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Prentis, Peter, White, Evelyn, Radford, Ian J., Lowe, Andrew J., & Clarke, Anthony R. (2007) *Can hybridization cause local extinction : the case for demographic swamping of the Australian native, *Senecio pinnatifolius*, by the invasive, *S. madagascariensis*?* New Phytologist, 176(4), pp. 902-912.

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

3

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19 Total word count 6467, Summary 176, Introduction 872, Materials and Methods,
20 1834, Results 878, Discussion 1274 (19.7 %), References 1349

21 3 tables, 4 figures, 1 supplementary table, 2 supplementary figures

1 **Summary**

- 2 • The outcome of interspecific hybridization between native and invasive
3 species depends on the relative frequencies of parental taxa and viability of
4 hybrid progeny.
- 5 • We investigated individual and population level consequences of hybridization
6 between the Australian native, *Senecio pinnatifolius*, and the exotic *S.*
7 *madagascariensis*, with AFLP markers and used this information to simulate
8 the expected outcome of hybridization.
- 9 • A high frequency (range 8.3-75.6 %) of hybrids was detected in open
10 pollinated seeds of both species, but mature hybrids were absent from
11 sympatric populations indicating that sympatric populations represent tension
12 zones. A hybridization advantage was observed for *S. madagascariensis*,
13 where significantly more progeny than expected were sired based on
14 proportional representation of the two species in sympatric populations.
15 Simulations indicated *S. pinnatifolius* could be replaced in sympatric
16 populations if hybridization was density dependent.
- 17 • For this native-exotic pair, prezygotic isolating barriers are weak, but low
18 hybrid viability maintains a strong postzygotic barrier to introgression. Due to
19 asymmetric hybridization, *S. pinnatifolius* appears under threat from
20 demographic swamping, and local extinction is possible where it occurs in
21 sympatry with *S. madagascariensis*.

22

- 1 **Key words:** Amplified fragment length polymorphism (AFLP), demographic
- 2 swamping, hybridization, hybrid viability, invasive species, *Senecio*, triploid.

1 **Introduction**

2 The importance of hybridization in the evolution and speciation of plants has
3 long been recognised (Rieseberg *et al.*, 1995; Arnold, 1997; Rieseberg *et al.*, 2003;
4 Abbott & Lowe, 2004; Hegarty & Hiscock, 2005; Buggs & Pannell, 2006). Natural
5 hybridization can result when divergent lineages, or species formed in allopatry,
6 change ranges and come into reproductive contact, potentially forming a secondary
7 contact zone (Anderson, 1949; Lagercrantz & Ryman, 1990; Cruzan, 2005; Hoskin *et*
8 *al.*, 2005). However, hybridization can also occur following biological invasions, if
9 introduced species are sufficiently closely related to native species. As global trade
10 and passenger travel continues to accelerate (Hanfling & Kollmann, 2002), it seems
11 probable that alien plant invasions will continue at an alarming rate, leading to
12 increasing contact and hybridization between previously allopatric species (Abbott,
13 1992; Abbott & Lowe, 2004). In contrast to natural range changes, biological
14 invasions are more likely to form extensive zones of contact, potentially accelerating
15 the eventual outcome of hybridization (Wolf *et al.*, 2001).

16 Hybridization between natives and exotics can have several outcomes,
17 including; enhanced weediness in hybrid offspring (Morrell *et al.*, 2005; Whitney *et*
18 *al.*, 2006), evolution of new hybrid lineages (Lowe & Abbott, 2004) and decline or
19 even extinction of hybridizing species (Wolf *et al.*, 2001). The latter is the most
20 potentially destructive outcome of interspecific hybridization, and can occur via two
21 main potential mechanisms (Wolf *et al.*, 2001). First, introgressive hybridization, the
22 transfer of genes between species via fertile or semi-fertile hybrids, may produce

1 hybrid derivatives of superior fitness that displace one or both pure conspecifics
2 causing decline through genetic assimilation. Secondly, if hybrids are sterile or
3 display reduced fitness, the population growth rate of the hybridising taxa may
4 decrease below that required for replacement of one or both parental species, resulting
5 in demographic swamping.

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

15 *Senecio*, one of the largest genera of flowering plants, is known worldwide for
16 its globally important weed species (Holm *et al.*, 1997) and the widespread
17 occurrence of interspecific hybridization between native and introduced taxa (Abbott,
18 1992; Lowe & Abbott, 2004; Kadereit *et al.*, 2006). *Senecio madagascariensis*
19 (fireweed), a native of southern Africa and Madagascar, was introduced to Australia
20 more than 80 years ago and is now an aggressive weed in its invasive range (Radford,
21 1997). In Australia, molecular genetic and morphological studies have demonstrated a
22 close affinity between fireweed and Australian native *Senecio* species, including *S.*

1 *pinnatifolius* (formerly *S. lautus*) (Scott et al. 1998). Although *S. madagascariensis*
2 ($2n = 2x = 20$) and *S. pinnatifolius* ($2n = 4x = 40$) differ in ploidy, empirical and
3 experimental crossing studies have established that both species can serve as paternal
4 and maternal parents of synthetic hybrids (Radford, 1997). Under greenhouse
5 conditions, synthetic triploid hybrids between the two species exhibit low viability
6 and low fertility (Radford, 1997). Despite low fertility, triploid hybrids can still act as
7 a genetic bridge between diploid and tetraploid taxa, as demonstrated by Lowe &
8 Abbott (2000). Thus, despite the low fertility of *S. madagascariensis* x *S. pinnatifolius*
9 F1 triploid hybrids, they could enable introgression of *S. madagascariensis* genes into
10 *S. pinnatifolius* (or vice-versa), but this remains untested in the field.

11 Populations of *S. madagascariensis* exist in sympatry with populations of the
12 native *S. pinnatifolius* across many regions of Australia's east coast. Within this area,
13 *S. madagascariensis* and *S. pinnatifolius* grow in close physical proximity, have
14 flowering periods that overlap and are pollinated by the same insect species (White,
15 2007). Hybrid formation has also been observed in sympatric populations of the two
16 species in the field (Radford, 1997), but may be restricted to certain variants of *S.*
17 *pinnatifolius*, such as tableland, headland and dune.

18 To examine in greater detail the outcome of hybridization between the native
19 *S. pinnatifolius* and the invasive *S. madagascariensis*, comparisons were made at
20 population (sympatric vs allopatric populations) and individual (sympatric
21 populations) levels to investigate contemporary and long-term outcomes of
22 hybridization. The molecular marker used to address these questions was amplified

1 fragment length polymorphisms (AFLP). Four primary questions are addressed in this
2 paper: (i) what is the viability of hybrids in the field? - Comprising a comparison of
3 frequencies of hybrids in open pollinated seed of both species with the occurrence of
4 adult stage hybrids in sympatric populations. (ii) Do hybrids or stabilised
5 introgressants persist in the field and to what hybrid class do they belong? (iii) Does
6 hybridization influence the level of genetic diversity or differentiation within
7 sympatric compared to allopatric populations of these hybridising species? (iv) Can
8 we estimate the likely outcome of hybridization between this native-invasive pair
9 under a number of hybridization scenarios, and is *S. pinnatifolius* at risk of genetic
10 assimilation and/or demographic swamping in sympatric populations?

11

1 **Materials and Methods**

2 *Study species*

3 *Senecio madagascariensis* (fireweed), a diploid annual weed from South
4 Africa, was first recorded in Australia in 1918 and has since invaded large areas of
5 farmland and grassland in south-eastern Australia (Radford *et al.*, 1995; Radford,
6 1997). Closely related to *S. madagascariensis*, is a group of sub-species belonging to
7 the Australian native *S. pinnatifolius* complex (formerly known as the *S. lautus*
8 complex prior to Radford *et al.*, 2004). *S.pinnatifolius* is an herbaceous perennial
9 tetraploid (Ornduff, 1964; Ali, 1966; Radford *et al.*, 2004), and exhibits similar
10 geographic range to *S. madagascariensis* in the eastern states, but generally occurs in
11 smaller, more scattered populations than the exotic (Radford, 1997; Radford &
12 Cousens, 2000). *Senecio pinnatifolius* var. *tableland* (also known as *Senecio lautus*
13 *ssp. lanceolatus*), the focus of this study, inhabits disturbed areas and pasture usually
14 close to the edge of rainforest or moist eucalypt forest and flowers between February
15 and June in south-eastern Australia (Radford & Cousens, 2000, supplementary Fig.1).
16 There is a four month period of overlap between the flowering periods of the native
17 and exotic *Senecio*, the latter flowers between the months of March and December in
18 Australia (Radford & Cousens, 2000). Previous studies have indicated that both
19 species are self-incompatible and insect-pollinated (Ali, 1966; Lawrence, 1985;
20 Radford, 1997). The two species are superficially morphologically similar (differing
21 in plant size, bract number and time to senescence), both producing similar-sized

1 yellow capitula which occur in clusters on the plant: floral visitors move freely
2 between the two species when they grow together in the field (White, 2007).

3

4 *Study sites and sample collections*

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

9 To determine the number of mature hybrid plants, as well as genetic diversity
10 for each species when they grow in sympatry, leaf material was collected from
11 approximately 45 plants (minimum 43, maximum 47) across the morphological range
12 exhibited by mature flowering plants of each species from two sympatric sites. Plants
13 from which leaf material was collected were identified as either *S. pinnatifolius* or *S.*
14 *madagascariensis* using morphological features, including bract number and leaf
15 morphology, following Ali (1969) and Nelson (1980). Despite repeated searches over
16 two consecutive flowering seasons, no obvious hybrids (ie. plants with intermediate
17 morphology) were observed in the field. In addition, ~ 20 seeds per plant were
18 collected from a subset of 10 plants of each species from which leaf material was
19 collected (a total of ~ 200 seeds per species for each of the two populations).

20 All allopatric and sympatric populations sampled occurred within the “Border
21 Ranges”, a group of linked mountain ranges running along the eastern portion of the
22 Queensland/New South Wales State border (population locations are indicated in

1 Table 1). All sites, regardless of location, occurred within a similar altitudinal range
2 (between 550 m and 700 m ASL), had similar types of neighbouring vegetation
3 (pasture and moist eucalypt forest or rainforest), and were surveyed during May when
4 both species were flowering. Allopatric populations were separated by at least five
5 km from the nearest known population of the other species. In sympatric populations,
6 *S. pinnatifolius* grew along the rainforest edges, and in nearby creek beds, while *S.*
7 *madagascariensis* inhabited adjacent pasture, with considerable mixing of the species
8 at the interface of the populations.

9 All leaf samples from allopatric and sympatric populations were transported
10 on ice, then frozen and stored at -80°C until DNA extractions were performed. Seeds
11 were germinated on moist filter paper until they reached approximately 20 mm in
12 height, at which point they were removed, frozen and stored at -80°C . Germination
13 percentages for *S. pinnatifolius* and *S. madagascariensis* in both sympatric sites were
14 generally quite low; particularly for *S. madagascariensis* ($< 35\%$ at both sites), and
15 numbers of resulting progeny for each species for each site are shown in Table 1.

16

17 *Relative densities of plants and flowers*

18 Relative plant and capitula densities of each species at each site were
19 determined using the using the Point Centred Quarter (PCQ) method (Krebs, 1989),
20 using 30 random plants of each species at each site as ‘centre points’. A Chi-square
21 test was used to determine whether the proportion of hybrids sired in the progeny of
22 each species was concordant with capitula densities of each species at each site.

1

2 *AFLP profiling*

3 Total cellular DNA was extracted from 0.1 g of plant material per sample
4 according to the protocol of Doyle & Doyle (1987) with slight modifications. DNA
5 was quantified visually on ethidium bromide stained agarose gels and samples were
6 diluted with 0.5 × TE buffer to obtain concentrations between 100 and 200 ng/μL.

7 AFLP restriction/ligation was performed following the protocol of Prentis *et*
8 *al.* (2004). AFLP PCR was performed following the method of Zawko *et al.* (2001),
9 using two primer pairs: E-AAG/M-AG and E-AAG/M-GA, where the selective EcoRI
10 primer was Hex labeled (Geneworks). The fluorescently labeled amplified products
11 were analysed by gel electrophoresis (5% acrylamide gels), using a Gelscan GS2000
12 (Corbet Research) with a TAMRA 500 size standard (Applied Biosystems). To
13 confirm reproducibility, five adult samples of each *Senecio* species were run blindly
14 six times from different extractions for both primer combinations and ambiguous loci
15 were not scored. This information was also used to produce an error rate of fragment
16 mis-scoring for both primer combinations. At an individual locus, bands of similar
17 size and intensity were considered to be homologous, following previous studies of
18 closely related species (Rieseberg, 1996; O'Hanlon & Peakall, 2000). AFLP profiles
19 were scored for the presence and absence of bands between 50 and 500 base pairs in
20 size.

21

22 *Data analysis – population level*

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

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

14

15 *Data analysis – individual level*

16 Principal coordinates analysis (PCOA) was used to examine clustering of
17 individual *S. pinnatifolius* and *S. madagascariensis* genotypes from both sympatric
18 and allopatric sites using GENALEX (Peakall & Smouse, 2006). To assign
19 individuals to their most likely species of origin, or hybrid status, the assignment
20 method of Duchesne & Bernatchez (2002) in AFLPOP was used. The assignment
21 method utilises multilocus AFLP data to test the likelihood that an individual
22 genotype (G) is a pure species or interspecific hybrid based on population-level allele

1 frequencies. If the frequency of an AFLP fragment was 0, $\log(0)$ was replaced by
2 $\log(\epsilon)$, where ϵ was chosen as 0.001. Individuals are assigned to species or hybrid
3 populations displaying the highest log-likelihood for G; however allocation of
4 genotypes were only made if minimal log-likelihood difference (MLD) was ≥ 1 for
5 mature individuals. This means a genotype is 10 times more likely to originate from a
6 particular population than any other candidate population. A MLD of 0 was used to
7 allocate progeny genotypes to parental species or hybrid swarms, as many individuals
8 were unassigned at higher MLD stringency levels. The MLDs chosen here are similar
9 to most previous studies (Potvin & Bernatchez, 2001; Campbell *et al.*, 2003; He *et*
10 *al.*, 2004).

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

17 First generation, F1 parental backcrosses and F2 hybrid populations were
18 simulated between all pairs of allopatric populations of *S. pinnatifolius* and *S.*
19 *madagascariensis*. Mature sympatric individuals of the two species were then
20 assigned to either allopatric populations or simulated hybrid swarms. Seed collections
21 from sympatric sites were also allocated to their species of origin or simulated
22 interspecific hybrid status using the same assignment method as above.

1

2 *Risk posed by demographic swamping*

3 Using a simulation approach, we calculated annual viable seed production of both
4 species in sympatric sites under a number of hybridization scenarios. The scenarios
5 used were no hybridization, maximum hybridization (all seeds produced during
6 synchronous flowering were hybrids), fixed level hybridization (based on actual levels
7 of hybridization observed in field-collected progeny in this study), linear density
8 dependent hybridization and threshold density dependent hybridization. Simulations
9 were parameterized using information from Radford, (1997), Radford & Cousens,
10 (2000) and the current study. Parameters used in the model were monthly capitula
11 production for both species in allopatric sites (see fig. 1) to estimate the proportion of
12 total yearly capitula produced during synchronous (S) and non-synchronous (N)
13 flowering, annual seed production (A), percentage germination under field conditions
14 (G), percentage establishment of both species in *S. pinnatifolius* var. tableland habitat
15 (E), and hybridization rate (H) (see supplementaryTable. 1 for values). The
16 hybridization rate (H) was calculated for each month, based on flowering synchrony
17 data from field observations (Radford & Cousens, 2000, supplementary Fig. 1), the
18 proportion of *S. madagascariensis* (Pm) in a population is used to estimate the
19 proportion of hybrid seed produced separately for both *S. madagascariensis* and *S.*
20 *pinnatifolius* using the density dependent relationships outlined in the linear and
21 power (threshold) relationships. Linear density dependent relationships were fitted
22 based on the assumption that $H = (1 - \text{observed } H) \text{ at } P_m = (1 - \text{observed } P_m)$, for

1 each site and species independently (Equations for lines of best fit, O'Reillys': *S.*
2 *madagascariensis* $y = (-0.101(\text{Pm})) + 0.103$, *S. pinnatifolius* $y = (0.739(\text{Pm})) + 0.13$;
3 Queen Mary Falls: *S. madagascariensis* $y = (-0.532(\text{Pm})) + 0.766$, *S. pinnatifolius* $y =$
4 $(0.894(\text{Pm})) + 0.053$). Threshold density dependent relationships were fitted using the
5 same principle but with hybridization data from both sites combined for both species
6 (see supplementary Fig. 2). Annual viable seed production (AVSP) was then
7 calculated for both species using the following equation; $\text{AVSP} = ((\text{Pr S} \times \text{A}) \times (1 - \text{H})$
8 $\times \text{G} \times \text{E}) + ((\text{Pr N} \times \text{A}) \times \text{G} \times \text{E})$. Simulations were run independently for each
9 sympatric site based on the actual levels of hybridization recorded in open pollinated
10 progeny at that site for the fixed rate hybridization scenario. The principal simplifying
11 assumptions of our model include (1) flowering time in sympatric populations is
12 similar to allopatric populations (2) rates of hybridization are density dependent and
13 (3) all hybrids are not viable.

14

1 **Results**

2

3 *Genetic diversity and population differentiation*

4 The two AFLP primer pair combinations produced 176 fragments for the 718
5 individuals screened, of which 88% were polymorphic between the two species. The
6 error rate of mis-scoring estimated from blind running of five individuals of each
7 *Senecio* species six times from different extractions was 1.7% and 1.9% for the primer
8 pairs 33-49 and 33-55, respectively. Mean genetic diversity within *S.*
9 *madagascariensis* and *S. pinnatifolius* populations was $H_E = 0.212 (\pm 0.017)$ and $H_E =$
10 $0.223 (\pm 0.014)$, respectively. Genetic diversity was similar between allopatric (0.229
11 ± 0.008) and sympatric (0.220 ± 0.010) populations for *S. pinnatifolius* ($T_3 = 0.697$; P
12 > 0.5). Similarly, no statistical difference for genetic diversity between allopatric
13 (0.223 ± 0.002) and sympatric (0.194 ± 0.008) populations of *S. madagascariensis*
14 was detected (equal variances not assumed; $T_{1,125} = 3.323$; $P > 0.1$), although a trend
15 toward lower genetic diversity in sympatric populations was observed for both
16 species.

17 A global F_{ST} analysis (0.493 , $P < 0.001$) detected very pronounced
18 differentiation between the species (average pairwise $F_{ST} = 0.607$, see Table 2 for
19 pairwise F_{ST} comparisons), and was much larger than for average within species
20 comparisons (average pairwise $F_{ST} = 0.218$). Global F_{ST} values of 0.270 ($P < 0.001$)
21 were calculated for *S. madagascariensis*, and 0.161 ($P < 0.001$) for *S. pinnatifolius*.

22 The NJ phenogram illustrated that *S. pinnatifolius* and *S. madagascariensis*

1 populations formed two distinct and strongly differentiated clusters, whether from
2 allopatric or sympatric populations (Fig. 1). Within each of the species clusters,
3 sympatric populations were more similar genetically to each other than they were to
4 allopatric populations (*S. pinnatifolius*: sympatric – allopatric comparisons $F_{ST} = 0.18$,
5 $P < 0.001$, sympatric – sympatric comparisons $F_{ST} = 0.15$, $P < 0.001$; *S.*
6 *madagascariensis*: sympatric – allopatric comparisons $F_{ST} = 0.30$, $P < 0.001$,
7 sympatric – sympatric comparisons $F_{ST} = 0.23$, $P < 0.001$), although the pattern was
8 more pronounced in *S. madagascariensis*. This pattern of clustering was also
9 confirmed in the individual PCOA (Figure 2), where the first two axes accounted for
10 81.1 % of the total variation, with the species differentiating axis 1 explaining greater
11 than 73.7 % of the total variation. Separation of individuals from sympatric and
12 allopatric populations of both species was parallel to PCOA axis 2 and not the species
13 differentiating axis (1).

14 Principal coordinates analysis (Figure 2) and assignment tests indicated a total
15 absence of mature hybrids in the field. All mature individuals sampled from the two
16 sympatric populations were assigned to either pure *S. pinnatifolius* or *S.*
17 *madagascariensis* groups, and not to simulated hybrid swarms between the two
18 species. The probability of incorrectly assigning mature individuals was extremely
19 low, since all allocated individuals had simulation P values of < 0.001 .

20 The assignment method detected F1 hybrid progeny amongst seed collected
21 from *S. pinnatifolius* and *S. madagascariensis* plants in each of the sympatric sites.
22 The level of hybrid progeny in the seeds differed quite markedly between the species

1 at O'Reilly's (% F1 hybrids: *S. pinnatifolius* = 8.3%; *S. madagascariensis* = 75.6%;
2 Figure 3 a), but was more similar at Queen Mary Falls (% F1 hybrids: *S. pinnatifolius*
3 = 15.6%; *S. madagascariensis* = 10.2%; Figure 3 b).

4

5 *Relative densities of plants and flowers*

6 The native *S. pinnatifolius* was the dominant species at both sympatric sites,
7 both in terms of plant and flower density: it had more than three-fold the plant density
8 and approximately 19-fold the flower density of *S. madagascariensis* (Table 1). At
9 both sites the rate of hybrid seed production by *S. pinnatifolius* was significantly
10 higher than would be expected if it was occurring proportionally to the relative
11 densities of *S. pinnatifolius* and *S. madagascariensis* flowers (O'Reillys': $\chi^2 = 5.43$,
12 $df = 1$, $p < 0.05$; Queen Mary Falls: $\chi^2 = 102.48$, $df = 1$, $p < 0.01$). *Senecio*
13 *madagascariensis* contributed only five percent of capitula in each of the sympatric
14 populations, but approximately 15 and 8.5 % of *S. pinnatifolius* progeny were
15 identified as F1 hybrids at Queen Mary Falls and O'Reillys' sites respectively. In
16 contrast, the rates of hybridization in *S. madagascariensis* seed were significantly
17 lower than expected from floral density (O'Reillys': $\chi^2 = 57.76$, $df = 1$, $p < 0.05$;
18 Queen Mary Falls: $\chi^2 = 1375.14$, $df = 1$, $p < 0.01$). Where *S. pinnatifolius* makes up
19 95 % of capitula at both sites, but only 10 and 75 % of *S. madagascariensis* progeny
20 were recognized as hybrids at Queen Mary Falls and O'Reillys' sites respectively.

21

22 *Risk posed by demographic swamping*

1 Distinctly different outcomes of AVSP were observed under the five hybridization
2 simulation scenarios. Simulations indicated that with no hybridization, *S. pinnatifolius*
3 would produce far greater viable seed than *S. madagascariensis* (271:182; Table 3),
4 but that this advantage in viable seed production was substantially decreased at
5 maximum hybridization (84:58; Table 3). If fixed rate hybridization was assumed (i.e.
6 independent of floral density), AVSP was still substantially greater in *S. pinnatifolius*
7 than *S. madagascariensis* at both QM Falls (241:170; Table 4) and O'Reillys'
8 (256:89; Table 3). Simulations based on density dependent hybridization
9 demonstrated that the proportion of *S. madagascariensis* in sympatric populations
10 needed to reach > 70 -80 % for linear density dependent hybridization and > 25 % for
11 threshold density dependent hybridization to produce more viable seed than *S.*
12 *pinnatifolius* (Fig. 4 a, b & c).
13
14

1 **Discussion**

2 *The incidence of hybridization and fate of hybrids*

3 Hybridization between *S. madagascariensis* and *S. pinnatifolius* occurs very
4 frequently in the wild (see also; Radford, 1997), with a large number of F1 hybrid
5 seed produced by both species in sympatric sites. In fact, the level of hybridization
6 recorded in open pollinated seed is two orders of magnitude greater than that recorded
7 between another well characterized native-exotic *Senecio* species pair, *S. vulgaris* ($2n$
8 $= 4x = 40$) and *S. squalidus* ($2n = 2x = 20$) (Marshall & Abbott, 1980). It is also an
9 order of magnitude higher than between *S. vulgaris* and the recent neo-species *S.*
10 *eboracensis* ($2n = 4x = 40$). Lowe & Abbott (2004) suggest that the low frequency of
11 hybridization between *S. eboracensis* and *S. vulgaris* was influenced by niche
12 separation, differences in flowering phenology and *S. eboracensis* being more
13 attractive to pollinators than *S. vulgaris*. Given that habitat differentiation between *S.*
14 *madagascariensis* and *S. pinnatifolius* is weak, there is a substantial overlap in their
15 flowering time, and that they are pollinated by the same insect species, the high level
16 of hybridization observed here is not unexpected. The frequency of hybridization in
17 our study suggests that prezygotic barriers are weak and do not act as a barrier to gene
18 flow between the species.

19 Despite the high proportion of hybrid seed collected from both species,
20 mature hybrids were totally absent from sympatric populations sampled in this study.
21 These results suggest that there is a very strong postzygotic reproductive barrier
22 between the study species. Effects of interploidal hybridization on offspring fitness

1 can be severe, often resulting in progeny that are highly sterile (Hardy *et al.*, 2001;
2 Lowe & Abbott, 2004; Pannell *et al.*, 2004; Buggs & Pannell, 2006). However
3 sterility is not the only consequence of interploidal hybridization in this study, since
4 no hybrids, sterile or otherwise, developed to maturity in sampled populations. A lack
5 of mature hybrids indicates that the fitness of interspecific hybrids must also be much
6 reduced, but further study is required to estimate at which life history stage hybrids
7 are selected against in sympatric populations.

8 Since the rapid spread of *S. madagascariensis*, hybrid zones between *S.*
9 *pinnatifolius* and *S. madagascariensis* have formed in many areas of eastern Australia
10 (Radford, 1997). Documented zero or near zero fitness of F1 hybrids in sympatric
11 populations indicates that contact zones formed between *S. pinnatifolius* and *S.*
12 *madagascariensis* represent tension zones. Theoretical models predict that tension
13 zones can be formed and maintained by a balance between dispersal of parent types
14 into areas of contact and subsequent selection against hybrid progeny (Barton &
15 Hewitt, 1989), although tension zones may also be maintained by positive frequency-
16 dependent selection (Buggs & Pannell, 2006). Areas of contact between diploid and
17 tetraploid *Centaurea jacea* in Belgium (Hardy *et al.*, 2000; 2001), and diploid and
18 hexaploid *Mercurialis annua* in northern Spain (Pannell *et al.*, 2004), appear to be
19 other good examples of tension zones in mixed ploidy plant populations.

20

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

1 Overall the level of differentiation between the species was very high (0.61)
2 and much higher than among populations within species (0.218). Although there were
3 no immediately obvious impacts of hybridization, populations in areas of sympatry
4 showed increased differentiation from conspecific allopatric populations and this
5 pattern was more pronounced in *S. madagascariensis*. Although genetic diversity was
6 not significantly different between allopatric and sympatric sites for either species,
7 there was a trend toward lower genetic diversity in sympatric sites for both species.

8 The pattern of increased differentiation between allopatric and sympatric
9 populations appears unrelated to introgression, since the PCOA axis of differentiation
10 was perpendicular to the axis differentiating the two species. Erosion of alleles in
11 early flowering *S. madagascariensis*, or late flowering *S. pinnatifolius* genotypes,
12 might change allele frequencies in sympatric populations and may be responsible for
13 the observed pattern of differentiation. An erosion of alleles may also explain a trend
14 toward lower genetic diversity at sympatric sites however further work is warranted
15 on this topic.

16

17

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

19 In areas of contact between *S. pinnatifolius* and *S. madagascariensis* in
20 tableland-variant habitat, simulations demonstrated *S. pinnatifolius* was not at risk
21 from demographic swamping when no hybridization occurred or when levels of
22 hybridization were constant and not affected by the proportion of *S.*

1 *madagascariensis*. However, *S. madagascariensis* displays a hybridization advantage
2 at both surveyed field sites, where it sires significantly more progeny than expected
3 from floral densities, and *S. pinnatifolius* significantly less. Thus hybridization
4 between the species is asymmetric, a phenomenon commonly reported in hybrid
5 zones (Rieseberg & Wendel, 1993; Arnold, 1997; Burgess *et al.*, 2005). Simulations
6 based on density-dependent asymmetric hybridization between the species, indicate
7 that the proportion of *S. madagascariensis* need only reach 25 % to cause the
8 demographic decline of *S. pinnatifolius* in sympatry (fig. 4 c). Under these
9 circumstances, an invasive species does not necessarily have to outnumber a native to
10 have an impact on the demography of an interfertile native through hybridization. In
11 fact, invasive species may be rare relative to a native plant, but may nevertheless pose
12 a threat to the native due to superior male fitness (eg. production of a greater number
13 of pollen grains), resulting in the invader siring a disproportionately higher proportion
14 of progeny (Anttila *et al.*, 1998). As a result, asymmetric hybridization in favour of an
15 invasive species can contribute to the decline and extinction of native species (Wolf *et*
16 *al.*, 2001). Hence, if *S. madagascariensis* increases numerically in areas of contact, it
17 may cause the extinction of *S. pinnatifolius* from east coast areas of Australia. Given
18 that *S. madagascariensis* is least fit in *S. pinnatifolius* var. tableland habitat, the
19 extinction of *S. pinnatifolius* throughout the range of other habitats it occupies is even
20 more probable and is the likely reason for the rapid historical spread of
21 *S. madagascariensis* into *S. pinnatifolius* habitat (R. McFadyen, pers. comm.). In

1 addition, pollen and seed dispersal by *S. madagascariensis* into hybrid zones from
2 neighbouring pure populations may further advantage the invasive over the native.

3 However, two factors may impede the displacement of *S. pinnatifolius*. First,
4 *S. madagascariensis* may be driven to local extinction in areas of contact during
5 colonisation, if it cannot establish within a few generations. Given that the O'Reillys'
6 contact zone has existed for between 20-35 generations however, it would seem that
7 this outcome is unlikely as *S. madagascariensis* can reproduce in the absence of *S.*
8 *pinnatifolius* for approximately 6 months annually and is likely to continually disperse
9 back into areas of contact after local extinctions. Second, natural selection against
10 maladaptive hybridization may lead to reproductive character displacement (eg.
11 flowering time divergence) and "avoidance" of the negative consequences associated
12 with interspecific fertilizations. Although the conditions under which reinforcing
13 natural selection may occur are limited (Pannell *et al.*, 2004; Hoskin *et al.*, 2005), it is
14 most likely to occur when contact zones are extensive, exposing a high proportion of
15 individuals to selection. As *S. pinnatifolius* and *S. madagascariensis* form extensive
16 contact zones in Eastern Australia, reinforcement may impede the displacement of *S.*
17 *pinnatifolius*, but this scenario needs to be investigated theoretically and empirically
18 to be supported.

19 The destructive force of interspecific hybridization is not uncommon in
20 hybridizing plant species (Wolf *et al.*, 2001; Buggs & Pannell, 2006). Adequate
21 molecular data, however, in open pollinated progeny and mature individuals from
22 sympatric populations are often lacking, meaning the actual level of hybridization and

1 the impact on native or rare species may be underestimated. Without this information
2 conservation strategies for the protection of hybridizing species may not be effective.
3 This data, in combination with ecological approaches, will provide the baseline for
4 comprehensive long-term studies into the consequences of hybridization on native
5 species.

6

1 **Acknowledgements**

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

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1 **Figure legends**

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

5
6 **Supplementary Fig. 2.** Graphs depicting the rate of hybridisation (threshold) as it
7 varies with the proportion of *Senecio madagascariensis* in populations for a) *S.*
8 *madagascariensis* and b) *Senecio pinnatifolius*

9
10 **Fig. 1** Unrooted neighbour-joining phenogram based on pairwise F_{ST} distances among
11 AFLP profiles for *Senecio pinnatifolius* (*Sp*) and *Senecio madagascariensis* (*Sm*) in
12 sympatric (^S) and allopatric (^A) sites, i.e. Hampton (H), Swanfels 1 (S1), Swanfels 2
13 (S2), Beechmont (B), Tamborine (T), Springbrook (S), Queen Mary Falls (QM) and
14 O'Reillys' (O).

15
16 **Fig. 2.** Principal coordinates analysis depicting clustering of *Senecio pinnatifolius* and
17 *Senecio madagascariensis* in sympatric and allopatric sites.

18
19 **Fig. 3.** Percentage of plants of each species, capitula produced by each species and
20 hybrid and non-hybrid F1 progeny produced by *Senecio pinnatifolius* (*Sp*) and
21 *Senecio madagascariensis* (*Sm*) plants in two sympatric populations; a) Queen Mary
22 Falls and b) O'Reillys'.

1

2 **Fig. 4.** Graph depicting annual viable seed production of *Senecio pinnatifolius* (closed
3 symbols) and *Senecio madagascariensis* (open symbols) in sympatric sites derived
4 under the following simulations a) linear density dependent hybridization at
5 O'Reillys' b) linear density dependent hybridization at Queen Mary Falls, and c)
6 threshold density dependent hybridization

1 Table 1: Population locations and relative densities of plants and flowers of native *Senecio pinnatifolius* (*Sp*) and exotic *Senecio*
 2 *madagascariensis* (*Sm*)

	Population	Location	Relative densities (<i>Sp:Sm</i>)		Sample sizes	
			Plants	Capitula	Parents	Progeny
Allopatric	Hampton	East of Hampton, Northern Darling Downs (27° 22'S, 152°10'E)			45	
	Swanfels 1	North of Killarney, Southern Darling Downs (28° 07'S, 152° 23'E)	100 : 0	100 : 0	42	
	Swanfels 2	North of Killarney, Southern Darling Downs (28° 08'S, 152° 23'E)			45	
Allopatric	Beechmont	Near Beechmont, Gold Coast Hinterland (28° 07'S, 153° 10'E)			45	
	Tamborine	Mt Tamborine, Gold Coast Hinterland (27° 58'S, 153°12'E)	0 : 100	0 : 100	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	<i>Sp</i> : 45; <i>Sm</i> : 45	<i>Sp</i> : 109; <i>Sm</i> : 49
	O'Reillys'	Near Lamington National Park, Gold Coast Hinterland (28°13'S, 153° 07'E)	0.84 : 0.16	0.97 : 0.03	<i>Sp</i> : 43; <i>Sm</i> : 47	<i>Sp</i> : 72; <i>Sm</i> : 41

3

- 1 Table 2. Pairwise F_{ST} estimates for both *Senecio pinnatifolius* (Sp) and *Senecio madagascariensis* (Sm) in sympatric (S) and allopatric (A)
- 2 populations, abbreviations as follows; Hampton (H), Swanfels 1 (S1), Swanfels 2 (S2), Beechmont (B), Tamborine (T), Springbrook (S), Queen
- 3 Mary Falls (QM) and O'Reillys' (O). *** represents significance at the $P < 0.001$ level.

	Sp^{AH}	Sp^{AS1}	Sp^{AS2}	Sp^{SQMF}	Sp^{SO}	Sm^{AB}	Sm^{AT}	Sm^{AS}	Sm^{SQMF}	Sm^{SO}	4
Sp^{AH}	-	***	***	***	***	***	***	***	***	***	***
Sp^{AS1}	0.130	-	***	***	***	***	***	***	***	***	5
Sp^{AS2}	0.163	0.110	-	***	***	***	***	***	***	***	6
Sp^{SQMF}	0.149	0.118	0.155	-	***	***	***	***	***	***	7
Sp^{SO}	0.228	0.212	0.190	0.151	-	***	***	***	***	***	
Sm^{AB}	0.573	0.594	0.577	0.581	0.597	-	***	***	***	***	8
Sm^{AT}	0.6010	0.614	0.588	0.607	0.618	0.238	-	***	***	***	9
Sm^{AS}	0.592	0.596	0.567	0.593	0.607	0.207	0.241	-	***	***	
Sm^{SQMF}	0.621	0.631	0.603	0.614	0.628	0.297	0.294	0.274	-	***	10
Sm^{SO}	0.639	0.645	0.608	0.628	0.637	0.310	0.334	0.269	0.237	-	11

1 Table 3. Annual viable seed production produced by *Senecio pinnatifolius* (*Sp*) and *Senecio madagascariensis* (*Sm*) in sympatric populations in
 2 tableland variant habitat for a range of different hybridization scenarios, abbreviations as follows; Queen Mary Falls (QM) and O'Reillys' (O).
 3 The values reported for linear and threshold hybridization scenarios are the range of viable seed produced when the proportion of *S.*
 4 *madagascariensis* in sympatric populations is 0.05 and 0.95 respectively.

Species	Total seed	Post germination	Post establishment	Maximum hybridization	Fixed rate (O)	Fixed rate (QM)	Linear (O)	Linear (QM)	Threshold
<i>S. p</i>	505	338	271	84	256	241	253-140	240-146	215-90
<i>S. m</i>	422	304	182	58	89	176	96-151	171-182	166-181

5

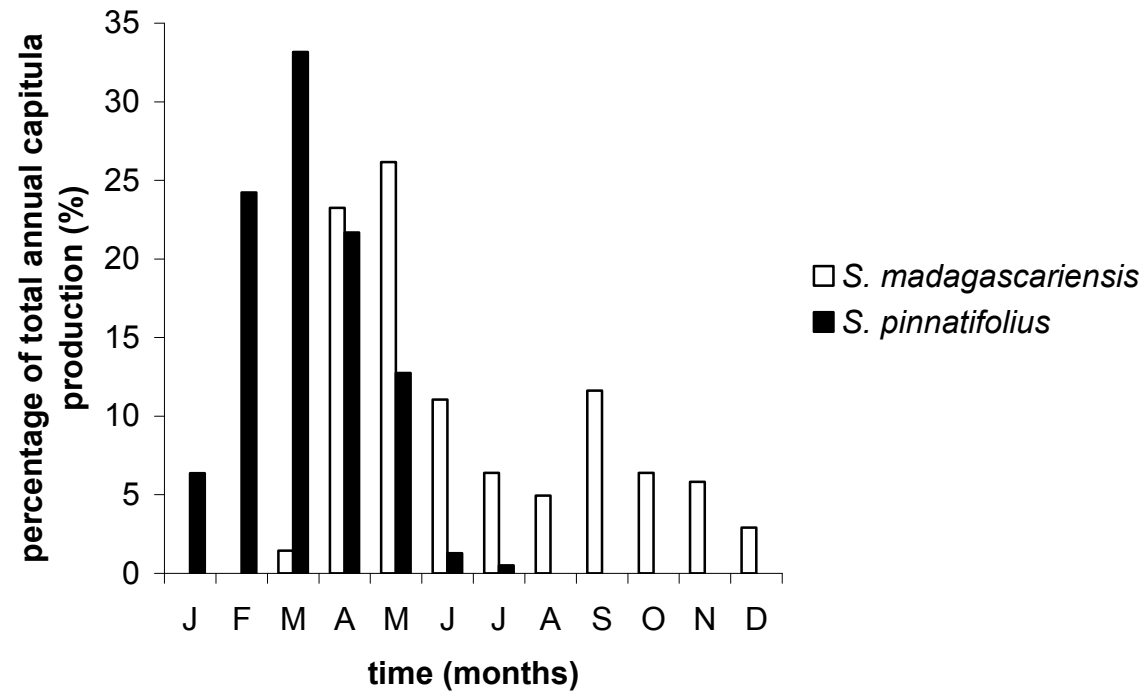
6

1 Supplementary Table 1. Values for demographic variables used to parameterize the simulation study and the source of reference of this data

Demographic variables	<i>S. pinnatifolius</i>	<i>S. madagascariensis</i>	Source of reference
Annual seed production (A)	505	442	Radford & Cousens, (2000)
Germination under field conditions (G)	0.67	0.72	Radford & Cousens, (2000)
Establishment in <i>Senecio pinnatifolius</i> habitat (E)	0.80	0.60	Radford & Cousens, (2000)
Hybridization rate (H)	Variable	Variable	This study; Radford, 1997
Synchronous flowering (S)	0.69	0.68	Radford, 1997
Non-synchronous flowering (N)	0.31	0.32	Radford, 1997

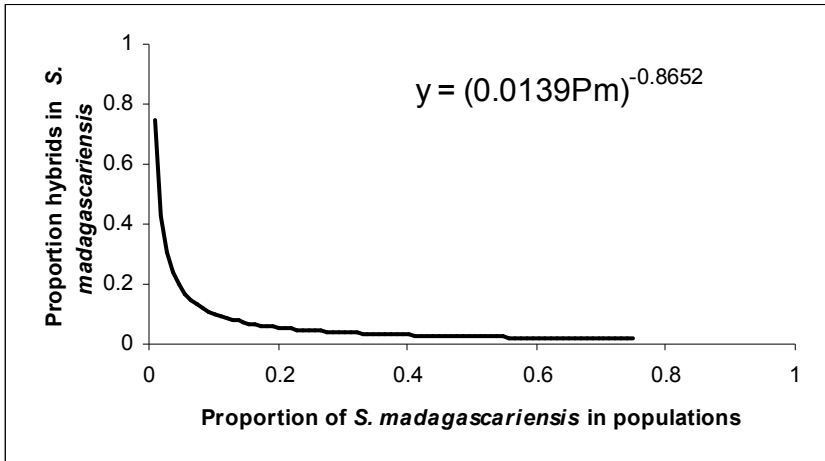
2
3
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S1

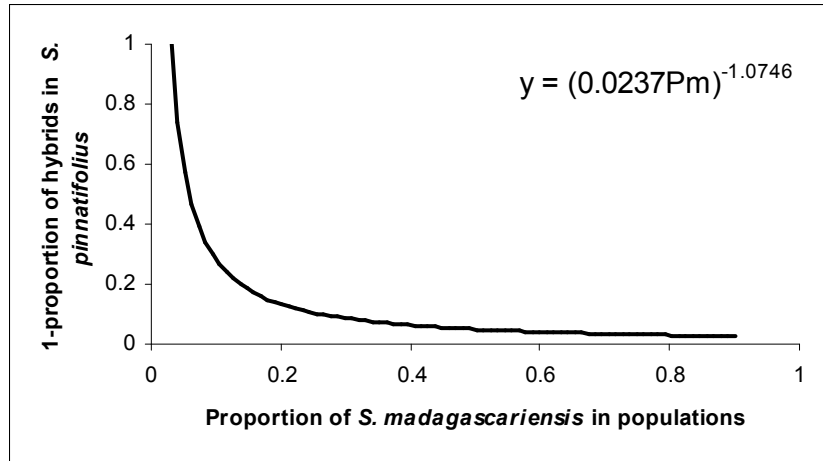


1
2
3

S2 (a)



S2 (b)



1

2

1

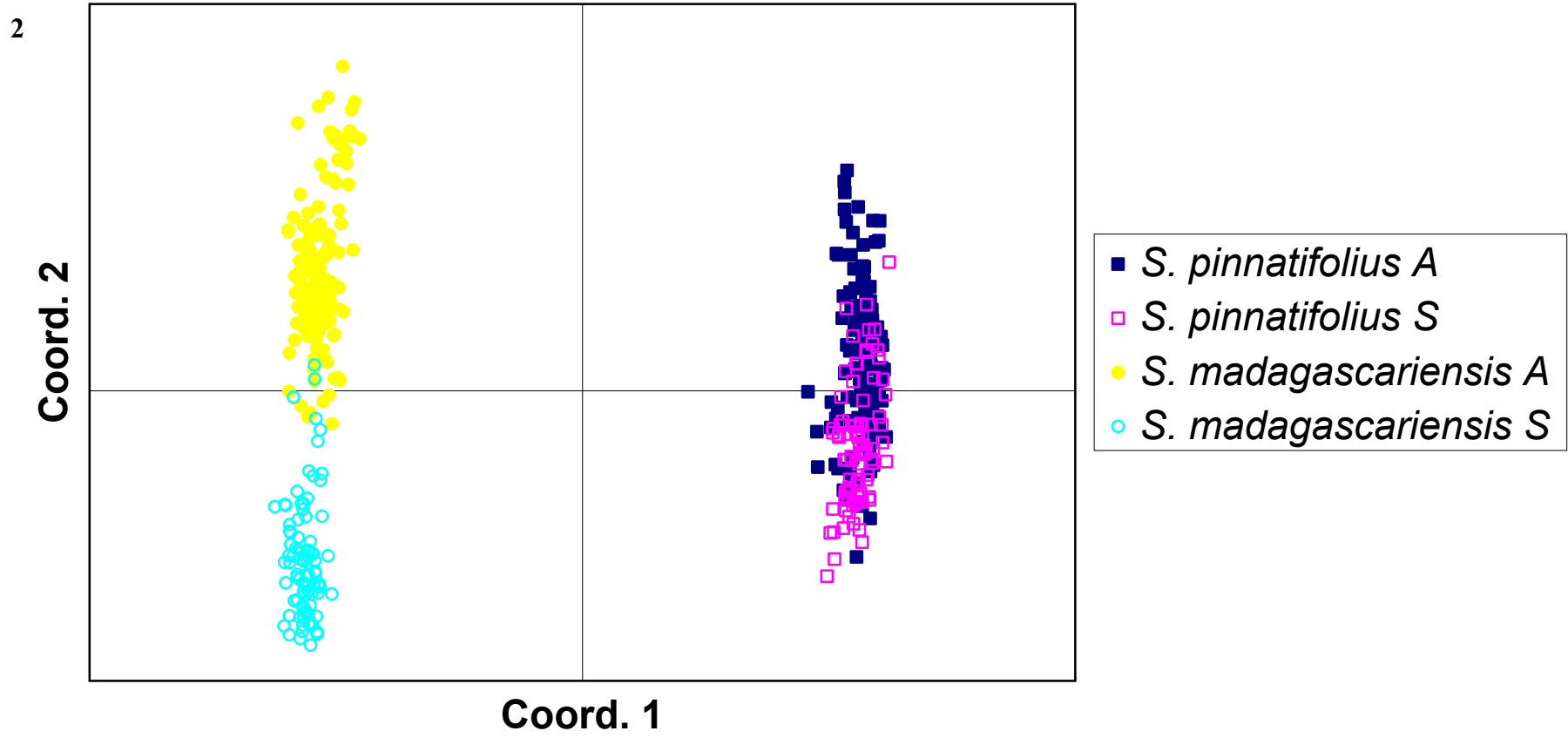
O (*Sp*)^S S2 (*Sp*)^A S1 (*Sp*)^A
QM (*Sp*)^S H (*Sp*)^A

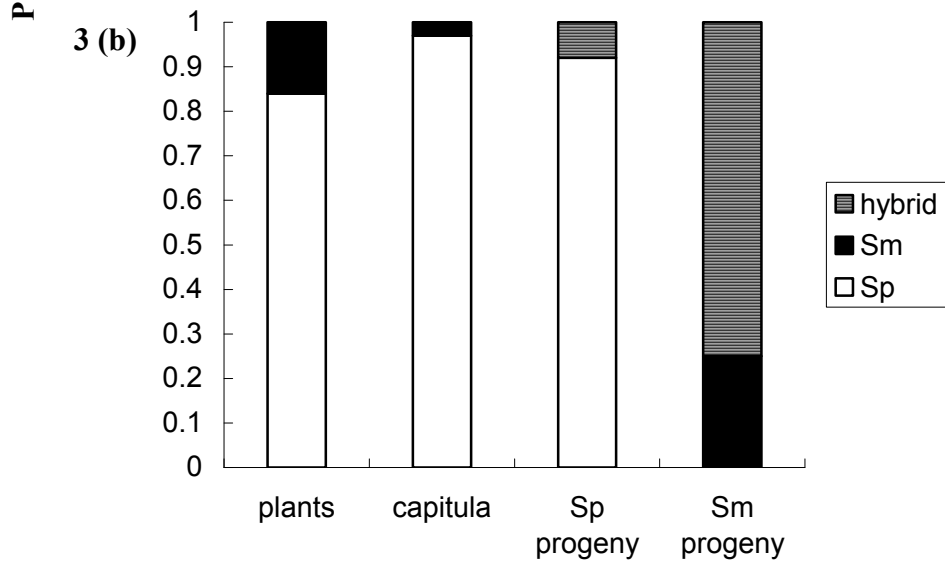
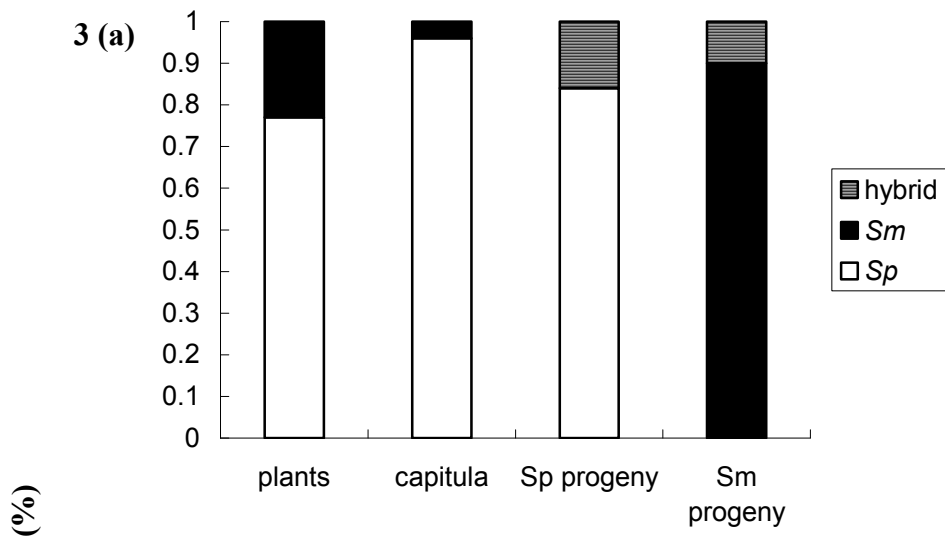
B (*Sm*)^A
QM (*Sm*)^S O (*Sm*)^S T (*Sm*)^A S (*Sm*)^A

0.1

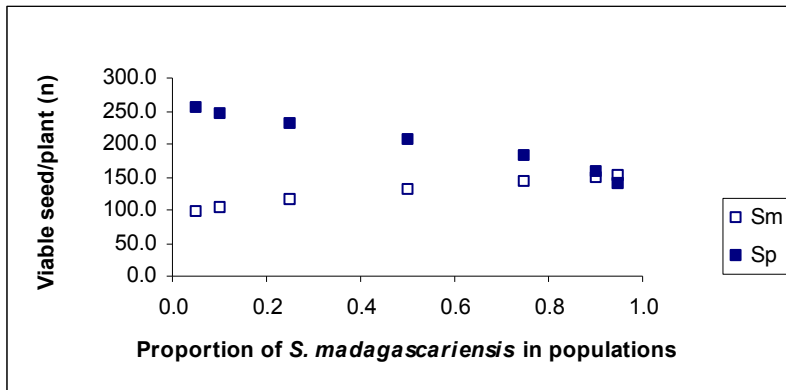
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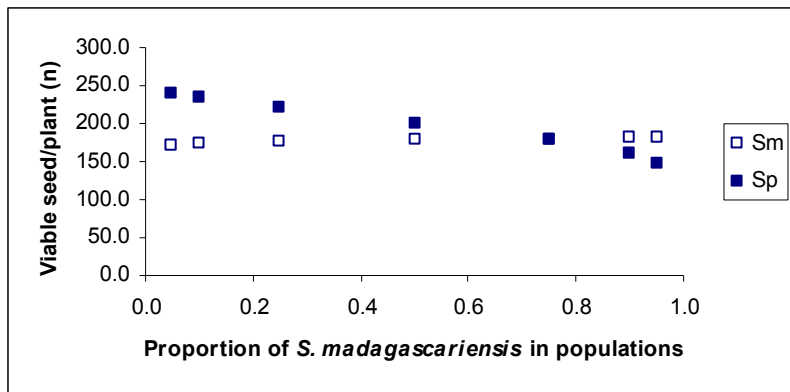




4 (a)



4(b)



4(c)

