion of hybrid seeds produced separately for both *S. madagascariensis* and *S. pinnatifolius* using linear frequency-dependent relationships outlined in brackets below. Linear density-dependent relationships were fitted based on the assumption that $H = (1 - \text{observed } H)$ at $P_m = (1 - \text{observed } P_m)$, for each site and species independently. (Equations for lines of best fit: O'Reillys': *S. madagascariensis*, $y = (-0.101 P_m) + 0.103$; *S. pinnatifolius*, $y = (0.739 P_m) + 0.13$; Queen Mary Falls: *S. madagascariensis*, $y = (-0.532 P_m) + 0.766$; *S. pinnatifolius*, $y = (0.894 P_m) + 0.053$.) Estimates were calculated independently for each sympatric site based on the actual levels of hybridization recorded in open pollinated progeny at that site for the fixed rate hybridization scenario. The principal simplifying assumptions of our estimates include the following: the flowering time in sympatric populations is similar to that in allopatric populations; rates of hybridization are frequency dependent; and all hybrids are not viable.

Results

Reciprocal crossing experiments

Achenes were successfully produced for both interspecific and intraspecific crosses, regardless of which species was the pollen or seed parent. Although the mean number of seeds produced from interspecific crosses was lower than that recorded for intraspecific crosses, differences in seed production were not statistically significant (Table 2). Similarly, no statistical difference in percentage seed germination (viability) was found

Table 2 Seed viability (% germination) and amount of seed produced (mean seed produced per capitulum) from intra- and interspecies reciprocal crosses between *Senecio pinnatifolius* and *Senecio madagascariensis*

Experimental crosses	Number of crosses (n)	Mean seeds produced per capitulum ($\pm$ SE)	% seed germination ($\pm$ SE)	Statistical significance
Seed produced				
Within species	10	46 ($\pm$ 9.09)		
Between species	13	26 ($\pm$ 7.19)		$P = 0.180$
Seed viability				
Within species	8		70.4 ($\pm$ 6.07)	
Between species	11		75.2 ($\pm$ 11.9)	$P = 0.502$

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between the seeds produced from interspecific and intraspecific crosses (Table 2).

Relative densities of plants and flowers

The native *S. pinnatifolius* was the dominant species at both sympatric sites, in terms of both plant and flower frequencies: it had more than 3-fold the plant frequency and approx. 19-fold the flower frequency of *S. madagascariensis* (Table 1). At both sites the rate of hybrid seed production by *S. pinnatifolius* was significantly higher than would be expected if it was occurring proportionally to the relative frequencies of *S. pinnatifolius* and *S. madagascariensis* flowers (O'Reillys': $\chi^2 = 5.43$, d.f. = 1, $P < 0.05$; Queen Mary Falls: $\chi^2 = 102.48$, d.f. = 1, $P < 0.01$). *Senecio madagascariensis* contributed only 5% of capitula in each of the sympatric populations, but approx. 15 and 8.5% of *S. pinnatifolius* progeny were identified as F1 hybrids at the Queen Mary Falls and O'Reillys' sites, respectively. By contrast, the rates of hybridization in *S. madagascariensis* seed were significantly lower than expected from floral frequency (O'Reillys': $\chi^2 = 57.76$, d.f. = 1, $P < 0.05$; Queen Mary Falls: $\chi^2 = 1375.14$, d.f. = 1, $P < 0.01$). *Senecio pinnatifolius* made up 95% of capitula at both sites, but only 10 and 75% of *S. madagascariensis* progeny were recognized as hybrids at the Queen Mary Falls and O'Reillys' sites, respectively.

Genetic diversity and population differentiation

The two AFLP primer pair combinations produced 176 fragments for the 718 individuals screened, of which 88% were polymorphic between the two species. The error rate of misscoring estimated from blind running of five individuals of each *Senecio* species six times for different extractions was 1.7 and 1.9% for the primer pairs 33–49 and 33–55, respectively. Mean (±SE) genetic diversity within *S. madagascariensis* and *S. pinnatifolius* populations was $H = 0.257$ (± 0.007) and $H = 0.277$ (± 0.014), respectively. Genetic diversity was similar between allopatric (0.283 ± 0.013) and sympatric (0.270 ± 0.015) populations for *S. pinnatifolius* (t-test $T_3 = 0.648$, $P > 0.5$). However, a significant difference in genetic diversity between allopatric (0.271 ± 0.003) and sympatric (0.239 ± 0.010) populations of *S. madagascariensis* was detected ($T_3 = 3.968$, $P = 0.02$).

Global F_{ST} analyses detected pronounced differentiation among populations of both species, with F_{ST} values of 0.271 ($P < 0.001$) for *S. madagascariensis* and 0.162 ($P < 0.001$) for *S. pinnatifolius*. The NJ phenograms (Fig. 1) illustrated that, for each species, sympatric populations were more similar genetically to each other than they were to allopatric populations (*S. pinnatifolius*: sympatric–allopatric comparisons $F_{ST} = 0.18$, $P < 0.001$; sympatric–sympatric comparisons $F_{ST} = 0.15$, $P < 0.001$; *S. madagascariensis*: sympatric–allopatric comparisons $F_{ST} = 0.30$, $P < 0.001$; sympatric–sympatric comparisons $F_{ST} = 0.23$, $P < 0.001$), although the pattern was more
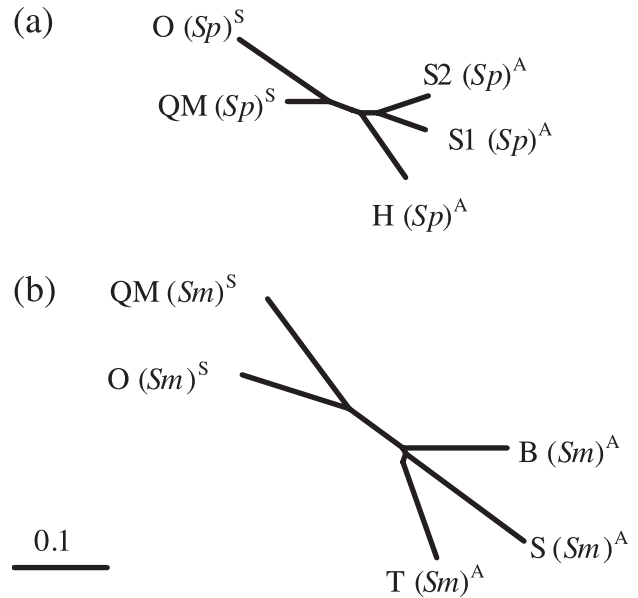

Fig. 1 Unrooted neighbour-joining phenogram based on pairwise F_{ST} (f-statistic) distances among amplified fragment length polymorphism (AFLP) profiles for (a) *Senecio pinnatifolius* (*Sp*) and (b) *Senecio madagascariensis* (*Sm*) in sympatric (S) and allopatric (A) sites; Hampton (H), Swanfels 1 (S1), Swanfels 2 (S2), Beechmont (B), Tamborine (T), Springbrook (S), Queen Mary Falls (QM) and O'Reillys' (O).
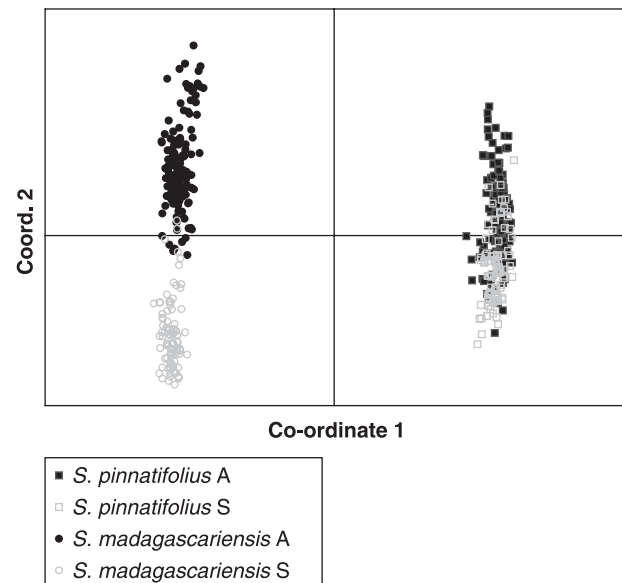
Fig. 2 Principal co-ordinates analysis depicting clustering of *Senecio pinnatifolius* and *Senecio madagascariensis* in sympatric (S) and allopatric (A) sites.

pronounced in *S. madagascariensis*. This pattern of clustering was also confirmed in the individual PCOA (Fig. 2), where the first two axes accounted for 81.1% of the total variation, with the species-differentiating axis 1 explaining > 73.7% of
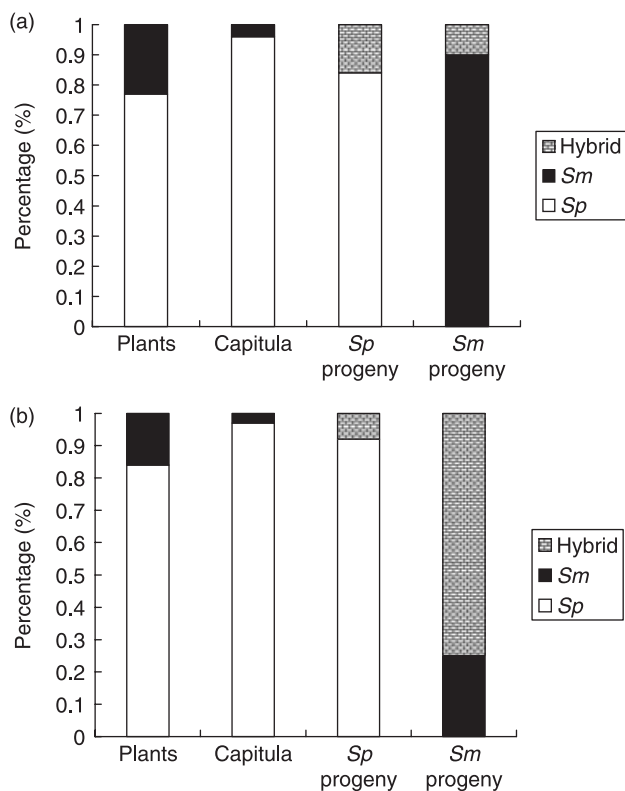
Fig. 3 Percentage of plants of each species, capitula produced by each species and hybrid and nonhybrid F1 progeny produced by *Senecio pinnatifolius* (*Sp*) and *Senecio madagascariensis* (*Sm*) plants in two sympatric populations: (a) Queen Mary Falls and (b) O'Reillys'.

the total variation. Separation of conspecific individuals from sympatric and allopatric populations of both species was unrelated to introgression, as individuals did not occur intermediate between the two species.

Principal co-ordinates analysis (Fig. 2) and assignment tests indicated a total absence of mature hybrids in the field. All mature individuals sampled from the two sympatric populations were assigned to either pure *S. pinnatifolius* or *S. madagascariensis*

groups, and not to simulated hybrid swarms between the two species. The probability of incorrectly assigning mature individuals was extremely low, as all allocated individuals had simulation P values of < 0.001.

The assignment method detected F1 hybrid progeny amongst seeds collected from *S. pinnatifolius* and *S. madagascariensis* plants in each of the sympatric sites. The proportion of hybrid progeny in the seeds differed quite markedly between the species at O'Reillys' (% F1 hybrids: *S. pinnatifolius*, 8.3%; *S. madagascariensis*, 75.6%; Fig. 3a), but was more similar at Queen Mary Falls (% F1 hybrids: *S. pinnatifolius*, 15.6%; *S. madagascariensis*, 10.2%; Fig. 3b).

Risk posed by hybridization

Estimates of the annual viable seed production (AVSP) were found to favour the native *S. pinnatifolius* under all hybridization scenarios (Table 3), except under linear frequency-dependent relationships, where the proportion of *S. madagascariensis* in sympatric populations strongly influenced the outcome (Fig. 4). The number of seeds expected to become viable adults of each species in a year estimated under no hybridization was greater for *S. pinnatifolius* (274) than for *S. madagascariensis* (252). *Senecio pinnatifolius* also produced a greater amount of viable seed than *S. madagascariensis*, when estimates were based on the fixed hybridization rates observed in this study, but this trend was stronger at O'Reillys' (259 and 124, respectively) than at Queen Mary Falls (244 and 235, respectively). Estimates of maximum possible hybridization also indicated that *S. pinnatifolius* (85) would produce more viable seeds than *S. madagascariensis* (81), but only by four seeds in a generation. Calculations based on linear frequency-dependent relationships produced estimates for seven different proportions of *S. madagascariensis* (5, 10, 25, 50, 75, 90 and 95%) in both sympatric sites (Fig. 4a,b). At the Queen Mary Falls and O'Reillys' sites, the proportion of *S. madagascariensis* in a mixed population needed to reach 10% and ~60%, respectively, for *S. madagascariensis* to produce more viable seeds than *S. pinnatifolius* in a generation.

Table 3 Annual viable seed production by *Senecio pinnatifolius* (*Sp*) and *Senecio madagascariensis* (*Sm*) in sympatric populations in Tableland variant habitat for a range of different hybridization scenarios

Species	Total seeds	Post germination	Post establishment	Maximum hybridization	Fixed rate		Linear	
					O	QM	O	QM
Sp	505	338	274	85	259	244	256–141	241–148
Sm	422	304	252	81	124	235	133–208	237–251

The values reported for density-dependent linear hybridization are the number of viable seeds produced in a year when the proportion of *S. madagascariensis* in sympatric populations is 0.05 and 0.95, respectively.
QM, Queen Mary Falls; O, O'Reillys'.
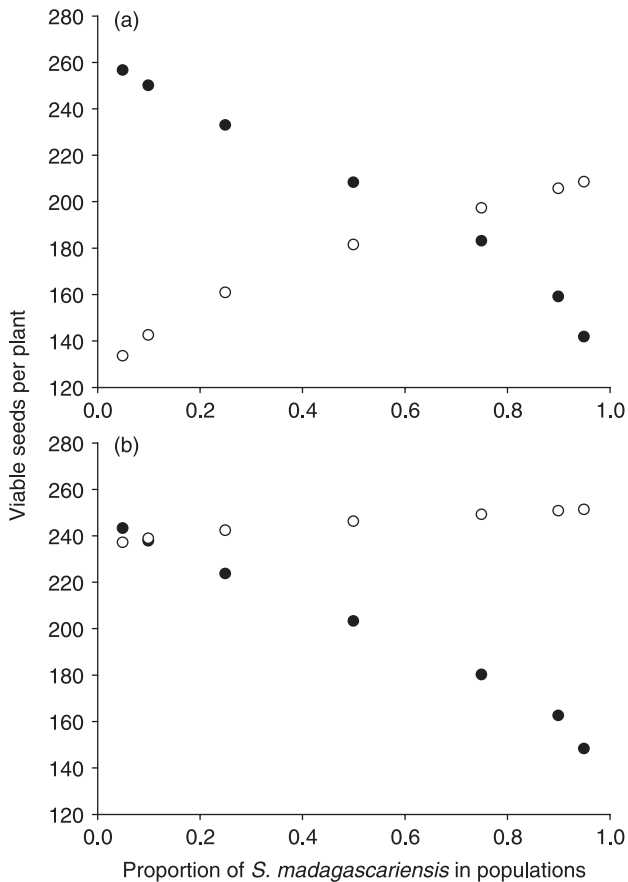
Fig. 4 Annual viable seed production of *Senecio pinnatifolius* (closed symbols) and *Senecio madagascariensis* (open symbols) in sympatric sites derived using linear density-dependent hybridization relationships (a) at O'Reillys' and (b) at Queen Mary Falls. Calculations based on linear density-dependent relationships produced estimates for seven different proportions of *S. madagascariensis* (5, 10, 25, 50, 75, 90 and 95%) in both sympatric sites.

Discussion

The incidence of hybridization and the fate of hybrids

Hybridization between *S. madagascariensis* and *S. pinnatifolius* occurs very frequently in the wild, with a large number of F1 hybrid seeds produced by both species in sympatric sites. Observed levels of hybridization in this study were in the same range as those reported previously between *S. madagascariensis* and *S. pinnatifolius* (Radford, 1997). In fact, the level of hybridization recorded in open pollinated seed is four orders of magnitude greater than that recorded in another well-characterized native–exotic *Senecio* species pair, *Senecio vulgaris* ($2n = 4x = 40$) and *Senecio squalidus* ($2n = 2x = 20$) (Marshall & Abbott, 1980). It is also an order of magnitude higher than that between *S. vulgaris* and the recent neo-species *Senecio eboracensis* ($2n = 4x = 40$). Lowe & Abbott (2004) suggest that the low frequency of hybridization between *S. eboracensis*

and *S. vulgaris* was influenced by niche separation, differences in flowering phenology and the greater attraction of *S. eboracensis* to pollinators. Given that habitat differentiation between *S. madagascariensis* and *S. pinnatifolius* is weak, that there is a substantial overlap in their flowering time, and that they are pollinated by the same insect species, the high level of hybridization observed here is not unexpected. The frequency of hybridization in our study suggests that prezygotic barriers are weak and do not prevent gene flow between the species.

Despite the high proportion of hybrid seeds collected from both species, mature hybrids were totally absent from sympatric populations sampled in this study. These results suggest that there is a very strong postzygotic reproductive barrier between the study species. Effects of interploidal hybridization on offspring fitness can be severe, often resulting in progeny that are highly sterile (Hardy *et al.*, 2001; Lowe & Abbott, 2004; Pannell *et al.*, 2004; Buggs & Pannell, 2006). However, sterility cannot be the only consequence of interploidal hybridization for the study species, as no hybrids, sterile or otherwise, developed to maturity in the sampled populations. A lack of mature hybrids indicates that the viability of interspecific hybrids must also be much reduced. Given that in this study the percentage germination of hybrid seeds was the same as that of nonhybrid seeds, the reduced viability of hybrids must occur after germination but before maturity. Further study is required to estimate the exact life history stage at which hybrids are selected against. Hybrids grown in pots were found to be of low vigour compared with either parental species (Radford, 1997), suggesting outbreeding depression, which may explain the absence of mature hybrids in the field.

Since the rapid spread of *S. madagascariensis*, hybrid zones between *S. pinnatifolius* and *S. madagascariensis* have formed in many areas of eastern Australia (Radford, 1997). An absence of mature F1 hybrids in sympatric populations indicates that contact zones formed between *S. pinnatifolius* and *S. madagascariensis* may represent tension zones. Theoretical tension zone models assume that hybrid fitness is independent of environment and intrinsically low as the result of genetic incompatibilities, but that low hybrid fitness is balanced by the continual dispersal of parent types into areas of contact (Barton & Hewitt, 1989). Tension zones may also be maintained by positive frequency-dependent selection (Buggs & Pannell, 2006). Areas of contact between diploid and tetraploid *Centaurea jacea* in Belgium (Hardy *et al.*, 2000, 2001), and diploid and hexaploid *Mercurialis annua* in northern Spain (Pannell *et al.*, 2004), appear to be other good examples of tension zones in mixed ploidy plant populations.

Long-term population impacts of hybridization – genetic diversity and differentiation

Overall levels of genetic diversity (H_E) were higher in the native *S. pinnatifolius* than in the exotic *S. madagascariensis*. Genetic diversity was significantly lower in allopatric compared with

sympatric sites for *S. madagascariensis*, but no significant difference was detected for *S. pinnatifolius*. The level of differentiation among populations within species was pronounced (*S. madagascariensis*, F_{ST} = 0.271; *S. pinnatifolius*, F_{ST} = 0.162). Although there were no immediately obvious impacts of hybridization on differentiation, populations in areas of sympatry showed increased differentiation from conspecific allopatric populations and this pattern was more pronounced in *S. madagascariensis*.

The pattern of increased differentiation between allopatric and sympatric populations appears to be unrelated to introgression, as the PCOA axis of differentiation was perpendicular to the axis differentiating the two species. A loss of alleles in nonviable hybrids of early-flowering *S. madagascariensis* or late-flowering *S. pinnatifolius* genotypes might change allele frequencies in sympatric populations and may be responsible for the observed pattern of differentiation. Similarly, a loss of alleles in nonviable hybrids may also explain lower genetic diversity of *S. madagascariensis* at sympatric sites; however, further work is warranted on this topic.

What does the future hold for *S. pinnatifolius*?

In areas of contact between *S. pinnatifolius* and *S. madagascariensis*, calculations demonstrated that *S. pinnatifolius* was not at risk from demographic swamping when no hybridization occurred or when levels of hybridization were constant and not affected by the proportion of *S. madagascariensis*. However, *S. madagascariensis* displayed a hybridization advantage at both surveyed field sites, where it sired significantly more progeny than expected based on capitulum frequencies, and *S. pinnatifolius* significantly less. Thus hybridization between the species is asymmetric, a phenomenon commonly reported in hybrid zones (Rieseberg & Wendel, 1993; Arnold, 1997; Burgess *et al.*, 2005). Estimates based on frequency-dependent asymmetric hybridization between the species indicate that the proportion of *S. madagascariensis* need only reach between 10 and 60% to produce more viable seeds than *S. pinnatifolius* in sympatry (Fig. 4). Under these circumstances, an invasive species does not necessarily have to outnumber a native to have an impact on the demography of an interfertile native through hybridization. In fact, invasive species may be rare relative to a native plant, but may nevertheless pose a threat to the native because of superior male fitness (e.g. production of a greater number of pollen grains), resulting in the invader siring a disproportionately higher proportion of progeny (Anttila *et al.*, 1998). As a result, asymmetric hybridization in favour of an invasive species can contribute to the decline and extinction of native species (Wolf *et al.*, 2001). Thus, if *S. madagascariensis* increases numerically in areas of contact, it may cause the decline of *S. pinnatifolius* from east coast areas of Australia.

Three factors may impede the decline of *S. pinnatifolius*. First, *S. madagascariensis* may be driven to local extinction in areas of contact during colonization, if it cannot establish within a few generations. Given that *S. madagascariensis* can reproduce in the absence of *S. pinnatifolius* for 6 months annually and the O'Reillys' contact zone has existed for between 14 and 25 generations (first recorded by Scott, 1994), this outcome is unlikely. Secondly, natural selection against maladaptive hybridization may lead to reproductive character displacement (e.g. flowering time divergence) and 'avoidance' of the negative consequences associated with interspecific fertilizations. Reinforcing natural selection is most likely when contact zones are extensive, exposing a high proportion of individuals to selection (Pannell *et al.*, 2004; Hoskin *et al.*, 2005). As *S. pinnatifolius* and *S. madagascariensis* form extensive contact zones, reinforcement may act to impede displacement of *S. pinnatifolius*. Thirdly, *S. pinnatifolius* variants may have physiological and morphological adaptations to specific environments, which allow variants to out-perform *S. madagascariensis* in their native habitat (Radford & Cousens, 2000).

The destructive force of interspecific hybridization is not uncommon in hybridizing plant species (Wolf *et al.*, 2001; Buggs & Pannell, 2006). However, adequate molecular data from open pollinated progeny and/or mature individuals are often lacking, meaning that the actual level of hybridization and its impact on native or rare species are underestimated. Without this information, conservation strategies for the protection of hybridizing species cannot be effective. In combination with ecological approaches, we encourage the use of molecular data to provide a baseline for comprehensive long-term studies of the consequences of hybridization for native species.

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Supplementary Material

The following supplementary material is available for this article online:

Fig. S1 Bar graph depicting the monthly proportion of annual capitulum production (%) in *Senecio pinnatifolius* and *Senecio madagascariensis* based on Radford (1997).

Table S1 Values for demographic variables used to parametrize the simulation study and the source of reference of this data.

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