Reproductive biology of the Magenta Lilly Pilly *(Syzygium paniculatum)* **and its implications for conservation**

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TABLE OF CONTENTS

Abstract

The Magenta Lilly Pilly (*Syzygium paniculatum*), endemic to a narrow strip along the New South Wales coast, is currently listed as vulnerable at both state and national levels. At present management of the species focuses on minimizing currently known threats, such as weed invasion, while little is known about the reproductive biology of the species. *S. paniculatum* is the only recorded polyembryonic Australian species of *Syzygium;* polyembryony being the development of multiple (and often asexual) embryos in one seed. Nuclear microsatellite markers were used to investigate the genetic outcome of polyembryony on the reproductive and population biology of the species focusing particularly on the population located on The Entrance Peninsular. Low within-population diversity was found, with low heterozygosity levels and a low level diversity indices when compared to other rare or rainforest species. Multiple embryos from single seeds were found to be identical to the mother. Multiple embryos

germinated and survived but one seedling was always significantly taller than all others in the seed but was not considered sexual. It was concluded that the rare *S. paniculatum* is an apomictic clonal species with extremely low genetic diversity.

1 Introduction

1.1. *Syzygium paniculatum*: a brief description

Syzygium paniculatum Gaertn., commonly known as Magenta Lilly Pilly, is a small to medium tree in the family Myrtaceae. *S. paniculatum* is endemic to New South Wales, occurring along a narrow, linear, coastal strip in five separate geographical areas: Jervis Bay, Coalcliff, Botany Bay, Central Coast and Seal Rocks (Figure 1). At Jervis Bay and Coalcliff, *S. paniculatum* grows in remnant stands of littoral rainforest in grey soils over sandstone. At Botany Bay, the Central Coast, and Seal Rocks, the species can be found growing in remnant littoral rainforest patches and riverside gallery rainforests on sandy soils, stabilized dunes, gravels, silts or clays. Plants produce clusters of white flowers at the ends of branches, usually between November and February. The fruits are deep magenta, maturing from March to June, and containing a single seed, which is often polyembryonic (containing more than one embryo, see Figures 2, 3, 4 & 5).

S. paniculatum is of conservation significance as it is listed as endangered under NSW Threatened Species Conservation Act 1995 (TSC Act) and vulnerable under Federal legislation. If appropriate conservation of *S. paniculatum* is to take place, to increase population numbers and protect the species from extinction, natural populations need to be studied to determine genetic diversity, breeding strategy and fitness potential of offspring.

An understanding of the reproductive biology of this species is likely to be crucial because the polyembryonic seeds may have arisen through asexual apomixis.

If polyembryony in *Syzygium paniculatum* is a product of apomixis, it is likely to have substantial implications for any recovery plan because apomixis may reduce genetic diversity within each population and increase genetic divergence between populations.

Although uncommon in native vegetation communities, the species is widely cultivated and a number of cultivars are on sale commercially. Genetic distinctiveness and reproductive biology could also have important implications for potential interactions between cultivars and natural populations.

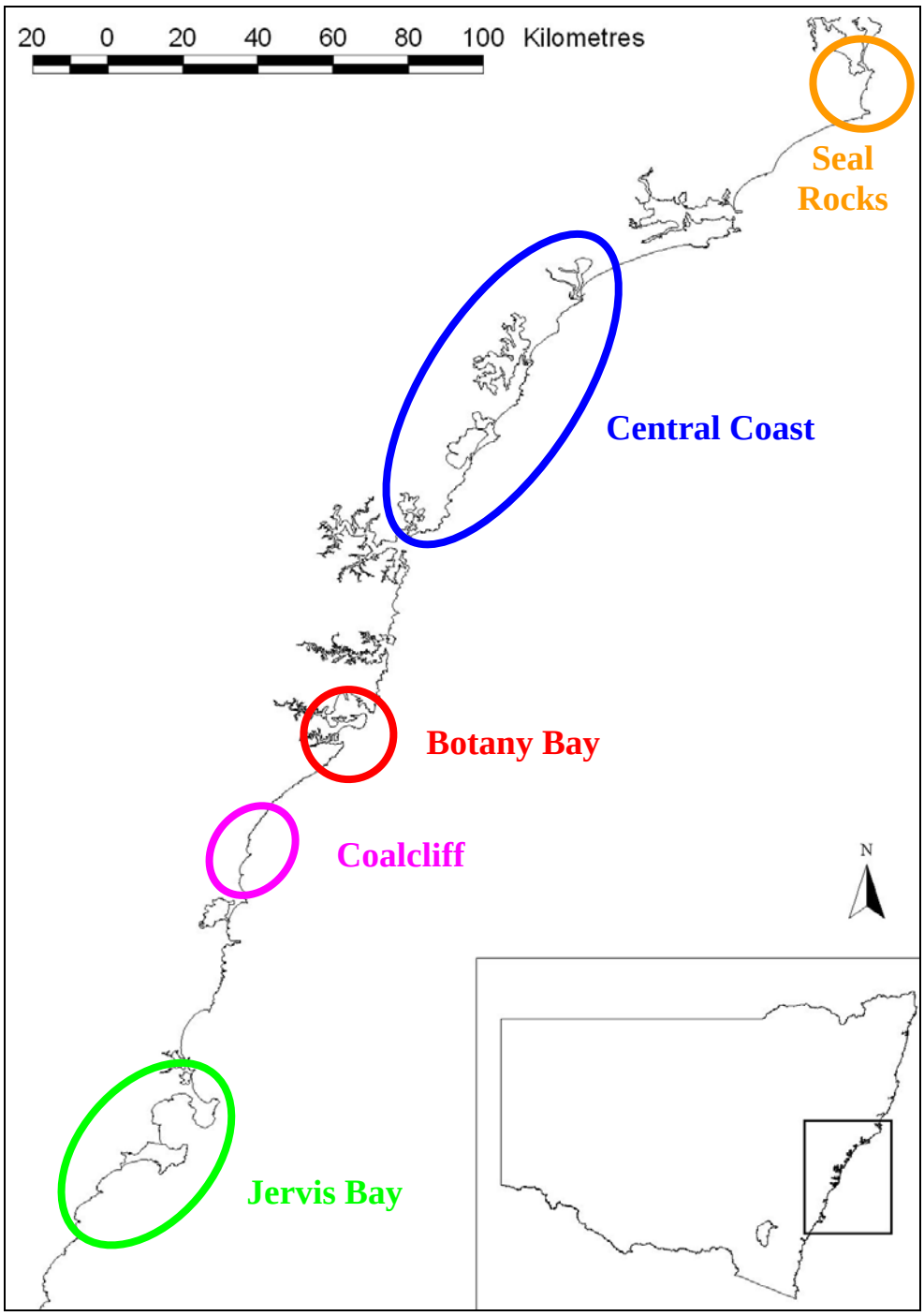


Figure 1. Distribution of *S. paniculatum* along the NSW coast and indicates the five geographically separated areas in which *Syzygium paniculatum* occurs; Seal Rocks (orange), Central Coast (blue), Botany Bay (red), Coalcliff (pink) and Jervis Bay (green).

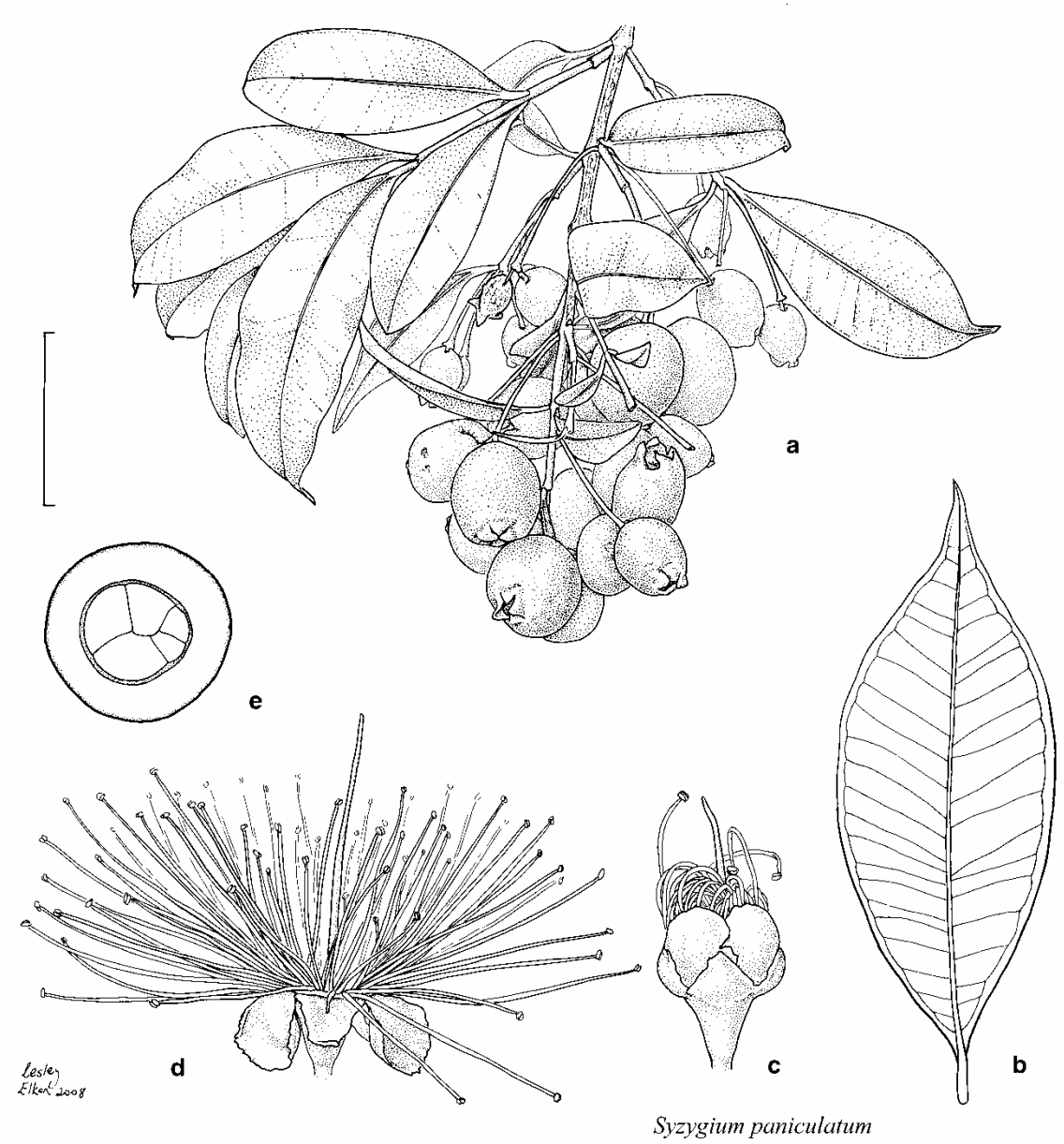


Figure 2. Botanical illustration of *S. paniculatum*. **a.** fruiting branch – scale bar 3cm, **b.** leaf venation – scale bar 2.5cm, **c.** opening bud – scale bar 1cm, **d.** flower side-view – scale bar 1cm, **e.** transverse section of fruit showing seed containing multiple embryos – scale bar 1.5cm. (L. Elkan 2008 © Botanic Gardens Trust)

1.2 Threatened species: population size and diversity

The fragmentation and isolation of habitats, due to human disturbance or geological change, has resulted in the reduction in population size of many species of plants. Small

population size is often a characteristic of threatened species (Frankham, 1996) and has been found to be positively correlated with genetic variation (Leimu *et al.*, 2006). Reduced population size can lead to reduced evolutionary potential due to genetic drift and inbreeding (Frankham, 1999). Genetic drift and inbreeding may result in marked genetic effects for populations such as reduced genetic diversity through changes in allele frequencies and loss of heterozygosity (Allendorf & Luikart, 2007; Frankham, 1996; Frankham, 1999). Thus the potential for rapid erosion of genetic variation may make small populations poor candidates for conservation efforts (Lesica & Allendorf, 1992). However, it is not always the case that rare species with reduced population sizes have low diversity (Gitzendanner & Soltis, 2000; Lesica & Allendorf, 1992; Lewis & Crawford, 1995; Ranker, 1994). Because genetic diversity is more likely to depend on the life history and evolutionary history of species, it cannot be assumed that all rare species will maintain low levels of genetic diversity (Gitzendanner & Soltis, 2000).

1.3 Polyembryony, clonality and *Syzygium paniculatum*

There are a number of different processes by which multiple embryos (polyembryony) can arise in plant seeds (Stebbins, 1941; Naumova, 1992; Ozias-Askins, 2006; Richards, 2003). Polyembryony can occur through the division of a fertilized diploid zygotic embryo (cleavage polyembryony), resulting in the development of multiple yet identical sexually derived embryos. It can also arise through various forms of apomixis, where additional asexual embryos develop from diploid cells, usually alongside the fertilized diploid zygotic embryo. The latter may result in the development of both sexual and asexual embryos (if a zygote develops and survives), or only asexually derived embryos (if the zygote fails to develop or develops and subsequently degenerates or is out-competed). Apomixis can therefore, in some cases, result in the production of high levels of clonal individuals in a

population, who have inherited their entire genome from their mother. This has the potential to drastically reduce the genetic diversity in the population or across the species if the clonal offspring of one mother happen to predominate. Also, if sexual reproduction is reduced (in facultative apomicts) or virtually non-existent (in obligate apomicts), the rate at which new combinations of alleles are made will also be reduced, in effect, hindering potential increase in genetic diversity. Apomicts can acquire genetic variability through means such as somatic mutation or somatic recombination; however most of the genetic variability found within apomictic species is a product of past sexuality (Richards, 2003).

Polyembryony has not been recorded for any other Australian species of *Syzygium*, but has been observed in a number of Asian species: *S. malaccense* (L.) Merr. & L.M.Perry, *S. jambos* (L.) Alston, and *S. cumini* (L.) Skeels. In *S. jambos*, Roy (1953) described the embryos as deriving from diploid cells in the nucellus, the maternal tissue surrounding the embryo sac. In Myrtaceae, formation of triploid endosperm (after pollination) is a source of nutrition to developing sexual embryos and this is also the case for nucellar embryos (Roy, 1961). This pollination requirement suggests that polyembryony in *Syzygium paniculatum* is not brought on by male sterility or lack of pollination. Sahai & Roy (1962) report the formation of a zygote that degenerates soon after fertilization so that only asexually derived embryos remain.

Understanding the immediate genetic outcome of polyembryony in *S. paniculatum*, will help in gaining an understanding of the effect that polyembryony has on population biology; both in *S. paniculatum,* as well as in other species. To determine which type of cells asexual embryos are derived from in *S. paniculatum*, would require a detailed embryological study and this was beyond the scope of this study. Regardless which type of maternal tissue gives rise to asexual embryos, they do not undergo meiosis and hence there is no

recombination to facilitate chromosomal crossover and embryos will be genetically identical to the maternal plant, except in rare cases of somatic mutation or mitotic crossover. By using suitable molecular techniques to study the population genetics of the species and the genotypes of offspring, it may be possible to define the reproductive biology of *S. paniculatum* as either sexual or asexual, or both. If embryos or offspring show genetic evidence of recombination, reproduction in *S. paniculatum* may be defined as sexual. If all offspring are found to be identical to the mother, reproduction in the species may be defined as asexual and the species could be regarded as clonal.

Figure 3. Fruit, seed and embryos of *S. paniculatum*. From left to right: the magenta coloured fruit; a single seed with seed coat left on; three embryos all dissected from a single seed.

Figure 4. Five embryos of varying size, dissected from a single *S. paniculatum* seed, collected from Abrahams Bosom. Each square in the scale bar represent 1 cm.

Figure 5. Polyembryonic seed of *S. paniculatum* with seed coat removed (left) and the same seed dissected to reveal five embryos and an under-developed embryo (right).

1.4 Clonality, fitness and *Syzygium paniculatum*

The process of clonality can be seen as having a fitness trade-off between proliferation of provisionally fit clonal genotypes and the ability to generate potentially fitter

sexually derived genotypes. Plants that maintain purely clonal reproductive habits have the potential for high genetic costs and disadvantages in changing environments (Callaghan et al., 1992). In some species, such as *Syzygium jambos*, polyembryony has been suggested to be so prolific that sexual reproduction is suppressed altogether (Sahai & Roy, 1962). If polyembryony in *S. paniculatum* is also found to be a completely clonal process, with all embryos identical to the parent plant, the number of potential new genotypic combinations will be limited. This could make the species even more vulnerable to future environmental change than originally thought. Even if some sexual reproduction is occurring alongside clonal reproduction, the consequence could still be a dramatic reduction in genetic diversity (Richards, 2003; Silvertown, 2008).

1.5 Ploidy in *Syzygium paniculatum*

Polyploidy is known to be linked to polyembryony and apomixis. It is thought that polyploidy may serve as a barrier for sexual reproduction (Andrew *et al.*, 2003; Lo *et al.*, 2009; Ozias-Askins, 2006; Van Der Hulst *et al.*, 2003; Van Dijk & Van Damme, 2000) whilst duplicate genes may be expressed asynchronously causing multiple embryos to arise (Carman, 1997). Alternatively, it has also been suggested that the occurrence of apomixis can actually promote polyploidy (Richards, 2003).

It is possible that polyembryonic *S. paniculatum* may also be a polyploid. In the Myrtaceae, diploid ($2n$=22) is the most common ploidy level but polyploidy has been documented, mainly in fleshy-fruited species (Da Costa & Forni-Martins, 2006 & 2007). The polyembryonic species, *Syzygium jambos*, has been found to be tetraploid ($2n$=44) (Oginuma *et al.*, 1993; Roy, 1953; Singhal *et al.*, 1985). However, diploid apomicts do exist (Bicknell, 1997) so polyploidy in *S. paniculatum* is speculative at best and needs to be investigated.

1.6 Applicability of nuclear microsatellite markers

The most suitable molecular markers for population studies are nuclear microsatellite markers (nSSRs) since they show co-dominance, simple Mendelian inheritance, high rate of polymorphism and mutation, abundance throughout the genome, reproducibility and relative ease of screening (Rossetto, 2001). They are also suitable for studying parentage, when the reproductive biology of a species is essentially unknown, but clonality is considered a possibility (Jones *et al.*, 2009).

1.7 Project outline

This research project will focus on the largest and better documented population (Payne 1991) located on the North Entrance Peninsula (c. 33º 18' 30″S, 151º 31' 10″E). The population will be sampled at three hierarchical levels in order to obtain information on the occurrence of apomixis, and on its potential effect on native populations as well as on the horticultural trade.

We will test mature trees as a representation of genetic diversity across the population. We will test embryos to determine the likely origin (whether sexual, asexual reproduction or both, occur in *S. paniculatum)* We will also test embryos to assess germinability and fitness and the likely survival of apomictic vs. zygotic embryos within controlled conditions (i.e. information relevant to the horticultural trade) and within natural conditions (i.e. within the representative individuals sampled; information relevant to the conservation of this rare species).

Highly informative molecular tools (microsatellite loci) will be used to obtain the relevant genetic information without the need for complex morphological and

developmental investigations. Co-dominant and highly variable molecular tools such as microsatellites (SSR) are particularly suited to these investigations as changes in allelic frequencies and heterozygosity measures can differentiate between outcrossed, selfed and apomictic progenies.

This is a unique approach, not been attempted before on a rare tree and likely to provide relevant information for conservation and management. For instance, cultivated material could include greater levels of apomixis than natural populations, and since *S. paniculatum* is a popular garden plant, dispersal of cultivated material onto native populations could potentially result in significant losses of natural diversity.

This project will provide enhanced understanding of genetic diversity at the population level, indication as to whether apomictically derived embryos can be used in propagating individuals with a particular genotype rather than vegetative propagation, and results relevant for the development of conservation and management strategies for wild populations and cultivated plants

2 Materials and Methods

2.1 Obtaining and analyzing diversity data

2.1.1 Sample collection

Leaf sample collections were made at the main study site, The Entrance, with the aim of collecting over 30 individuals across the site.

In addition to leaf collections from The Entrance, collections were also made for ten other populations of *Syzygium paniculatum* (Figure 6). At two populations, Captain Cook Drive and Wamberal Lagoon this included wild juveniles. . Additional fruit samples were also collected at Abrahams Bosom, Towra Point and Cams Wharf, to supplement those collected at the Entrance (as fruit set was generally low in the two years the project was running and the proposed targets could not be achieved). Collections at additional populations was work extra to the AFF funded project, and was conducted as part of an Honours project (Katie Thurlby, UNSW) which is currently in preparation for publication.

2.1.2 DNA preparation

Total genomic DNA was extracted from dried leaf tissue and frozen embryo tissue using the Qiagen® DNeasy® 96 Plant Kit protocol. Nine nuclear microsatellite markers were developed for *S. paniculatum* and were used to determine genotypes of adults in wild populations as a measure of diversity and to study the parentage or breeding system through offspring genotypes. Preliminary genotyping results showed extremely low variation, so primers were also tested in eight diverse species of the tribe Syzygieae, including the polyploid, *Syzygium jambos*.

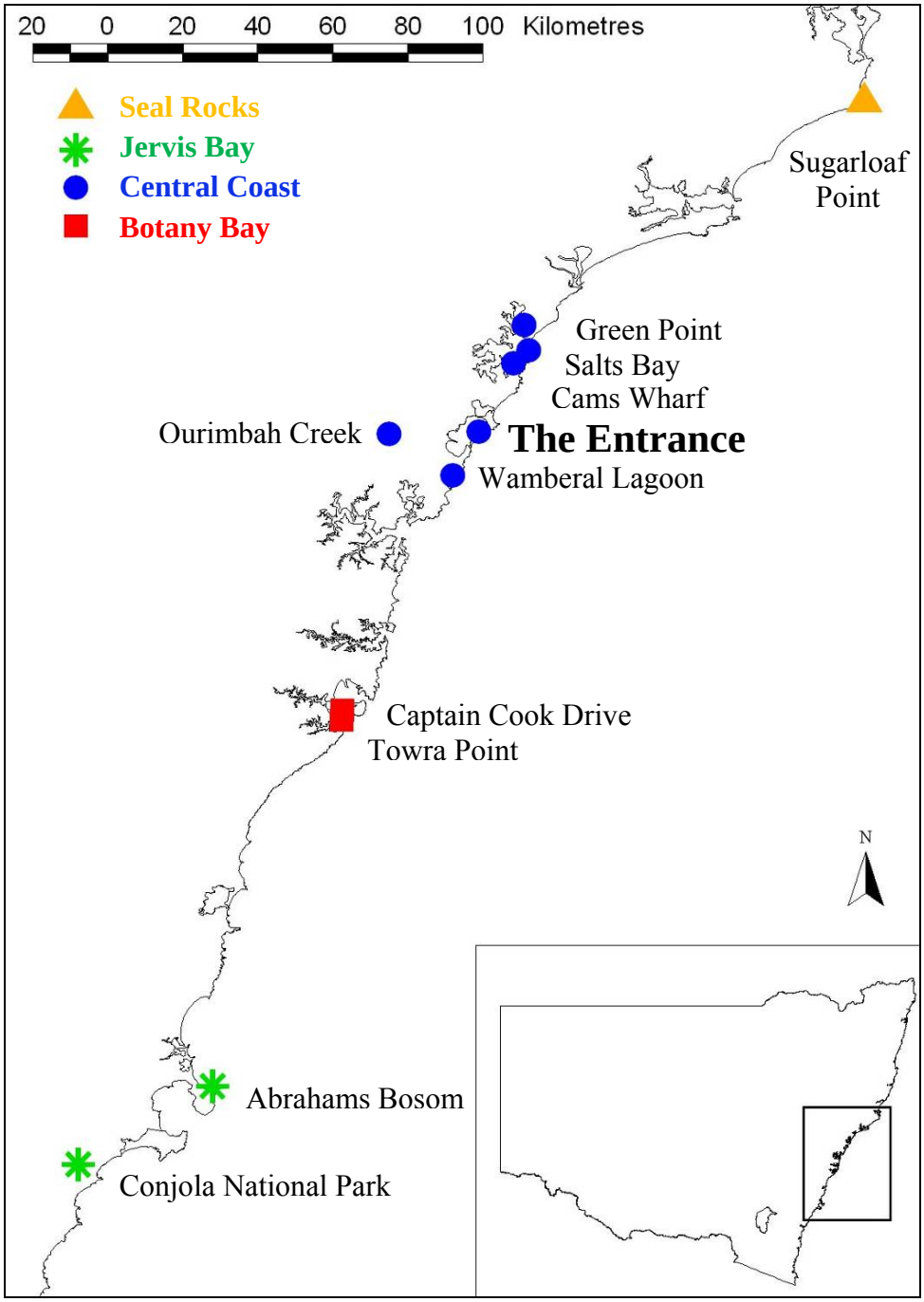


Figure 6. Populations sampled are grouped according to the geographical area by coloured symbols: Seal Rocks (orange triangle), Central Coast (blue circle), Botany Bay (red square), Jervis Bay (green star).

2.1.3 Ploidy identification

Genotyping traces were analysed in Genemapper 4.0 (Applied Biosystems) to look for similarities between species in microsatellite peak patterns, which may serve as an indicator for ploidy level. Genotyping traces of the known tetraploid *Syzygium jambos* and assumed diploid species, *S. corynanthum* and *S. francisii*, were compared with genotyping traces of *S. paniculatum*. Chromosome counts were also attempted, however time and resources did not allow for optimization of the necessary techniques and as such, chromosome counts are yet to be completed.

2.1.4 Analysis of diversity data

The preliminary results for ploidy identification indicated that the species might be tetraploid; having four copies of the genome rather than the usual two, making a co-dominant analysis approach difficult. Standard analysis packages such as GenAlEx (Peakall & Smouse, 2005) require determination of microsatellite allele copy number. In diploid species, determining allele copy number is simple because a heterozygote will yield two peaks (two different microsatellite lengths) in a genotyping trace, whilst a homozygote will yield one peak (two copies of the one microsatellite length). For tetraploid species, with two copies of a microsatellite from each parent, determining allele copy number is not always possible. For this reason, diversity statistics were calculated using the program ATETRA (Van Puyvelde et al., 2009) which is designed specifically for analysing tetraploid data. Use of ATETRA required an assumption of ploidy level. After preliminary testing, tetraploidy was inferred for *Syzygium paniculatum* however, ploidy should ideally be confirmed by chromosome counts.

Also, following other studies of clonal polyploid species (Andrew *et al.*, 2003; Samadi *et al.*, 1999) and to confirm results obtained through ATETRA, a dominant binary approach was also taken. This approach uses all the alleles present in the population to construct a genetic barcode for each individual by scoring alleles across all microsatellite loci as present or absent. Data could then be used in the program GenAlEx (Peakall & Smouse, 2005) to perform various data analyses.

2.2 Obtaining and analyzing germination and reproduction data

Fruit samples were collected from some individuals at The Entrance and were dissected and used for both the reproduction and germination trials. Fruit samples were also collected from Abrahams Bosom, Towra Point and Cams Wharf, for use in the Honours project (Katie Thurlby, UNSW) to supplement the data obtained at The Entrance.

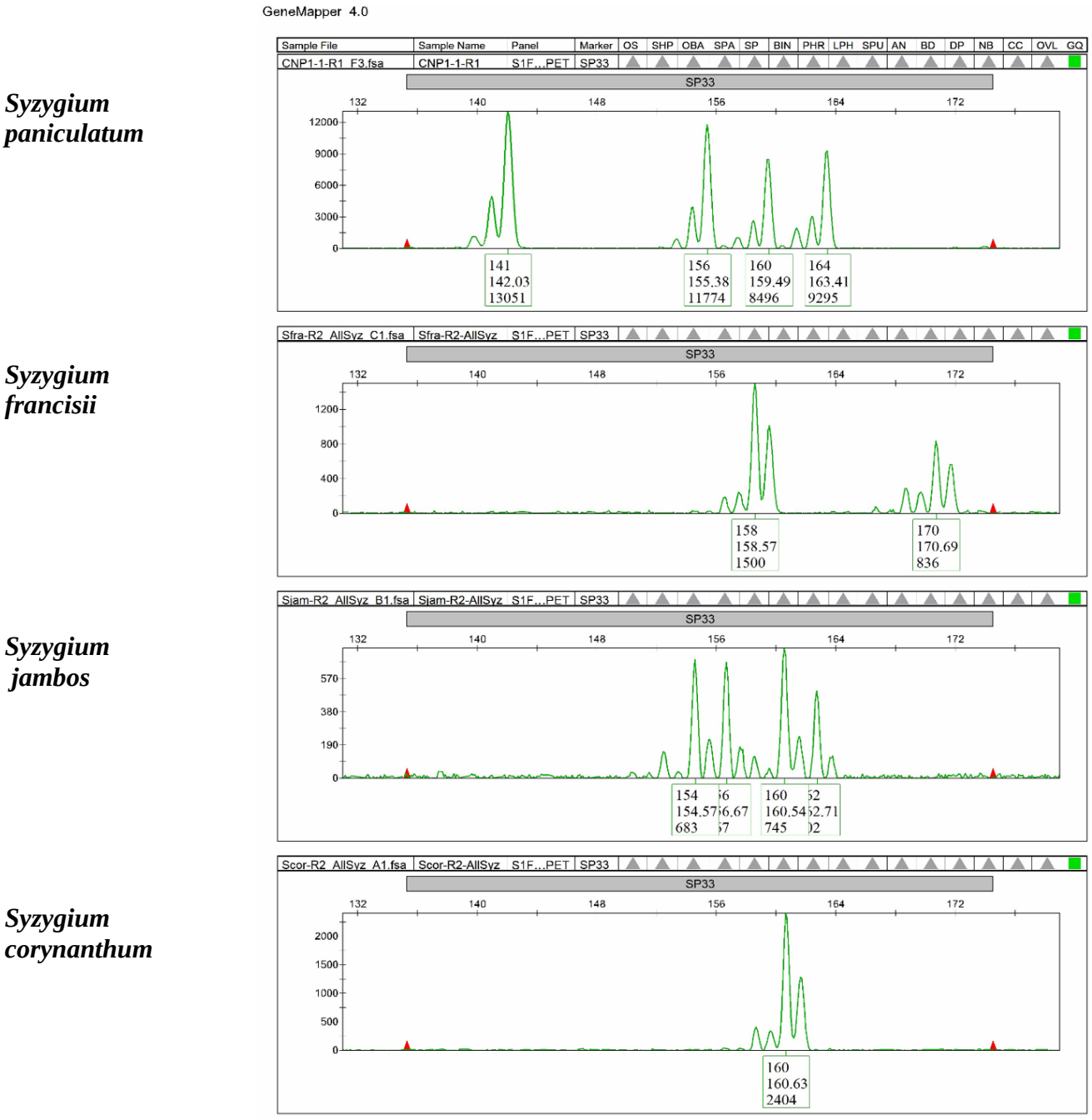
The germination and reproduction trials were designed to investigate the fitness and parentage of embryos. Both seeds with single and multiple embryos were germinated. The first embryo to germinate from each polyembryonic seed was recorded and measurements of seedling leaf number and height were taken every three to six weeks. Statistical analysis was then performed.

Leaf samples were then taken from germinated seedlings, DNA extracted and genotyping conducted. Genotypes of each individual seedling were compared to other individual seedlings originally belonging to the same seed (family groups) to look for allelic differences which may suggest the occurrence of either sexual reproduction or recombination. Embryo material was also collected and DNA extracted however, the DNA obtained from embryos was not of satisfactory quality for use in genotyping and as such, only leaf material from the germinated embryos could be successfully genotyped.

3 Results

3.1. Ploidy

The genotyping data for *Syzygium paniculatum* showed four allele peaks at the SP33 microsatellite locus and three allele peaks at the SP38, SP54 and SP86 loci, suggesting polyploidy (possibly tetraploid). *Syzygium jambos* (known tetraploid) also showed four alleles at marker SP33. The two other *Syzygium* species, S. *corynanthum* and S. *francisii*, showed normal diploid patterns of one (homozygous) and two (heterozygous) peaks respectively (Figure 7).



Syzygium paniculatum

Syzygium francisii

Syzygium jambos

Syzygium corynanthum

Figure 7. Genotyping traces for one microsatellite locus (SP33). *Syzygium paniculatum* (complete homozygous tetraploid= 4 peaks), *Syzygium francisii* (assumed heterozygous diploid =2 peaks), *Syzygium jambos* (complete heterozygous tetraploid= 4 peaks) and *Syzygium corynanthum* (assumed homozygous diploid=1 peak).

3.2. Genetic Diversity

Both low heterozygoisty and extremely low genetic diversity were found at The Entrance. Of the 31 individuals genotyped across nine microsatellite loci, 30 possessed an identical genotype with the single remaining individual possessing a genotype that showed allelic differences at four of the nine loci. These differences were three instances of homozygosity (in loci that were heterozygous in all other samples) and one instance of a 2bp change. These allelic differences may be attributable to a rare sexual or selfing event, a remnant of past sexual reproduction or a genotyping error.

Between population data, obtained as part of the honours project (Katie Thurlby et al., unpubl), showed that genotypes were distributed along a geographic gradient. It also showed that the main genotype found at The Entrance is the most common genotype across the entire species, being present in 70.89% of individuals. The majority of divergences from the most common genotype occurred in northern populations. All statistical tests showed the southern populations (most of which contain only the most common genotype) grouped together with northern populations as outliers. Almost all variation between populations is explained by this southern grouping. Variation was not found to be related to current population size, as the largest populations (The Entrance and Wamberal Lagoon) exhibited proportionally less variation than smaller populations.

3.3. Reproduction

Embryo dissection confirmed polyembryony, with embryo number ranging from one to nine per seed. In most seeds there is one large embryo, with each subsequent embryo diminishing in size.

Leaves were taken from seedlings germinated ex-situ and family groups were genotyped. Embryos proved extremely difficult to genotype, probably due to the high carbohydrate levels in seeds. As fruit set was low, data was supplemented by including individuals from other populations collected as part of the aforementioned honours project. One seedling (single embryo in a seed) from The Entrance showed evidence of sexual origin (two loci heterozygous in the parent were homozygous in the offspring and two new alleles were present). Four seedlings germinated from two seeds (two embryos per seed) showed evidence for the sexual origin of one embryo in the seed (multiple new instances of homozygosity) and the asexual origin of the other (identical genotype to parent). This suggests that both sexual reproduction and asexual reproduction is occurring at The Entrance. In other populations tested there was also evidence for the sexual origin of embryos (single embryos or one embryo from a polyembryonic seed) as well as extensive evidence for asexual origin of embryos (including polyembryonic seeds showing no evidence of sexual origin).

Additionally, juveniles from two populations were sampled and genotyped. Of the eight wild juvenile leaf samples only one had a different genotype to the adults sampled at the same location. This individual had a 1bp change at one allele in one microsatellite locus. This single allele change most likely provides evidence of somatic mutation (rather than reproduction) as no changes in heterozygosity were found in the

genotype as would be expected if recombination had occurred. It is possible that the parent plant was not sampled, and as such, that the juvenile is also a single surviving germinant from an apomictic seed.

3.4. Germination Trial

From polyembryonic seeds, multiple embryos germinated and multiple seedlings survived (Figure 8 & 9). Embryos germinated successfully whether they were kept within the bounds of the seed coat or whether embryos were separated. One seedling was always taller than other seedlings arising from the same seed suggesting a possible fitness advantage and this was found to be statistically significant. Seedling height was highly correlated to embryo weight suggesting that embryo size is a determining factor in embryo height. When genotype was taken into account and all sexual embryos compared to all asexual embryos, it was found that sexual seedlings were significantly taller than asexual seedlings; however the test was no longer significant when the smaller embryos from polyembryonic seeds were removed from the test (leaving the largest embryo from a purely asexual seed and the sexual embryo). Seedling height was not correlated to embryo weight in sexual embryos but was found to be highly significant in polyembryonic asexual seedlings. Leaf number and branch number were also recorded but no statistically significant differences were found.

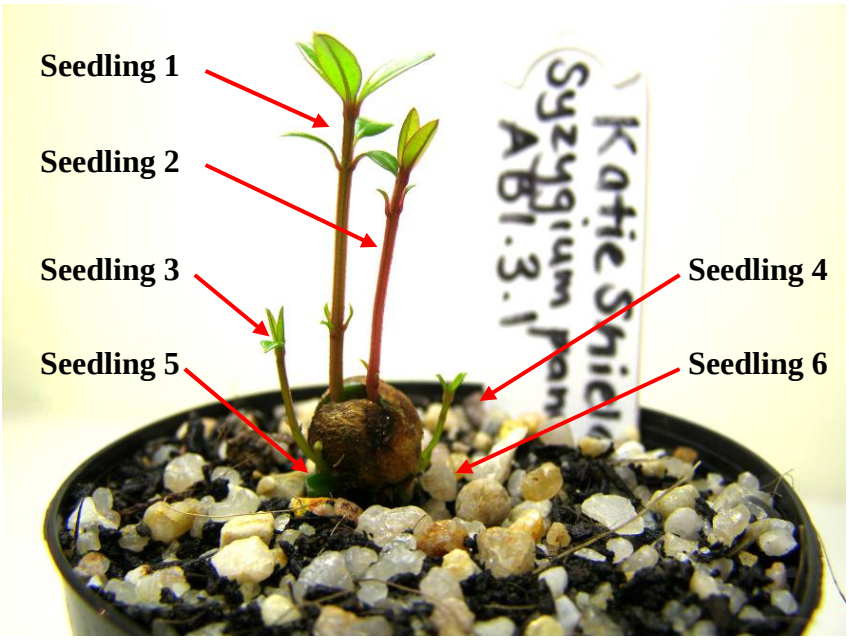


Figure 8. Multiple seedlings arising from a single seed.

Figure 9. Survival of the multiple seedlings (Figure 8) over time. Left to right: Weeks 12, 18, 21 and 27, (photographs not to same scale).

4 Discussion

4.1. Ploidy

The results suggest that because *Syzygium paniculatum* displays similar genotypic patterns to the tetraploid *S. jambos*, *S. paniculatum* is likely to be tetraploid; however, this will need confirmation through chromosome counts. As the multiple alleles were found at four loci, it is unlikely that the multiple alleles are caused by localized gene duplication and more likely that it is a result of whole genome duplication. Although our results do not exclude other higher ploidy levels, tetraploidy should be assumed to be most likely as it would require the least number of events to arise. It is not yet known whether *S. paniculatum* is an allopolyploid, or an autopolyploid.

There is a strongly documented link between polyploidy and apomixis (Bicknell & Koltunow, 2004; Roche *et al.*, 2001) and it is thought that polyembryony might be caused by duplicate genes being expressed asynchronously (Carman, 1997). It is possible that the polyembryony found in *S. paniculatum* is caused by the polyploid nature of the species; however this would need further investigation.

4.2 Diversity

The present study has shown that *S. paniculatum* has extremely low overall genetic diversity. Only nine genotypes were found among eleven populations across the entire geographic range of the species (Katie Thurlby unpubl. obs., 2011). *S. paniculatum* also showed rather low heterozygosity and genetic diversity when compared to other rare species (Katie Thurlby, unpubl. obs., 2011).

There are a number of explanations as to why The Entrance population possesses such extremely low diversity and shares an identical genotype with almost all southern populations of *S. paniculatum*. It is possible that The Entrance population represents one part of a large clonal stand of *S. paniculatum* existing in the southern distribution of the species. This clonal stand is likely to have been broken into smaller populations due to the occupation of *S. paniculatum* in specific costal habitats of littoral rainforest which has been largely cleared or modified for coastal development, agriculture and sand mining (Floyd, 1990). This clearing has reduced population numbers substantially and reduced habitat to small, highly vulnerable fragments (Payne, 1991).

Conversely, low levels of heterozygosity, extremely low within population diversity and almost no diversity, may be an indication of a founder effect. Clonal reproduction, through dispersible apomictic seed, would facilitate the fast spread of genetically identical individuals to colonize and expand populations with any new genetic diversity arising via somatic mutation. It is likely that The Entrance population, along with other southern populations of *S. paniculatum*, was colonized in such a way.

It is possible that The Entrance population of S. paniculatum arose as one of multiple hybridization events. Variability across populations of an apomictic species can be described by the multiple origins of asexual polyploids by hybridization (Soltis & Soltis, 2000). *S. paniculatum* may have arisen in such a manner at a number of different sites where the two parental species co-occur (or did co-occur). This would result in the pattern of unique genotypes which we see across the range of *S. paniculatum* (Katie Thurlby, pers. obs. 2011) and could also explain the existence of polyembryony and polyploidy in the species. However, none of the results obtained from this study are able to directly substantiate this idea and further studies are required.

4.3 Reproduction and Fitness

Reproduction in *Syzygium paniculatum* appears to be asexual or, clonal. This study has shown that embryos produced in seeds of *S. paniculatum* are of both sexual and asexual origin. This supports the view that *S. paniculatum* is a facultative apomictic species i.e. able to reproduce both sexually and asexually, which is more common than obligate apomixis (Asker & Jerling, 1992; Bicknell & Koltunow, 2004). Sexual embryos occurred both in monoembryonic seeds and polyembryonic seeds however most embryos from polyembryonic seeds showed no evidence of sexual origin. It is possible that a sexual embryo develops first in *all* seeds but is *sometimes* out-competed by the proliferation of multiple asexual embryos. If sexual reproduction occurs and the sexual embryo is not out-competed, a sexual individual can survive either along in a seed or alongside asexual embryos. This study found limited evidence for recent recombination in adult populations, suggesting that if there is a sexual embryo it never (or hardly ever) survives in-situ. Also, as a sexual event may occur in as little as 2% of progeny of apomictic species (Bicknell & Koltunow, 2004) the small samples sizes in this study may have resulted in a gross overestimate of the sexual reproduction in the species as a whole.

Apomixis can be influenced by environmental factors such as climate, nutritional supply or competition (Asker & Jerling, 1992) which suggest that environmental influences during particular seasons may result in a higher rate of sexual reproduction than others. Apomicts are often found in highly disturbed areas or where individuals are widely dispersed (Asker & Jerling, 1992), so it is possible that habitat disruption and habitat fragmentation may be an influencing factor for sexuality in *S. paniculatum*.

The results of this study did not find a significant fitness advantage for sexual individuals in terms of height; rather, they suggest that embryo size is a significant contributor to seedling height while embryo origin is not. Sexual reproduction does involve some advantages however,

because through sexual reproduction, species are equipped for relatively fast genetic changes should they be required by a changing environment conversely, the genotype of one sexual seed has one chance to survive whereas the identical genotype of multiple asexual seeds has multiple chances to survive. So it is possible that there underlying are trade-offs between the advantages and disadvantages of different reproductive modes.

4.4 Conservation Implications

The results of this study imply that *Syzygium paniculatum* may have some difficulty adapting to future environmental change. Until now, the species has survived with moderately low overall genetic diversity and extremely low (if any) variation between individuals. This survival is likely to be due to the ability of the species to reproduce prolifically by way of polyembryony and the theoretical fitness of the persistent genotypes in the current environment. It should however be noted, that whilst there is low between-individual variation in the species, the nature of apparent tetraploidy in *S. paniculatum* means that there is high within-individual variation. Nevertheless, the lack of variation between individuals and in the species as a whole, and the apparent asexual reproduction, means that the development of new variation is restricted to the slow process of somatic mutation.

The detection of some sexual reproduction within the species is positive as it means there is potential within the species to adapt genetically to future environmental change however the distinct lack of variation particularly at The Entrance suggests that such sexual individuals rarely survive in-situ.

It is unlikely that the reduced population sizes and low genetic variation found in *S. paniculatum* are completely due to human interference. However, human activity is likely to have

caused significant reduction in the range and population sizes of the species. From a conservation perspective, there is a need to improve environmental conditions and habitats where practicable, not only to preserve current genetic diversity but to give the species the best possible chance for the accumulation of variation in the future, be that by somatic mutation or sexual reproduction. This particularly applies to the northern populations over southern populations, where the majority of known genetic diversity has been found (Katie Thurlby, pers. comm. 2011). Appropriate activities would include habitat protection and management, weed management, and fire management plus on-going monitoring and surveying as well as more extensive research.

In addition to preserving habitats, in the interest of preserving allelic variation, it may be advisable to introduce each of the various different genotypes found across the species into cultivation to make sure that the variation found within the species is not diluted further by the cultivation of only the most common genotype.

Propagation of *S. paniculatum* via seed may be a viable mode of cultivating genetically identical plants as multiple embryos in each seed are easily germinated more reliably than through methods of vegetative cultivation and a high proportion of individuals survive beyond the seedling and sapling stages. Further studies would need to determine the rate of possible sexual events and the practicality of cultivating via seed for large scale production.

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