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


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Summary

- The outcome of interspecific hybridization between native and invasive species depends on the relative frequencies of parental taxa and viability of hybrid progeny.

- We investigated individual and population level consequences of hybridization between the Australian native, Senecio pinnatifolius, and the exotic S. madagascariensis, with AFLP markers and used this information to simulate the expected outcome of 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 indicating that sympatric populations represent tension zones. 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. Simulations indicated S. pinnatifolius could be replaced in sympatric populations if hybridization was density dependent.

- For this native-exotic pair, prezygotic isolating barriers are weak, but low hybrid viability maintains a strong postzygotic barrier to introgression. Due to asymmetric hybridization, S. pinnatifolius appears under threat from demographic swamping, and local extinction is possible where it occurs in sympatry with S. madagascariensis.
Key words: Amplified fragment length polymorphism (AFLP), demographic swamping, hybridization, hybrid viability, invasive species, *Senecio*, triploid.
Introduction

The importance of hybridization in the evolution and speciation of plants has long been recognised (Rieseberg et al., 1995; Arnold, 1997; Rieseberg et al., 2003; Abbott & Lowe, 2004; Hegarty & Hiscock, 2005; Buggs & Pannell, 2006). Natural hybridization can result when divergent lineages, or species formed in allopatri, change ranges and come into reproductive contact, potentially forming a secondary contact zone (Anderson, 1949; Lagercrantz & Ryman, 1990; Cruzan, 2005; Hoskin et al., 2005). However, hybridization can also occur following biological invasions, if introduced species are sufficiently closely related to native species. As global trade and passenger travel continues 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 (Morrell et al., 2005; Whitney et al., 2006), evolution of new hybrid lineages (Lowe & Abbott, 2004) and decline or even extinction of hybridizing species (Wolf et al., 2001). The latter 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 semi-fertile hybrids, may produce
hybrid derivatives of superior fitness that displace one or both pure conspecifics causing decline through genetic assimilation. Secondly, if hybrids are sterile or display reduced fitness, the population growth rate of the hybridising taxa may decrease below that required for replacement of one or both parental species, resulting in demographic swamping.

The potential for introgression is regulated 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). This can be particularly true 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 hybridising 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 distinguish between processes.

*Senecio*, one of the largest genera of flowering plants, is known worldwide 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 more than 80 years ago and is now an aggressive weed in its invasive range (Radford, 1997). In Australia, molecular genetic and morphological studies have demonstrated a close affinity between fireweed and Australian native *Senecio* species, including *S.*
*pinnatifolius* (formerly *S. lautos*) (Scott et al. 1998). Although *S. madagascariensis* (2n = 2x = 20) and *S. pinnatifolius* (2n = 4x = 40) differ in ploidy, empirical and experimental crossing studies have established that both species can serve as paternal and maternal parents of synthetic hybrids (Radford, 1997). Under greenhouse conditions, synthetic triploid hybrids between the two species exhibit low viability and low fertility (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 the low fertility of *S. madagascariensis* x *S. pinnatifolius* F1 triploid hybrids, they 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 (White, 2007). Hybrid formation has also been observed in sympatric populations of the two species in the field (Radford, 1997), but may be restricted to certain variants of *S. pinnatifolius*, such as tableland, headland and dune.

To examine in greater detail the outcome of hybridization between the native *S. pinnatifolius* and the invasive *S. madagascariensis*, comparisons were made at population (sympatric vs allopatric populations) and individual (sympatric populations) levels to investigate contemporary and long-term outcomes of hybridization. The molecular marker used to address these questions was amplified
fragment length polymorphisms (AFLP). Four primary questions are addressed in this
paper: (i) what is the viability of hybrids in the field? - Comprising a comparison of
frequencies of hybrids in open pollinated seed of both species with the occurrence of
adult stage hybrids in sympatric populations. (ii) Do hybrids or stabilised
introgressents persist in the field and to what hybrid class do they belong? (iii) Does
hybridization influence the level of genetic diversity or differentiation within
sympatric compared to allopatric populations of these hybridising species? (iv) Can
we estimate the likely outcome of hybridization between this native-invasive 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

*Seneecio madagascariensis* (fireweed), a diploid annual weed from South Africa, was first recorded in Australia in 1918 and has since invaded large areas of farmland and grassland in south-eastern Australia (Radford *et al.*, 1995; Radford, 1997). Closely related to *S. madagascariensis*, is a group of sub-species belonging to the Australian native *S. pinnatifolius* complex (formerly known as the *S. lautus* complex prior to Radford *et al.*, 2004). *S. pinnatifolius* is an herbaceous perennial tetraploid (Ornduff, 1964; Ali, 1966; Radford *et al.*, 2004), and exhibits 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 (also 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 Fig.1). There is a four month period of overlap between the flowering periods of the native and exotic *Senecio*, 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 approximately 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 approximately 45 plants (minimum 43, maximum 47) across the morphological range exhibited by mature flowering plants of each species from two sympatric sites. 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 (ie. plants with intermediate morphology) were observed in the field. In addition, ~20 seeds per plant were collected from a subset of 10 plants of each species from which leaf material was 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 ASL), 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 five 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 of the populations.

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 approximately 20 mm in height, at which point they were removed, frozen and stored at –80 °C. Germination percentages for *S. pinnatifolius* and *S. madagascariensis* in both sympatric sites were generally quite low; particularly for *S. madagascariensis* (< 35 % at both sites), and numbers of resulting progeny for each species for each site are shown in Table 1.

**Relative densities of plants and flowers**

Relative plant and capitula densities of each species at each site were determined using the using the Point Centred Quarter (PCQ) method (Krebs, 1989), using 30 random plants of each species at each site as ‘centre points’. A Chi-square test was used to determine whether the proportion of hybrids sired in the progeny of each species was concordant with capitula 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× TE buffer to obtain concentrations between 100 and 200 ng/µL.

AFLP restriction/ligation was performed following the protocol of Prentis et al. (2004). AFLP 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 EcoRI primer was Hex labeled (Geneworks). The fluorescently labeled amplified products were analysed by gel electrophoresis (5% acrylamide gels), using a Gelscan GS2000 (Corbet Research) with a TAMRA 500 size standard (Applied Biosystems). To confirm reproducibility, five adult samples of each Senecio species were run blindly six times from different extractions for both primer combinations and ambiguous loci were not scored. This information was also used to produce an error rate of fragment mis-scoring 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 base pairs in size.

Data analysis – population level
Genetic diversity within each population was quantified by calculating expected heterozygosity ($H_E$) using a Bayesian method with nonuniform prior distribution of allele frequencies (Zhivotovsky, 1999) in the program AFLPSURV (Vekemans et al., 2002). Estimates of average null allele frequencies and expected heterozygosity from dominant markers have been shown to be accurate using this method for outcrossing angiosperm species (Krauss, 2000), which is the case for both Senecio species in this study (Radford, 1997). A t-test was used to compare whether levels of genetic diversity were similar in sympatric and allopatric populations of both species.

Global $F_{ST}$ and pairwise $F_{ST}$, used to characterize the extent of population differentiation among all population pairs, were estimated in AFLPSURV. PHYLIP (Felsenstein, 2005) was used to construct a neighbour-joining (NJ) phenogram in TREEVIEW (Page, 1996) from the pairwise $F_{ST}$ matrix.

**Data analysis – individual level**

Principal coordinates 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 utilises 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 
\( \log(\epsilon) \), where \( \epsilon \) was chosen as 0.001. Individuals are assigned to species or hybrid 
populations displaying the highest log-likelihood for \( G \); however allocation of 
genotypes were only made if minimal log-likelihood difference (MLD) was \( \geq 1 \) for 
mature individuals. This means 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 
allocation 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 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 between 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. Seed collections 
from sympatric sites were also allocated to their species of origin or simulated 
interspecific hybrid status using the same assignment method as above.
Risk posed by demographic swamping

Using a simulation approach, we calculated annual viable seed production of both species in sympatric sites under a number of hybridization scenarios. The scenarios used 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), linear density dependent hybridization and threshold density dependent hybridization. Simulations were parameterized using information from Radford, (1997), Radford & Cousens, (2000) and the current study. Parameters used in the model were monthly capitula production for both species in allopatric sites (see fig. 1) to estimate the proportion of total yearly capitula produced during synchronous (S) and non-synchronous (N) flowering, annual seed production (A), percentage germination under field conditions (G), percentage establishment of both species in \textit{S. pinnatifolius} var. tableland habitat (E), and hybridization rate (H) (see supplementary Table. 1 for values). The hybridization rate (H) was calculated for each month, based on flowering synchrony data from field observations (Radford & Cousens, 2000, supplementary Fig. 1), the proportion of \textit{S. madagascariensis} (Pm) in a population is used to estimate the proportion of hybrid seed produced separately for both \textit{S. madagascariensis} and \textit{S. pinnatifolius} using the density dependent relationships outlined in the linear and power (threshold) relationships. Linear density dependent relationships were fitted based on the assumption that $H = (1 – \text{observed } H)$ at $Pm = (1 – \text{observed } Pm)$, for
each site and species independently (Equations for lines of best fit, O’Reilly’s’: S. madagascariensis $y = (-0.101(Pm)) + 0.103$, S. pinnatifolius $y = (0.739(Pm)) + 0.13$; Queen Mary Falls: S. madagascariensis $y = (-0.532(Pm)) + 0.766$, S. pinnatifolius $y = (0.894(Pm)) + 0.053$). Threshold density dependent relationships were fitted using the same principle but with hybridization data from both sites combined for both species (see supplementary Fig. 2). Annual viable seed production (AVSP) was then calculated for both species using the following equation; $AVSP = ((Pr S x A) x (1- H) x G x E) + ((Pr N x A) x G x E)$. Simulations were run 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 model include (1) flowering time in sympatric populations is similar to allopatric populations (2) rates of hybridization are density dependent and (3) all hybrids are not viable.
Results

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 mis-scoring estimated from blind running of five individuals of each Senecio species six times from different extractions was 1.7% and 1.9% for the primer pairs 33-49 and 33-55, respectively. Mean genetic diversity within *S. madagascariensis* and *S. pinnatifolius* populations was $H_E = 0.212 \pm 0.017$ and $H_E = 0.223 \pm 0.014$, respectively. Genetic diversity was similar between allopatric (0.229 ± 0.008) and sympatric (0.220 ± 0.010) populations for *S. pinnatifolius* ($T_3 = 0.697; P > 0.5$). Similarly, no statistical difference for genetic diversity between allopatric (0.223 ± 0.002) and sympatric (0.194 ± 0.008) populations of *S. madagascariensis* was detected (equal variances not assumed; $T_{1.125} = 3.323; P > 0.1$), although a trend toward lower genetic diversity in sympatric populations was observed for both species.

A global $F_{ST}$ analysis (0.493, $P < 0.001$) detected very pronounced differentiation between the species (average pairwise $F_{ST} = 0.607$, see Table 2 for pairwise $F_{ST}$ comparisons), and was much larger than for average within species comparisons (average pairwise $F_{ST} = 0.218$). Global $F_{ST}$ values of 0.270 ($P < 0.001$) were calculated for *S. madagascariensis*, and 0.161 ($P < 0.001$) for *S. pinnatifolius*. The NJ phenogram illustrated that *S. pinnatifolius* and *S. madagascariensis*
populations formed two distinct and strongly differentiated clusters, whether from allopatic or sympatric populations (Fig. 1). Within each of the species clusters, sympatric populations were more similar genetically to each other than they were to allopatic populations (S. pinnatifolius: sympatric – allopatic comparisons $F_{ST} = 0.18$, $P < 0.001$, sympatric – sympatric comparisons $F_{ST} = 0.15$, $P < 0.001$; S. madagascariensis: sympatric – allopatic comparisons $F_{ST} = 0.30$, $P < 0.001$, sympatric – sympatric comparisons $F_{ST} = 0.23$, $P < 0.001$), although the pattern was more pronounced in S. madagascariensis. This pattern of clustering was also confirmed in the individual PCOA (Figure 2), where the first two axes accounted for 81.1% of the total variation, with the species differentiating axis 1 explaining greater than 73.7% of the total variation. Separation of individuals from sympatric and allopatic populations of both species was parallel to PCOA axis 2 and not the species differentiating axis (1).

Principal coordinates analysis (Figure 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, since all allocated individuals had simulation $P$ values of < 0.001.

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

Relative densities of plants and flowers

The native *S. pinnatifolius* was the dominant species at both sympatric sites, both in terms of plant and flower density: it had more than three-fold the plant density and approximately 19-fold the flower density 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 densities of *S. pinnatifolius* and *S. madagascariensis* flowers (O’Reilly’s: $\chi^2 = 5.43$, df = 1, $p < 0.05$; Queen Mary Falls: $\chi^2 = 102.48$, df = 1, $p < 0.01$). *Senecio madagascariensis* contributed only five percent of capitula in each of the sympatric populations, but approximately 15 and 8.5 % of *S. pinnatifolius* progeny were identified as F1 hybrids at Queen Mary Falls and O’Reillys’ sites respectively. In contrast, the rates of hybridization in *S. madagascariensis* seed were significantly lower than expected from floral density (O’Reillys’: $\chi^2 = 57.76$, df = 1, $p < 0.05$; Queen Mary Falls: $\chi^2 = 1375.14$, df = 1, $p < 0.01$). Where *S. pinnatifolius* makes up 95 % of capitula at both sites, but only 10 and 75 % of *S. madagascariensis* progeny were recognized as hybrids at Queen Mary Falls and O’Reillys’ sites respectively.

Risk posed by demographic swamping
Distinctly different outcomes of AVSP were observed under the five hybridization simulation scenarios. Simulations indicated that with no hybridization, *S. pinnatifolius* would produce far greater viable seed than *S. madagascariensis* (271:182; Table 3), but that this advantage in viable seed production was substantially decreased at maximum hybridization (84:58; Table 3). If fixed rate hybridization was assumed (i.e. independent of floral density), AVSP was still substantially greater in *S. pinnatifolius* than *S. madagascariensis* at both QM Falls (241:170; Table 4) and O’Reillys’ (256:89; Table 3). Simulations based on density dependent hybridization demonstrated that the proportion of *S. madagascariensis* in sympatric populations needed to reach > 70 -80 % for linear density dependent hybridization and > 25 % for threshold density dependent hybridization to produce more viable seed than *S. pinnatifolius* (Fig. 4 a, b & c).
Discussion

The incidence of hybridization and fate of hybrids

Hybridization between *S. madagascariensis* and *S. pinnatifolius* occurs very frequently in the wild (see also; Radford, 1997), with a large number of F1 hybrid seed produced by both species in sympatric sites. In fact, the level of hybridization recorded in open pollinated seed is two orders of magnitude greater than that recorded between another well characterized native-exotic *Senecio* species pair, *S. vulgaris* (2n = 4x = 40) and *S. squalidus* (2n = 2x = 20) (Marshall & Abbott, 1980). It is also an order of magnitude higher than between *S. vulgaris* and the recent neo-species *S. 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 *S. eboracensis* being more attractive to pollinators than *S. vulgaris*. Given that habitat differentiation between *S. madagascariensis* and *S. pinnatifolius* is weak, 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 act as a barrier to gene flow between the species.

Despite the high proportion of hybrid seed 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 is not the only consequence of interploidal hybridization in this study, since no hybrids, sterile or otherwise, developed to maturity in sampled populations. A lack of mature hybrids indicates that the fitness of interspecific hybrids must also be much reduced, but further study is required to estimate at which life history stage hybrids are selected against in sympatric populations.

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). Documented zero or near zero fitness of F1 hybrids in sympatric populations indicates that contact zones formed between *S. pinnatifolius* and *S. madagascariensis* represent tension zones. Theoretical models predict that tension zones can be formed and maintained by a balance between dispersal of parent types into areas of contact and subsequent selection against hybrid progeny (Barton & Hewitt, 1989), although 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 the level of differentiation between the species was very high (0.61) and much higher than among populations within species (0.218). Although there were no immediately obvious impacts of hybridization, populations in areas of sympatry showed increased differentiation from conspecific allopatric populations and this pattern was more pronounced in *S. madagascariensis*. Although genetic diversity was not significantly different between allopatric and sympatric sites for either species, there was a trend toward lower genetic diversity in sympatric sites for both species.

The pattern of increased differentiation between allopatric and sympatric populations appears unrelated to introgression, since the PCOA axis of differentiation was perpendicular to the axis differentiating the two species. Erosion of alleles in 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. An erosion of alleles may also explain a trend toward lower genetic diversity 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* in tableland-variant habitat, simulations demonstrated *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* displays a hybridization advantage at both surveyed field sites, where it sires significantly more progeny than expected from floral densities, 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). Simulations based on density-dependent asymmetric hybridization between the species, indicate that the proportion of *S. madagascariensis* need only reach 25% to cause the demographic decline of *S. pinnatifolius* in sympatry (fig. 4c). 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 due to 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). Hence, if *S. madagascariensis* increases numerically in areas of contact, it may cause the extinction of *S. pinnatifolius* from east coast areas of Australia. Given that *S. madagascariensis* is least fit in *S. pinnatifolius* var. tableland habitat, the extinction of *S. pinnatifolius* throughout the range of other habitats it occupies is even more probable and is the likely reason for the rapid historical spread of *S.madagascariensis* into *S.pinnatifolius* habitat (R.McFadyen, pers. comm.). In
addition, pollen and seed dispersal by *S. madagascariensis* into hybrid zones from neighbouring pure populations may further advantage the invasive over the native. However, two factors may impede the displacement of *S. pinnatifolius*. First, *S. madagascariensis* may be driven to local extinction in areas of contact during colonisation, if it cannot establish within a few generations. Given that the O’Reillys’ contact zone has existed for between 20-35 generations however, it would seem that this outcome is unlikely as *S. madagascariensis* can reproduce in the absence of *S. pinnatifolius* for approximately 6 months annually and is likely to continually disperse back into areas of contact after local extinctions. Second, natural selection against maladaptive hybridization may lead to reproductive character displacement (eg. flowering time divergence) and “avoidance” of the negative consequences associated with interspecific fertilizations. Although the conditions under which reinforcing natural selection may occur are limited (Pannell *et al.*, 2004; Hoskin *et al.*, 2005), it is most likely to occur when contact zones are extensive, exposing a high proportion of individuals to selection. As *S. pinnatifolius* and *S. madagascariensis* form extensive contact zones in Eastern Australia, reinforcement may impede the displacement of *S. pinnatifolius*, but this scenario needs to be investigated theoretically and empirically to be supported.

The destructive force of interspecific hybridization is not uncommon in hybridizing plant species (Wolf *et al.*, 2001; Buggs & Pannell, 2006). Adequate molecular data, however, in open pollinated progeny and mature individuals from sympatric populations are often lacking, meaning the actual level of hybridization and
the impact on native or rare species may be underestimated. Without this information,

conservation strategies for the protection of hybridizing species may not be effective.

This data, in combination with ecological approaches, will provide the baseline for

comprehensive long-term studies into the consequences of hybridization on native

species.
Acknowledgements

Thanks to landholders Phil Curtis and the O’Reilly family for giving us permission to work on their land and Mike Duffy for assistance with fieldwork. We also gratefully acknowledge suggestions and comments made on previous versions of the manuscript by Rachel McFadyen (CEO Weed CRC) and Andy Shepherd (CSIRO).
References


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Morrell PL, Williams-Coplin TD, Lattu AL, Bowers JE, Chandler JM, Paterson AH. 2005. Crop-to-weed introgression has impacted allelic composition of


Figure legends

Supplementary Fig. 1. Bar graph depicting the monthly proportion of annual capitula production (%) in *Senecio pinnatifolius* and *Senecio madagascariensis* based on Radford, 1997.

Supplementary Fig. 2. Graphs depicting the rate of hybridisation (threshold) as it varies with the proportion of *Senecio madagascariensis* in populations for a) *S. madagascariensis* and b) *Senecio pinnatifolius*.

Fig. 1 Unrooted neighbour-joining phenogram based on pairwise $F_{ST}$ distances among AFLP profiles for *Senecio pinnatifolius* (Sp) and *Senecio madagascariensis* (Sm) in sympatric (S) and allopatric (A) sites, i.e. 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 coordinates analysis depicting clustering of *Senecio pinnatifolius* and *Senecio madagascariensis* in sympatric and allopatric sites.

Fig. 3. Percentage of plants of each species, capitula produced by each species and hybrid and non-hybrid F1 progeny produced by *Senecio pinnatifolius* (Sp) and *Senecio madagascariensis* (Sm) plants in two sympatric populations; a) Queen Mary Falls and b) O’Reillys’.
Fig. 4. Graph depicting annual viable seed production of *Senecio pinnatifolius* (closed symbols) and *Senecio madagascariensis* (open symbols) in sympatric sites derived under the following simulations a) linear density dependent hybridization at O’Reilly’s b) linear density dependent hybridization at Queen Mary Falls, and c) threshold density dependent hybridization
Table 1: Population locations and relative densities of plants and flowers of native *Senecio pinnatifolius* (*Sp*) and exotic *Senecio madagascariensis* (*Sm*)

<table>
<thead>
<tr>
<th>Population</th>
<th>Location</th>
<th>Relative densities (<em>Sp:Sm</em>)</th>
<th>Sample sizes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plants</td>
<td>Capitula</td>
</tr>
<tr>
<td>Hampton</td>
<td>East of Hampton, Northern Darling Downs (27° 22’S, 152°10’E)</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Swanfels 1</td>
<td>North of Killarney, Southern Darling Downs (28° 07’S, 152°23’E)</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Swanfels 2</td>
<td>North of Killarney, Southern Darling Downs (28° 08’S, 152°23’E)</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Allopatric</td>
<td></td>
<td>100 : 0</td>
<td>100 : 0</td>
</tr>
<tr>
<td>Beechmont</td>
<td>Near Beechmont, Gold Coast Hinterland (28° 07’S, 153° 10’E)</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Tamborine</td>
<td>Mt Tamborine, Gold Coast Hinterland (27° 58’S, 153°12’E)</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Springbrook</td>
<td>Springbrook Plateau, Gold Coast Hinterland (28° 11’S, 153°16’E)</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Allopatric</td>
<td></td>
<td>0 : 100</td>
<td>0 : 100</td>
</tr>
<tr>
<td>Queen Mary</td>
<td>Near Queen Mary Falls section of Main Range National Park, Southern</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Falls</td>
<td>Darling Downs (28° 20’S, 152°21’E)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O’Reillys’</td>
<td>Near Lamington National Park, Gold Coast Hinterland (28°13’S, 153°07’E)</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Sympatric</td>
<td></td>
<td>0.77 : 0.23</td>
<td>0.96 : 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Table 2. Pairwise $F_{ST}$ estimates for both *Senecio pinnatifolius* (Sp) and *Senecio madagascariensis* (Sm) in sympatric (S) and allopatric (A) populations, abbreviations as follows; Hampton (H), Swanfels 1 (S1), Swanfels 2 (S2), Beechmont (B), Tamborine (T), Springbrook (S), Queen Mary Falls (QM) and O’Reillys’ (O). *** represents significance at the $P < 0.001$ level.

<table>
<thead>
<tr>
<th></th>
<th>Sp$^{AH}$</th>
<th>Sp$^{AS1}$</th>
<th>Sp$^{AS2}$</th>
<th>Sp$^{SQMF}$</th>
<th>Sp$^{SO}$</th>
<th>Sm$^{AB}$</th>
<th>Sm$^{AT}$</th>
<th>Sm$^{AS}$</th>
<th>Sm$^{SQMF}$</th>
<th>Sm$^{SO}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sp$^{AH}$</td>
<td>-</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Sp$^{AS1}$</td>
<td>0.130</td>
<td>-</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Sp$^{AS2}$</td>
<td>0.163</td>
<td>0.110</td>
<td>-</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Sp$^{SQMF}$</td>
<td>0.149</td>
<td>0.118</td>
<td>0.155</td>
<td>-</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Sp$^{SO}$</td>
<td>0.228</td>
<td>0.212</td>
<td>0.190</td>
<td>0.151</td>
<td>-</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Sm$^{AB}$</td>
<td>0.573</td>
<td>0.594</td>
<td>0.577</td>
<td>0.581</td>
<td>0.597</td>
<td>-</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Sm$^{AT}$</td>
<td>0.601</td>
<td>0.614</td>
<td>0.588</td>
<td>0.607</td>
<td>0.618</td>
<td>0.238</td>
<td>-</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Sm$^{AS}$</td>
<td>0.592</td>
<td>0.596</td>
<td>0.567</td>
<td>0.593</td>
<td>0.607</td>
<td>0.207</td>
<td>0.241</td>
<td>-</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Sm$^{SQMF}$</td>
<td>0.621</td>
<td>0.631</td>
<td>0.603</td>
<td>0.614</td>
<td>0.628</td>
<td>0.297</td>
<td>0.294</td>
<td>0.274</td>
<td>-</td>
<td>***</td>
</tr>
<tr>
<td>Sm$^{SO}$</td>
<td>0.639</td>
<td>0.645</td>
<td>0.608</td>
<td>0.628</td>
<td>0.637</td>
<td>0.310</td>
<td>0.334</td>
<td>0.269</td>
<td>0.237</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 3. Annual viable seed production produced by *Senecio pinnatifolius* (*Sp*) and *Senecio madagascariensis* (*Sm*) in sympatric populations in tableland variant habitat for a range of different hybridization scenarios, abbreviations as follows; Queen Mary Falls (QM) and O’Reilly’s (O).

The values reported for linear and threshold hybridization scenarios are the range of viable seed produced when the proportion of *S. madagascariensis* in sympatric populations is 0.05 and 0.95 respectively.

<table>
<thead>
<tr>
<th>Species</th>
<th>Total seed</th>
<th>Post germination</th>
<th>Post establishment</th>
<th>Maximum hybridization</th>
<th>Fixed rate (O)</th>
<th>Fixed rate (QM)</th>
<th>Linear (O)</th>
<th>Linear (QM)</th>
<th>Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. p</em></td>
<td>505</td>
<td>338</td>
<td>271</td>
<td>84</td>
<td>256</td>
<td>241</td>
<td>253-140</td>
<td>240-146</td>
<td>215-90</td>
</tr>
<tr>
<td><em>S. m</em></td>
<td>422</td>
<td>304</td>
<td>182</td>
<td>58</td>
<td>89</td>
<td>176</td>
<td>96-151</td>
<td>171-182</td>
<td>166-181</td>
</tr>
</tbody>
</table>
Supplementary Table 1. Values for demographic variables used to parameterize the simulation study and the source of reference of this data

<table>
<thead>
<tr>
<th>Demographic variables</th>
<th>S. pinnatifolius</th>
<th>S. madagascariensis</th>
<th>Source of reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual seed production (A)</td>
<td>505</td>
<td>442</td>
<td>Radford &amp; Cousens, (2000)</td>
</tr>
<tr>
<td>Germination under field conditions (G)</td>
<td>0.67</td>
<td>0.72</td>
<td>Radford &amp; Cousens, (2000)</td>
</tr>
<tr>
<td>Establishment in <em>Senecio pinnatifolius</em> habitat (E)</td>
<td>0.80</td>
<td>0.60</td>
<td>Radford &amp; Cousens, (2000)</td>
</tr>
<tr>
<td>Hybridization rate (H)</td>
<td>Variable</td>
<td>Variable</td>
<td>This study; Radford, 1997</td>
</tr>
<tr>
<td>Synchronous flowering (S)</td>
<td>0.69</td>
<td>0.68</td>
<td>Radford, 1997</td>
</tr>
<tr>
<td>Non-synchronous flowering (N)</td>
<td>0.31</td>
<td>0.32</td>
<td>Radford, 1997</td>
</tr>
</tbody>
</table>
y = \((0.0139P_m)^{-0.8652}\)

Proportion of *S. madagascariensis* in populations

1 - Proportion of hybrids in *S. pinnatifolius*

S2 (a)

y = \((0.0237P_m)^{-1.0746}\)

Proportion of *S. madagascariensis* in populations

Proportion hybrids in *S. madagascariensis*
Proportion of *S. madagascariensis* in populations

Viable seed/plant (n)
4(b)

![Graph showing the proportion of S. madagascariensis in populations against viable seed/plant counts. The graph compares Sm and Sp populations.]