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ECOLOGICAL AND GENETIC PROCESSES UNDERLYING ALLEE EFFECTS AMONG TREE POPULATIONS IN THE CONTEXT OF DIVERGENT POPULATION HISTORIES

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To My Parents

"LORSQUE TU NE SAIS PAS OU TU VAS, REGARDE D'OU TU VIENS"
- AFRICAN PROVERB -

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SUMMARY

Rare plant species are vulnerable to genetic erosion and inbreeding associated with small population size and isolation due to increasing fragmentation, though the degree to which these problems undermine population viability remains debated. The critically endangered long-lived tropical tree *Medusagyne oppositifolia* is an endemic to the Seychelles with a naturally patchy distribution. The species comprises 90 extant trees in four populations, with only the largest (78 trees) having successful recruitment. Whether recruitment failure in the three small populations is linked to genetic problems associated with fragmentation is not known. It could be shown that genetic diversity is high in three populations, with only the smallest having relatively low diversity. All populations have high genetic differentiation and low pollen and seed dispersal distances (< 100 m). Inter-population pollination crosses from the large donor population to a small recipient population resulted in higher reproductive success relative to within-population crosses. These results demonstrate the potential for genetic and ecological rescue to support conservation of plant species with limited gene flow.

Historically widespread species with limited gene flow may be particularly vulnerable to the negative genetic effects of forest fragmentation and small population size. *Vateriopsis seychellarum* (Dipterocarpaceae) is a formerly widespread canopy tree of the Seychelles, but is now reduced to 132 adult individuals distributed in eleven sites. A genetic inventory of all adult trees and 317 sampled progeny shows that despite its restricted range, overall genetic diversity was relatively high but the juvenile cohort had significantly lower genetic diversity than adults. Overall low historical (< 150m) and contemporary gene flow (seeds disperse < 25 m, pollen disperse < 50 m) was detected. The molecular data confirm that two populations were derived from self-fertilised offspring from a single tree. These selfed progeny produce viable offspring. Despite extreme genetic bottlenecks self-compatibility may provide *V. seychellarum* with some resistance to the genetic consequences of habitat fragmentation, at least in the short term.

Glionnetia sericea is an endemic and vulnerable tree species restricted to habitats between 400m and 900m in the Seychelles. Due to highly mobile and specialized pollinators (*Agrius convolvuli* and *Cenophodes tamsi*) the potential for the species persistence in a fragmented landscape is high. The results show no decrease in genetic diversity from adult to seedling stage and no increase in genetic structuring despite habitat fragmentation. Historical and contemporary gene flow between populations is apparent despite low general seed dispersal abilities. Artificial cross pollinations between populations did not lead to an enhanced fruit or seed set compared to within-population crosses. Thus, the current and

historical gene flow due to a mobile pollinator seems to have maintained genetic diversity and population connectivity in a rare and restricted endemic of the Seychelles.

A literature review indicated that widespread species are more prone to negative genetic and ecological consequences of habitat fragmentation compared to historically fragmented species. Furthermore, negative effects to habitat fragmentation could also be associated with short distance gene flow in formerly widespread species. These finding could, in part, also be observed for *Medusagyne oppositifolia, Vateriopsis seychellarum* and *Glionnetia sericea*. The formerly widespread species, *Vateriopsis seychellarum* showed a significant loss in genetic diversity and increased genetic differentiation from adult to seedling stage due to short distance gene dispersal. The historically patchily distributed species *Glionnetia sericea* showed no significant negative response to habitat fragmentation but long-distance pollen flow. The historically fragmented species *Medusagyne oppositifolia* showed no negative response at least in its largest population. These findings demonstrate that population histories are important factors that need to be considered when exploring trees' responses to habitat fragmentation. The positive effects of long distance gene flow, underlines the need to protect whole ecosystems and not only species, especially for insect pollinated trees.

ZUSAMMENFASSUNG

Seltene Pflanzenarten sind durch verstärkte Fragmentierung ihrer Habitate anfällig für genetische Verarmung und Inzucht durch kleine und isolierte Populationen, auch wenn das Ausmass in welchem dies die Vitalität der Populationen minimiert noch unklar ist. Der vom Aussterben bedrohte, tropische Baum *Medusagyne oppositifolia* ist eine endemische Art der Seychellen mit einer natürlich fragmentierten Verbreitung. Die Art besteht aus 90 Individuen in vier Populationen, und nur die grösste Population (78 Bäume) hat Nachkommen. Ob das Fehlen von Nachkommen in den drei kleinen Populationen durch genetische Probleme, in Verbindung mit Habitat Fragmentierung, verursacht wird ist nicht bekannt. Es wurde gezeigt, dass die genetische Diversität, mit Ausnahme der kleinsten Population, hoch ist. Alle Populationen haben eine hohe genetische Differenzierung und eine geringe räumliche Pollen Verbreitung (< 100 m). Bestäubungsversuche mit Pollentransport von einer grossen zu einer kleinen Population ergaben einen höheren Fortpflanzungserfolg als Bestäubungsversuche innerhalb einer Population. Diese Ergebnisse zeigen, dass durch „Genetic Rescue" ein Beitrag zum Schutz von Baumarten mit geringen Genflussraten gemacht werden kann.

Historisch weit verbreitete Arten mit geringen Genflussraten könnten besonders von der Fragmentierung ihrer Habitate und kleinen Populationsgrössen gefährdet sein. *Vateriopsis seychellarum* (Dipterocarpaceae) ist solch eine historisch weit verbreitete Baumart der Seychellen, welche heute nur noch mit 132 adulten Individuen in elf Populationen vorkommt. Eine genetische Analyse aller adulten Bäume und 317 Nachkommen zeigte, dass trotz der kleinen Populationen die genetische Diversität relativ hoch war, die Nachkommen aber eine signifikant niedrigere genetische Diversität als adulte Bäume hatten. Insgesamt wurden geringe historische (< 150m) und rezente Genflussraten ermittelt (Samenverbreitung < 25 m, Pollenfluss < 50 m). Die genetischen Daten bestätigen dass zwei Populationen aus selbst-befruchteten Nachkommen eines einzigen Baumes stammen. Diese Nachkommen zeugen ihrerseits fertile Nachkommen. Trotz des starken genetischen Flaschenhalses könnte die Fähigkeit zur Selbst-Befruchtung *Vateriopsis seychellarum* wenigstens vorläufig eine gewisse Resistenz gegen Habitat Fragmentierung verschaffen.

Glionnetia sericea ist eine endemische und gefährdete Baumart der Seychellen, begrenzt auf Habitate in Höhen von 400 – 900 m. Durch sehr mobile spezialisierte Bestäuber (*Agrius convolvuli* and *Cenophodes tamsi*) hat diese Art das Potential in fragmentierten Landschaften zu überdauern. Die Ergebnisse zeigen keine Reduktion der genetischen Diversität oder Zunahme der genetischen Differenzierung trotz des fragmentierten Habitats. Es besteht historischer und rezenter Genfluss zwischen Populationen trotz geringer räumlicher Samenverbreitung. Bestäubungsversuche zwischen Populationen hat nicht zu einem erhöhten

Fortpflanzungserfolg geführt. Daher scheint es als haben die mobilen Bestäuber die genetische Diversität und Kohärenz der Populationen in dieser endemischen Seychellen Art erhalten.

Eine Literaturauswertung verdeutlichte, dass weit verbreitete Arten anfälliger für negative genetische und ökologische Folgen sind als historisch fragmentierte Arten. Des Weiteren konnten bei historisch weit verbreiteten Arten negative Effekte der Habitat Fragmentierung mit kurzen Genflussraten in Verbindung gebracht werden. Diese Ergebnisse konnten auch zum Teil in *Medusagyne oppositifolia, Vateriopsis seychellarum* und *Glionnetia sericea* beobachtet werden. Der ehemals weit verbreitete Baum *Vateriopsis seychellarum* zeigte durch geringe Genflussraten einen signifikanten Rückgang in der genetischen Diversität und erhöhte genetische Differenzierung der Nachkommen auf. Der natürlich fragmentierte Baum *Glionnetia sericea* zeigte keine negativen Effekte durch die Fragmentierung seiner Habitate, dafür aber ausgedehnte Pollenflussraten. Der ebenfalls historisch fragmentierte Baum *Medusagyne oppositifolia* zeigte in seiner grössten Population keine negativen Effekte. Diese Ergebnisse veranschaulichen, dass die historische Verbreitungsgeschichte von Arten ein wichtiger Faktor ist der bei der Betrachtung von Folgen der Habitat Fragmentierung beachtet werden muss. Die positiven Effekte von weiten räumlichen Genflussraten verdeutlicht die Notwendigkeit, besonders bei Insektenbestäubten Bäumen, ganze Ökosysteme zu schützen und nicht nur einzelne Arten.

General introduction

HABITAT FRAGMENTATION AND ITS ECOLOGICAL AND GENETIC CONSEQUENCES FOR TREE SPECIES

Anthropogenically induced habitat fragmentation represents a major long-term threat to global biodiversity and might influence species' capabilities to survive in their natural habitats (Frankham 1995; Ross *et al.* 2002; Young *et al.* 1996). Extensive deforestation, particularly in tropical areas, has led to reductions in population sizes of many species (Aldrich & Hamrick 1998; Benitez-Malvido & Martinez-Ramos 2003; Bruna & Kress 2002; Cayuela *et al.* 2006).

Forest tree species show a wide range of responses to forest fragmentation (Bacles & Jump 2011; Kramer *et al.* 2008), with species vulnerabilities depending on several reproductive and demographic factors (Ghazoul 2005). A fragmented environment is often shaped by the survival of species in small and isolated populations. Population isolation is known to lead to more frequent inbreeding, genetic erosion, and the loss of rare alleles which might negatively affect seed production and seedling viability (Cascante *et al.* 2002; Ghazoul *et al.* 1998; Sebbenn *et al.* 2011). The loss of reproductive individuals might also alter pollen movement among remaining trees and populations, and greatly reduce effective population size (Loveless & Hamrick 1984).

There is substantial evidence to support the relationship between population size, genetic diversity and plant survival (Frankham 1996; Leimu *et al.* 2006; O'Grady *et al.* 2004). Indeed, Honnay & Jacquemyn (2007), found a positive correlation between population size and genetic diversity. Other meta-analysis support this finding stating that population size is positively correlated with genetic diversity and fitness (Leimu *et al.* 2006; Reed 2005; Reed & Frankham 2003). Such Allee effects (declines in reproduction that is disproportionate to the decline in population size) contribute to reduced efficiency of pollination and seed dispersal processes (reviewed by Ghazoul 2005). Still, there seem to be controversies as to whether rare, widespread, endemic or non-endemic species will be more prone to negative genetic

consequences of habitat fragmentation. Common species and large populations were found to be as, or even more, susceptible to the loss of genetic diversity through habitat fragmentation compared to rare species and small populations (Angeloni *et al.* 2011; Honnay & Jacquemyn 2007). There is, however, variability on tree species' genetic responses to habitat fragmentation. Tree species have been suggested to be especially resistant to negative genetic effects following habitat fragmentation (Hamrick 2004), although Angeloni et al. (2011) demonstrated that tree species express more inbreeding depression following habitat fragmentation than shrubs and herbs. These examples show the need for more research exploring the diverse impacts of habitat fragmentation on different species.

The magnitude of consequences to habitat fragmentation may depend on multiple factors including: population history, mating system, ability to purge deleterious effects following inbreeding and gene dispersal ability. Only few studies have explored the importance of population distribution histories for a species' vulnerability to the negative consequences of habitat fragmentation (but see Angeloni *et al.* 2011; Brigham 2003; Leimu & Mutikainen 2005). For example inbreeding depression may be lower in populations that have been small for a long time and may have consequently purged deleterious alleles, whereas a recent reduction in population sizes may cause stronger inbreeding depression (Keller & Waller 2002; Lande & Schemske 1985). Thus, species that have survived in naturally small and isolated populations over long time periods may be less vulnerable to habitat fragmentation compared to species occurring in more continuous populations.

CONSERVATION GENETICS: THE ROLE OF RARITY, GENE FLOW AND GENETIC RESCUE FOR PLANT SURVIVAL

In a fragmented environment species with the ability of long-distance gene flow may be resistant to population isolation caused by habitat fragmentation (Couvet 2002). For example, due to long distance insect pollination, small, fragmented populations and even individual trees exchanged pollen and thus stayed connected in *Gomortega keule*, an endangered Chilean tree (Lander *et al.* 2010). Contrarily, species with limited gene dispersal such as the New Caledonian *Araucaria nemorosa* show early signs of genetic erosion when being reduced to small and fragmented populations (Kettle *et al.* 2007). There is further support for a fitness benefit for tree species with the ability for long distance gene flow in literature (Kramer *et al.*

2008; Petit & Hampe 2006). These findings suggest that species with limited gene dispersal may be vulnerable to inbreeding when populations are fragmented (Jones & Comita 2008; Kettle *et al.* 2011).

Genetic rescue describes the increase in fitness of small populations resulting from the alleviation of inbreeding depression by immigrants (Thrall *et al.* 1998). Indeed, studies on plants showed that pollen-mediated gene flow (Richards 2000) and immigrations (Newman & Tallmon 2001) can improve various fitness components. Outside-metapopulation crosses of *Parnassia palustris* led to a significantly higher seed set than within-metapopulation crosses, and this effect was more pronounced in small populations (Bossuyt 2007). Willi et al. (2007) conclude that the benefits of interpopulation outbreeding are likely to outweigh potential drawbacks (such as outbreeding depression), especially for populations that suffer from inbreeding. Thus, in threatened tree species with limited pollen and seed dispersal, whether it be recently fragmented tropical species (Collevatti *et al.* 2010; Collevatti & Hay 2011; Eckert *et al.* 2010) or chronically fragmented temperate species (Dubreuil *et al.* 2010), genetic rescue might be a good strategy to prevent population or even species extinction. So far, to our knowledge, only one other study have tested the potential of genetic rescue on tree species (Seltmann *et al.* 2009), an obvious lack of information considering that 8753 globally threatened tree species (be their rarity natural or anthropogenically caused) exist (Oldfield *et al.* 1998). Ongoing habitat fragmentation and logging, especially in tropical regions (Wright & Muller-Landau 2006), stresses the need for more research on the ecological and genetic consequences of species reductions to small population sizes. The role of long-distance gene flow, mating system and genetic rescue may determine the potential persistence of tree species in fragmented environments.

ISLANDS: BIODIVERSITY HOTSPOTS

Over the past centuries, islands have been of major interest to the scientific community (Darwin 1872; Wallace 1880) they are often small, provide simplicity and harbor inhabitants with special, extraordinary features (Grant 1998) and can serve as "natural laboratories" for fundamental ecological research (Emerson 2002; Whittaker 1998; Witt & Maliakal-Witt 2007). Numbers of endemic and immensely diverse taxa are found on Oceanic islands (Cowie 1996; Kier *et al.* 2009), endemism exceeding 95% (Cowie & Holland 2006), explaining the

high biodiversity found in these biota, fitting the description "Biodiversity hot-spots" (Myers *et al.* 2000; Whittaker 1998).

Anthropogenic influences, especially the introduction of invasive species and habitat loss, cause great damage to these vulnerable ecosystems and have contributed to the extinction of many endemic plant and animal species (Brooks 2002; Coote & Loeve 2003; Everett 2000; Fleischmann 1997; Reaser *et al.* 2007; Sax *et al.* 2002; Simberloff 1995). Indeed, of the recorded number of extinctions (over 800 plants and animals) since 1500, the majority was found to be island species (Frankham *et al.* 2004; Ricketts *et al.* 2005), even though estimated numbers of actual extinctions are much higher, with extinction rates of 100 – 1000 individuals per year (Pimm *et al.* 1995).

The ancient continental islands of the Seychelles consist of a scattered group of 115 granitic and coralline islands in the Western Indian Ocean with a total land area of 438 km². They are listed as a "Biodiversity hot-spot" with an internationally recognized unique flora (over 40% endemism) and designated a Centre of Plant Diversity by UNEP together with WWF, IUCN and WCMC (Myers *et al.* 2000; Stoddart 1984). The elevated endemism on the Seychelles can be explained through extended isolation, as they separated from the Indian subcontinent some 65 million years ago, and from Madagascar some 20–30 million years earlier (Briggs 2003).

Habitat fragmentation and degradation occurred over the past 200 years, further enforced by the introduction of invasive plant species (Fleischmann 1997; Porembski & Barthlott 2000), forcing endemic plant species to retreat to remote, isolated areas with less invasive species, such as granitic inselbergs, providing last refugia for these species. Of the 250 indigenous species in Seychelles, as many as 54 taxa (almost 21%) of the flora are now considered threatened according to the IUCN classification scheme (Carlström 1996). Recently, the threat status has been assessed for the 50 endemic woody plant species and subspecies of the Seychelles. 36 of these species are classified as threatened, eight as near threatened and one as extinct (IUCN 2011). Natural forests no longer exist, except as relict vegetation at the highest altitudes and on inselbergs, according to an INDUFOR study of 1993 (Küffer 2006).

OUTLINE OF THE THESIS

The last remnants of three highly endangered, endemic tree species from the Seychelles are explored: *Medusagyne oppsitifolia* (Ochnaceae), *Vateriopsis seychellarum* (Dipterocarpaceae) and *Glionnetia sericea* (Rubiaceae). These three species occur in fragmented and isolated populations of the Seychelles main Island (Mahé) and show divergent historical distributions. *Medusagyne oppositifolia* is likely to always have occurred in naturally patchy habitats, whereas *V. seychellarum* is thought to have had a widespread distribution. *G. sericea* provides an intermediate species. Ecological and genetic approaches were conducted to determine the genetic and ecological consequences of habitat fragmentation to individual plant fitness and population viability. A combination of field ecological procedures and molecular genetic techniques were used to achieve this aim.

Genetic studies, using neutral genetic markers (microsatellites), reveal the genetic structure and variability of the three species, explaining historic and recent gene flow. Moreover, paternity and parentage analysis of seeds and seedlings over the entire range of the three species allow determination of the magnitude of successful pollen and seed dispersal and reproductive success of populations. To resolve the potential for genetic rescue from between population pollinations (cross-pollination), artificial gene flow experiments were conducted and controlled in nursery experiments for *M. oppositifolia*.

Mahé, with typical inselberg habitats   Mist forest habitat on Mahé

Chapter 2: Genetic rescue of *Medusagyne oppositifolia*. Genetic and reproductive processes are explored in the critically endangered long-lived tropical tree *Medusagyne oppositifolia*. This species is failing to recruit in three of its four populations, recruitment only apparent in the largest population (78 adults). As all populations produce flowers and fruits we evaluate whether recruitment failure is linked to genetic problems associated with fragmentation, and if genetic rescue can mitigate such problems.

Small mature *M. oppositifolia*
tree at Mt. Sebert

Hermaphrodite *M. oppositifolia*
flowers

"Jellyfish tree" fruits

Chapter 3: Forest fragmentation *Vateriopsis seychellarum*. *Vateriopsis seychellarum* is a formerly widespread canopy tree of the Seychelles, now critically endangered and reduced to 132 known individuals, distributed in 11 populations. It is the sole representative of the Dipterocarpaceae family in the Seychelles. We use 10 microsatellite loci and a genetic inventory of all known adult trees (132) and sampled progeny (317) to investigate population history, fine-scale spatial genetic structure (FSGS), mating system and contemporary and historical gene dispersal.

Largest known *V. seychellarum*
tree at the Tea Plantation

Early fruits of *V. seychellarum*

Germinated *V. seychellarum*
seedling at Le Niol

Chapter 4: Gene flow *Glionnetia sericea*. Long-distance gene flow is predicted to counteract negative genetic effects of habitat fragmentation. The patchily distributed and endangered *Glionnetia sericea* remains in small and large populations on Mahé in inselbergs and mist forests habitats at altitudes between 400 m and 900 m asl. Due to its highly mobile pollinators the species may have been able to prevent population isolation despite strong habitat fragmentation. We explore this theory by use of 10 microsatellite loci, exploring contemporary and historical gene dispersal and genetic constitution of adult, seedling and seed cohorts.

G. sericea tree at Mt. Sebert	Young (white) and old (red) flower of *G. sericea*	Early fruiting stage of *G. sericea*
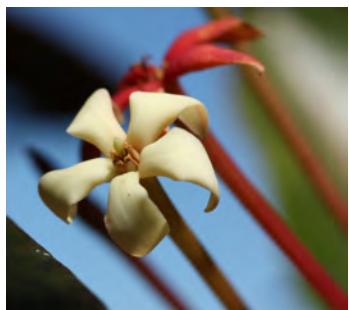
Chapter 5: Population histories and persistence in fragmentation. The importance of population history on tree species in the context of recent habitat fragmentation is evaluated. We explore whether species with a historically patchy distribution (such as *M. oppositifolia*) which have persisted in relatively small populations are less vulnerable to habitat fragmentation compared to species that were historically more widespread and were recently reduced to small population sizes (like *V. seychellarum*). *G. sericea* acts as a comparison with an intermediate species. Furthermore we discuss whether successful long-distance gene flow by pollen or seed will counteract negative genetic consequences of habitat fragmentation irrespective of the degree of habitat degradation or plant specific reproductive systems.

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Back from the brink: Potential for genetic rescue in a critically endangered tree

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ABSTRACT

Rare plant species are vulnerable to genetic erosion and inbreeding associated with small population size and isolation due to increasing habitat fragmentation. The degree to which these problems undermine population viability remains debated. We explore genetic and reproductive processes in the critically endangered long-lived tropical tree *Medusagyne oppositifolia*, an endemic to the Seychelles with a naturally patchy distribution. This species is failing to recruit in three of its four populations. We evaluate whether recruitment failure is linked to genetic problems associated with fragmentation, and if genetic rescue can mitigate such problems. *Medusagyne oppositifolia* comprises 90 extant trees in four populations, with only the largest (78 trees) having successful recruitment. Using 10 microsatellite loci we demonstrated that genetic diversity is high (H_E: 0.48 – 0.63; H_O: 0.56 – 0.78) in three populations, with only the smallest population having relatively low diversity (H_E: 0.26 and H_O: 0.30). All populations have unique alleles, high genetic differentiation, and significant within population structure. Pollen and seed dispersal distances were mostly less than 100 m. Individuals in small populations were more related than individuals in the large population, thus inbreeding might explain recruitment failure in small populations. Indeed, inter-population pollination crosses from the large donor population to a small recipient population resulted in higher reproductive success relative to within-population crosses. Our study highlights the importance of maintaining gene flow between populations even in species that have naturally patchy distributions. We demonstrate the potential for genetic and ecological rescue to support conservation of plant species with limited gene flow.

INTRODUCTION

A potentially negative consequence of habitat fragmentation for rare plants is restricted gene flow between remaining small populations which can lead to genetic depletion and reduced individual fitness (Angeloni *et al.* 2011; Charlesworth & Charlesworth 1999; Leimu *et al.* 2010). In small populations genetic drift and inbreeding can cause the fixation of recessive deleterious alleles leading to the loss of population genetic diversity and further reduction of fitness through inbreeding depression (Van Geert *et al.* 2008). A large body of empirical research has demonstrated causal links between reduction in genetic diversity, elevated inbreeding and reduced fitness in wild plant populations (reviewed by Leimu *et al.* (2006) and in tropical trees specifically by Lowe *et al.* (2005)). Understanding processes that lead to genetic depletion will help to identify possibilities for population and species management. Forest tree species show a wide range of responses to forest fragmentation (Bacles & Jump 2011; Kramer *et al.* 2008), with species vulnerabilities depending on several reproductive and demographic traits (Ghazoul 2005). For example, tree species that have limited gene dispersal may be vulnerable to inbreeding when populations are fragmented (Jones & Comita 2008; Kettle *et al.* 2011).

Genetic rescue is the supplementation of genetically impoverished populations with new individuals (or genotypes) with the purpose of alleviating genetic erosion (Thrall *et al.* 1998) and enhancing population viability (Pimm *et al.* 2006; Tallmon *et al.* 2004). Studies on annual and short-lived perennial plants have demonstrated that pollen-mediated gene flow between populations, and introductions of new individuals into a population, can also improve various fitness components and increase population viability, an effect that appears more pronounced in small populations (Bossuyt 2007; Newman & Tallmon 2001; Richards 2000; Tufto 2001; Willi *et al.* 2007). Despite the growing number of highly threatened long-lived tree species, few studies have investigated the potential for genetic rescue in trees (see Seltmann *et al.* (2009) for an exception), probably because many trees have a general propensity for extensive gene flow (Petit & Hampe 2006).

We explore these ideas in the context of the critically endangered (IUCN 2010) endemic jellyfish tree *Medusagyne oppositifolia* (Ochnaceae) which survives as only 90 adult trees on four isolated inselbergs (massive granitic outcrops) on the island of Mahé, Seychelles (Fig. 1). Only the single largest of these populations is recruiting, the remaining populations only consisting of very few individuals. This taxonomically unique species (Fay *et al.* 1997) is emblematic of the many threatened endemic plant species within the Seychelles and is

granted the highest conservation priority. Despite a naturally patchy distribution along inselbergs, populations of *M. oppositifolia* have been further reduced by anthropogenic influences (fires and invasive species). More generally, *M. oppositifolia* is representative of many threatened plant species and particularly of the 8753 (mostly tropical) tree species that have been evaluated as globally threatened (Oldfield *et al.* 1998). Many tropical tree species exhibit a high degree of habitat specialization, which often results in patchy distributions of more or less isolated subpopulations, each one of which contains relatively few individuals (Ghazoul & Shiel 2010). Such small populations are especially vulnerable to habitat degradation, which further isolates remaining populations to precariously small numbers to the point at which negative genetic effects might become relevant (Lowe *et al.* 2005).

The aim of this study was to determine the extent to which *M. oppositifolia* suffers from deleterious genetic consequences associated with small population size and population isolation. Further, we investigate the possibilities for genetic rescue to increase reproductive output and thus recruitment success in these small isolated populations. Our expectation is that prior to human impact, gene flow among populations provided a buffer against the consequences of small population size, but contemporary gene flow is insufficient to maintain connectivity between extant populations due to population decline. In this context, we address the following questions: i) Is *M. oppositifolia* genetically depauperate? ii) Are remnant populations genetically structured? iii) To what extent does contemporary realised gene flow link remnant populations? We answered these questions by conducting a genetic inventory of all known adult trees across the four populations, and approximately 25% of all juvenile *M. oppositifolia* at the largest population (and the only one with seedlings), through which we were able to determine realised gene flow by both pollen and seed, and assess historical gene dispersal.

Finally, we ask whether genetic rescue can increase fitness in a small isolated population. We address this using controlled pollination experiments to explore whether cross pollination from the large population to a small isolated population can increase reproductive fitness in terms of fruit set, seed set, germination rate and seedling survival compared to within-population crosses.

MATERIALS AND METHODS

Study species and populations

Medusagyne oppositifolia Baker (Ochnaceae) is a small but long-lived tree endemic to the main island of Mahé on the Seychelles, formerly belonging to the monospecific family Medusagynaceae but which has recently been assigned to Ochnaceae (Bremer *et al.* 2009; Fay *et al.* 1997). It is commonly known as the jellyfish tree as its fruit capsules resemble inverted jellyfish.

 Medusagyne oppositifolia is considered a habitat specialist of granitic inselbergs and has never been recorded in the surrounding matrix (although historical distribution records are few) (Matatiken 2006). The naturally patchy distribution of *M. oppositifolia* reflects the distribution of inselberg habitats as 'islands' within more extensive forest (Matatiken 2006). Since the human colonization of the Seychelles in the early 18th Century, the populations of many endemic species have declined in size. The inselberg habitats (compared to the surrounding forests) have remained relatively intact on Mahé, presumably owing to stressful xeromorphic and edaphically poor conditions, allowing many rare endemics to survive in last refugia. However, some populations on inselbergs have almost certainly been lost as a result of invasive species and fires (Didier Dogley, pers. comm.).

 Only 90 *M. oppositifolia* adult trees remain: 78 are found on the inselberg of Bernica (170 - 270 m a.s.l.), seven on Mt. Sebert (480m a.s.l.), three on Copolia (460m a.s.l.) and two on Mt. Jasmin (290 m a.s.l.) with each of these inselberg habitats having an approximate size of 1 hectare (Fig. 1). Although the trees from these small populations produce fruit, no seedlings or saplings have been located, and the causes for recruitment failure remain unclear. The only location where *M. oppositifolia* successfully regenerates is Bernica, where seedlings typically establish and grow within humus-filled small pits and clefts within the inselberg rock surface. Anecdotal reports suggest that single *M. oppositifolia* trees recently existed elsewhere on Mahé (Matatiken 2006) but extensive searches within the framework of this study did not reveal additional populations.

 Medusagyne oppositifolia is andromonoecious with male and hermaphroditic flowers occurring on mixed inflorescences and flowering synchronously. The flowers are visited and presumably pollinated by native and introduced bees (mainly *Apis mellifera*), wasps and flies (CN Kaiser-Bunbury, unpubl. data). Prior to this study, no information on the breeding system of this species was available. Each fruit contains around 40 small (2 mm long and 1

mm thick) winged seeds which are readily dispersed by wind. Recruitment is only apparent at Bernica which supported 156 seedlings and saplings in 2006 (Matatiken 2006).

Sampling and genetic analysis

In 2009 we collected leaf samples for DNA extraction from all known adults (90 trees) of *M. oppositifolia* at the four inselberg sites, and from 39 seedlings at Bernica. We restricted our collection of leaf material to seedlings that were judged to be sufficiently large to tolerate the sampling. Leaf material was immediately dried and stored in silica gel. DNA was extracted from the leaves using the QIAGEN DNeasy 96 Plant Kit, following the manufacturer's protocol. All samples were screened at a total of ten (B107, C107, D12, D118, D6, B112, A9, B8a, A4, A7) nuclear microsatellite loci, details of which are described in Finger *et al.* (2009). Fragment analysis was conducted using an ABI3730 sequencer and genotyped using Genemapper 3.5 software (Applied Biosystems). No significant linkage disequilibrium was observed for any pair of loci after Bonferroni correction. Therefore, further analyses were performed on multi-locus data from all ten microsatellites.

Assessment of genetic diversity and inbreeding

Presence of null alleles (Selkoe & Toonen 2006) was tested using the program MICRO-CHECKER (Van Oosterhout *et al.* 2004). Number of alleles (N_A), number of effective alleles (N_E), allele frequencies and the number of private alleles (P_A) were calculated using GenAlEx 6 (Peakall & Smouse 2006). Allelic richness (R_S) was calculated using FSTAT 2.9.3.2 (Goudet 1995). Tests of linkage disequilibrium, observed and expected heterozygosities (H_O, H_E) and inbreeding coefficients (F_{IS}) using 10,000 permutations, were calculated with Arlequin 3.5 (Excoffier *et al.* 2005). Because this study includes a complete sample of all individuals there is no sampling bias in the comparison between populations (Leberg 2002). For comparison of the values of N_E, H_O, H_E, and R_S an analysis of variance was applied in R, version 2.10.1 (R Development Core Team 2010).

Assessment of genetic structure over the species range

Overall F_{ST} values were calculated with Arlequin 3.5. Differentiation between populations was assessed using pairwise F_{ST} values calculated using FSTAT. To test for the presence of geographical groupings of related samples, we applied a Bayesian cluster analysis to all individuals using the software STRUCTURE (Pritchard *et al.* 2000). The batch run function was used to carry out a total of 100 runs, 10 each for one to 10 clusters (K1 to K10). For each run the burn-in and simulation length was 150,000 and 500,000, respectively. Since the log

probability values for the different K values have been shown to be of little reliability in other cases, the more refined ad hoc statistic ΔK based on the rate of change in the log probability of data between successive K values (Evanno *et al.* 2005) was used. It is calculated as $\Delta K = ([mL(K + 1) - 2mL(K) + mL(K - 1)])/\text{SD}L(K)$, where $L(K)$ is the logarithm of the probability that K is the correct number of clusters, m is the mean and SD is the standard deviation.

Assessment of within population spatial genetic structure and relatedness among individuals

Spatial genetic structure within the large population (Bernica) was assessed using a Mantel test and 10,000 permutations using SPAGEDI 1.3 (Hardy & Vekemans 2002), to test the null hypothesis of no correlation between spatial distance and multilocus kinship (*F*). To visualize results of the FSGS, *F* values were assigned over a set of 15 distance classes and plotted against the distance. Distance classes ranged from 0-10 m to 200-280 m with 10 m intervals from 0-100 m, 20 m intervals from 100-140 m, 30 m intervals from 140-200 and a 280 m interval from 200-280. Multilocus kinship coefficients *F* (Loiselle *et al.* 1995) were also calculated for all possible pairwise combinations for all adults in all populations and mean kinship coefficients for each population estimated.

Estimating realised gene flow using parentage analysis

Using multilocus genotypes (10 loci) of the 39 seedlings and all 90 adult trees we applied a maximum likelihood exclusion analysis in CERVUS 3.0, to assign the two most likely candidate parents (Kalinowski *et al.* 2007; Marshall *et al.* 1998). Simulations of paternity were run using the allele frequencies of all adult reproductive trees and the following settings: 10000 cycles; minimum number of loci typed 5, unknown parents; all surviving adults across the species range were set as candidate parents for seedlings; 1% for proportion of loci mistyped, and 87 percent for proportion of loci genotyped. The proportion of candidate parents sampled was set at 99 percent, which was justifiable given that we had genotyped all known individuals. Assignment was based upon the 95% and 80% confidence level of the critical LOD score. Pollen dispersal distance was deduced for each seedling based on the distance between the most likely parent trees as assigned by CERVUS. Seed dispersal was calculated as the distance between the seedlings to their closest assigned parent trees (Bacles *et al.* 2006; Dow & Ashley 1998; Nakanishi *et al.* 2009), based on the assumption that seed dispersal is less than pollen dispersal. For comparison, we also calculated the seed dispersal distances taking the more distal parent as mother tree.

Evidence of genetic rescue through pollination experiments

To test the effects of inter-population pollen dispersal on *M. oppositifolia* fitness, experimental crosses should ideally be conducted between all extant populations. However, asynchronous flowering periods among populations, and limited site and tree accessibility, constrained our experiment to one recipient population. In 2009, on three trees at the Mt. Sebert population, 5–10 inflorescences each with 10–30 flowers at bud stage were enclosed within Delnet™ pollination bags. Ten bags were used on each of two trees (1-005 and 1-007), and four bags on the third tree (1-006). Within each bag four treatments were applied: 1. Selfing, using pollen sourced from flowers of the same tree; 2. Within-population crosses using pollen sourced from another tree at Mt. Sebert; 3. Between-population crosses using a random mix of pollen donors (four or five trees) at Bernica; and 4. Unmanipulated (non-pollinated) flowers. As a control we determined seed set of unmanipulated flowers outside pollination bags and therefore openly accessible to pollinators. Pollen was transferred by brushing the viable stigmas of hermaphrodite flowers with anthers of male flowers. Flowers were individually labelled to distinguish between treatments. In total, 466 flowers were pollinated for between-population, within-population crosses and selfed treatment, 795 were not pollinated and 23 fruits from 2 trees were collected for the controls. The pollination bags were retained until the fruits were ripe for collection. Mature seeds proved to be either viable (developed and full) or non-viable (developed but empty). To determine fitness values for each pollination treatment we recorded fruit set (developed fruits as a proportion of treated flowers), viable seeds (viable seeds as a proportion of all developed seeds) and seed set (viable seeds as a proportion of the number of ovules).

Viable seeds were subsequently sown at the Biodiversity Centre nursery on Mahé and germination recorded. In total, 5218 seeds were sown: 18 seeds from the selfed treatment, 1780 from within-population, 2296 from between-population crosses, 391 from non-pollination and 733 from controls. Seeds were germinated in coarse and unfertilised soil. We used trays for seed germination, and re-potted individual seedlings at a minimum height of 2 cm. Germination date and date of death were recorded to calculate seedling survival.

Validation of pollination treatments with paternity analysis

In field pollination experiments, unintended pollen transfer may result in some degree of contamination. To quantify the degree of contamination and to validate our treatments for genetic rescue, we genotyped all germinated seedlings from our pollination experiment and assigned paternity. We used maximum likelihood in CERVUS 3.0 (as outlined above) to

assign the most likely candidate father with all adult trees as candidates, given the known mother trees. We used the genetically corrected treatments determined by the paternity analysis as the basis for the analysis of genetic rescue.

Data analysis

Statistical analyses of the pollination experiments were carried out in R, version 2.10.1 (R Development Core Team 2010). We used generalized linear mixed-effects models (GLMM) with a binomial error distribution to analyse fruit and seed set. GLMM can account for the nested experimental design, and we included bags nested in trees as a random effect in the model (Bolker *et al.* 2009). We applied the lmer function from the lme4 package (Bates 2005). Due to the different number of flowers and fruits per tree and treatment the data for seed set were unbalanced. Our analysis is robust for unbalanced data (Bolker *et al.* 2009) by using the "cbind" function which calculates fruit and seed set weighted by sample sizes. We ran two sets of models: a main effect model with Treatment as fixed effect to determine the level of selfing, and a full model with Treatment (only within- and between-population crosses) and Tree ID as fixed effects to look at the question of genetic rescue. Model selection was based on the Akaike Information Criterion (AIC), and lowest AIC values defined minimum adequate models. After running the analysis we decided to remove the seeds from the selfed and the non-pollination treatments as this increased the power of our model and we were mainly interested in the comparison of within- and between-population crosses.

As only 23 fruits from two trees could be collected as a control group, we randomly sampled seed set of 23 fruits of treatments of within- and between-population crosses without replacement and repeated the sampling 10000 times according to the bootstrap method (Efron & Tibshirani 1998). Mean seed set of control fruits was compared to the bootstrapped mean and its estimated confidence intervals of the two treatments.

Survival rates of germinated and genotyped seedlings in the nursery were calculated using the survival rate analysis in R in the package survival (R Development Core Team 2010). We used censored data, 1 defining the seedlings that died during the germination experiment and 0 for individuals that were still alive at the end of the experiment. A parametric Weibull survival regression (indicating that hazard for death decreases with age) was applied.

RESULTS

Genetic diversity and inbreeding

At the species level the ten loci yielded between two and 15 alleles, with a total number of 86 alleles. A comparison of genetic diversity over all loci and populations is given in Table 1. No significant inbreeding (F_{IS}) was detected overall (0.014, p = 0.316 SE). H_E values ranged from 0.26 ($\pm$ 0.09 SE) in the Mt. Jasmin population to 0.63 ($\pm$ 0.05 SE) in Bernica. Allelic richness, based on two diploid individuals ranged from 1.70 ($\pm$ 0.26 SE) in Mt. Jasmin to 2.73 ($\pm$ 0.13 SE) in Copolia. Bernica contained the greatest allelic diversity and highest numbers of private alleles (20) jointly with Mt. Sebert.
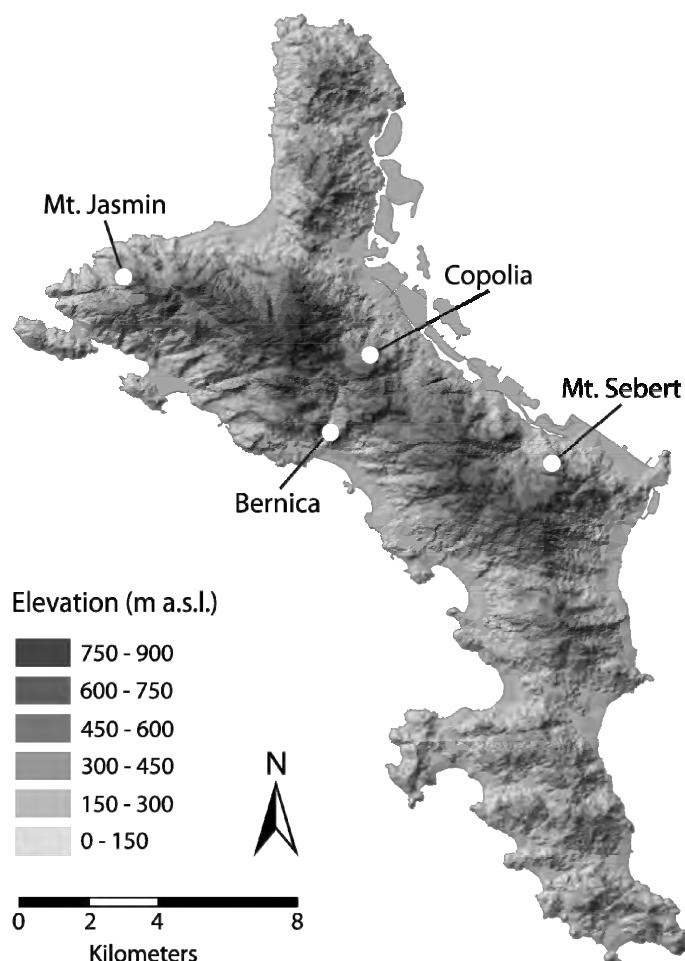
Figure 1: Map of the Seychelles main Island Mahé, with the four known *Medusagyne oppositifolia* populations. Number of adult individuals for Mt. Jasmin = 2, Mt. Sebert = 7, Copolia = 3, Bernica = 78.

Table 1: Genetic variability of 10 microsatellite loci estimated for all populations of *Medusagyne oppositifolia*. Abbreviations: n: number of genotyped individuals; N_A: Mean number of alleles; N_E: effective number of alleles; H_O: observed heterozygosity; H_E: expected heterozygosity; R_S: Allelic richness, based on two diploid individuals; P_A: total number of private alleles (mean frequency); F_{IS}: Inbreeding coefficient; ± SE. Analysis of variance for N_E, H_O, H_E and R_S: * = p < 0.05, ** = p < 0.01, *** = p < 0.001.

POP ID	n	N_A	N_E**	H_O**	H_E***	R_S**	P_A	F_{IS}
Mt. Jasmin	2	1.70 (±0.26)	1.59 (±0.22)	0.30 (±0.13)	0.26 (±0.09)	1.70 (±0.26)	2 (0.38±0.13)	0.20ns
Mt. Sebert	7	3.10 (±0.35)	2.27 (±0.29)	0.56 (±0.08)	0.48 (±0.07)	2.11 (±0.18)	5 (0.27±0.06)	-0.08ns
Copolia	3	3.20 (±0.20)	2.76 (±0.20)	0.78 (±0.06)	0.62 (±0.03)	2.73 (±0.13)	3 (0.19±0.03)	-0.04ns
Bernica	78	6.10 (±0.91)	3.18 (±0.46)	0.63 (±0.05)	0.63 (±0.05)	2.42 (±0.15)	20 (0.09±0.02)	0.02ns
Bernica Seedlings	39	5.00 (±0.76)	3.06 (±0.42)	0.66 (±0.05)	0.62 (±0.05)	2.39 (±0.15)	0	-0.06ns
Mean	-	3.82 (±0.33)	2.57 (±0.17)	0.59 (±0.04)	0.52 (±0.03)	-	-	-0.01ns

Genetic differentiation

The overall F_{ST} value was 0.15 (p < 0001), showing that ca. 15% of the total molecular variance was among populations and 85% was within populations. Mean pairwise genetic distances (Nei 1978) were significantly different among most populations (Table 2) but considerably higher between the geographically most distant Mt. Sebert and Mt. Jasmin (0.36, p = 0.058; 13.5 km between populations), and lowest between the geographically close sites Bernica and Copolia (0.10, p = 0.008; 2.5 km). The STRUCTURE analysis identified four or five distinct genetic clusters (K4, K5) as the most likely (and biologically relevant) solutions, where both K4 and K5 indicated a sub-structuring within Bernica. The analysis also discriminated the other three populations, which is consistent with the current population geographic structure (see Fig. S1a and b, Supporting Information).

Table 2: Pairwise F_{ST} values between populations, * = p < 0.05.

	Mt. Jasmin	Mt. Sebert	Copolia	Bernica
Mt. Jasmin	0	0.36	0.24	0.24*
Mt. Sebert		0	0.15*	0.14*
Copolia			0	0.10*
Bernica				0

Within population fine-scale spatial genetic structure and kinship

Significant fine-scale spatial genetic structure (FSGS) was detected in adult trees at Bernica (Fig. 2), as indicated by significant regression of kinship against spatial distance. Kinship coefficients (F) are significantly different from zero within pairs of trees up to 50 m apart ($p < 0.001$). The average individual kinship coefficient over all individuals in Bernica was not significantly different to 0 ($F = 0.004 \pm 0.119$ SE). In Copolia average individual kinship coefficient was higher ($F = 0.17 \pm 0.008$ SE) across three pairs, and even higher values were found in the Mt. Sebert ($F = 0.41 \pm 0.134$ SE; 21 pairs) and Mt. Jasmin ($F = 0.57$; 1 pair) populations (see Fig. 2).
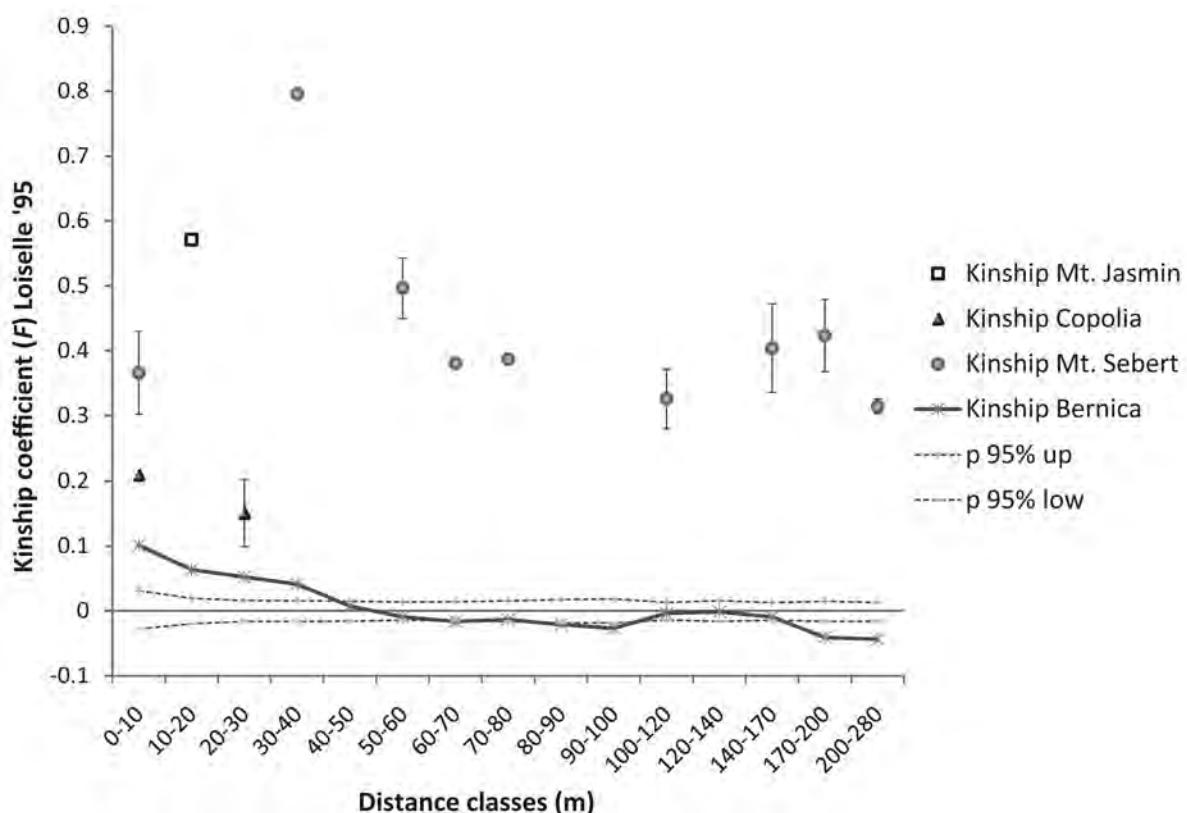
Figure 2: Correlogram of average kinship coefficient (F) of adult individuals of *Medusagyne oppositifolia* at Bernica for 15 distance classes with intervals of 10, 20, 30 and 80m. The solid line represents the average F values. The dotted lines represent the 95% (two-tailed) CI of the average F distribution calculated from 10000 permutations of spatial distance among pairs of adults. Single dots represent the pairwise kinship coefficients of individual pairs at Mt. Jasmin, Copolia and Mt. Sebert. If error bars (SE) are present more than one pair was observed at that distance class.

Contemporary pollen and seed dispersal

Using parentage assignment we were able to assign all of our sampled seedlings (39) with 95% confidence. Realised pollen dispersal ranged between 2.9 m to 207 m, with nearly half of all seedlings resulting from mating events of less than 25 metres (median = 18.94 m,

Interquartile range = 0) (Fig. 3a). Assuming that the nearest parent is always the maternal tree, 81.6% of seedlings on Bernica occurred within 50 m of the mother tree, with an overall dispersal distance of 36.5 m (median, Interquartile range = 13.2). Even under the less plausible scenario where the maternal tree is always the furthest parent, 76% of all seedlings occurred within 100 m (Fig. 3b).
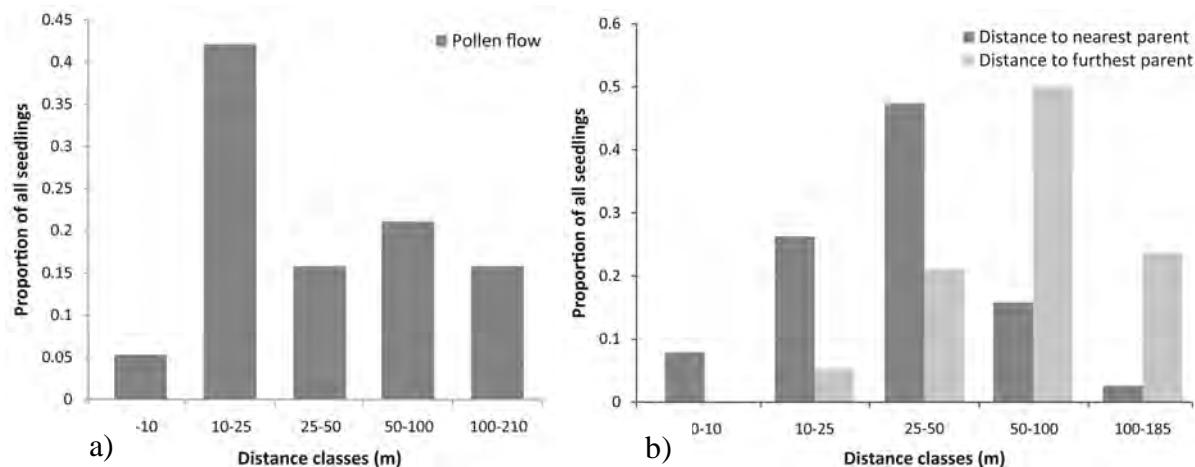
Figure 3: (a) Frequency distributions of realised pollen dispersal distances and (b) seed dispersal distances in the Bernica population of *M. oppositifolia*, calculated from n = 39 seedlings and parent pairs. In dark grey assuming that the nearest parent tree is the morther tree, in light grey the scenario that the furthest parent tree is the mother tree.

Genetic rescue using pollination experiments

Fruit and seed set

Fruit set was significantly higher in between-population crosses (0.86 ± 0.06 SE) than within-population crosses (0.72 ± 0.09 SE; z = -1.93, p = 0.05). Fruit set for selfed and no-pollination treatments was low (0.16 ± 0.07 SE and 0.10 ± 0.03 SE, respectively).

Seed set values obtained from between-population crosses (0.37 ± 0.02 SE) and within-population crosses (0.32 ± 0.02 SE) over all trees were not significantly different (z = -1.61, p = 0.10), though one of the trees within-population crosses resulted in very low seed set, resulting in a significant (p < 0.001) tree interaction effect which is likely to have confounded the comparison. Mean seed set of between- and within-population crosses were not significantly different to the open-pollinated control group (Controls: 0.46; between-population crosses: 0.44, bootstrapped 95% CI 0.35 – 0.52; within-population crosses: 0.48; bootstrapped 95% CI 0.40 – 0.55). Seed set for selfed and no-pollination was low (0.003 ± 0.001 SE and 0.01 ± 0.01 SE, respectively).

Viable seeds (as a proportion of developed seeds) was highest from between-population crosses (0.77 ± 0.03 SE) and significantly greater than within-population crosses (0.52 ± 0.03 SE) across all trees ($z = -10.7$, $p < 0.0001$; full details in Supporting Information, Table S1). Unlike for seed set, there was no significant tree interaction effect with treatment. The mean viable seed of the control group was also lower than that from between-population crosses (Controls: 0.79; between-population crosses: 0.92, bootstrapped 95% CI 0.86 – 0.96) and similar to treatment within population-crosses (Controls: 0.79; within-population crosses: 0.77; bootstrapped 95% CI 0.72 – 0.83). Viable seed set for selfed and no-pollination was low (0.03 ± 0.01 SE and 0.07 ± 0.01 SE, respectively).

Germination and survival rates

Germination rates were low for all seeds collected at Mt. Sebert. Seeds from between-population crosses (49 seedlings, 2% germination rate) and within-population crosses (32 seedlings, 2%) had lower germination probability than seeds from selfed treatments (4 seedlings, 22%; $p < 0.001$), although this has to be set in the context that very few selfed seeds were produced. Mean survival in days since germination were 190 ± 18.5 SE for between-population crosses, 155 ± 14.8 SE for within-population crosses and 101 ± 60.1 SE days for selfed treatments, with differences only significantly different for between-population and within population crosses (between- and within-population: $z = -1.95$, $p = 0.05$; between-population and selfed: $z = -1.71$, $p = 0.09$, within-population and selfed $z = -0.82$, $p = 0.41$). No seeds of non-pollinated fruits and control group germinated (details in SI Table 1).

DISCUSSION

This study provides evidence that genetic erosion and inbreeding are leading to an elevated risk of extinction in a rare tree species with isolated populations and limited gene flow, despite a naturally patchy distribution. Our results suggest that genetic rescue may help to mitigate these effects leading to an enhancement of viability in the offspring of threatened tree species which have limited gene flow.

Is Medusagyne oppositifolia *genetically depauperate?*

The relatively high degree of genetic diversity found in three small *M. oppositifolia* populations suggests that despite their small size and isolation these populations are important for conservation of species-wide genetic diversity (see also Schneller & Holderegger (1996)). This may be a general feature of similarly isolated and endangered species, as the patterns of genetic diversity observed among adult populations of *M. oppositifolia* are comparable with naturally isolated, rare, tropical, and endemic island tree species (Born *et al.* 2008; Dutech *et al.* 2004; Kettle *et al.* 2007; Sebbenn *et al.* 2011; Ueno *et al.* 2005). Similar results have also been found among temperate tree species. For example, high levels of genetic diversity remain within the 67 extant individuals of the Minorcan cork oak (Lorenzo *et al.* 2009). High genetic diversity can also be retained within extant populations of formerly widespread species that have been subject to recent and rapid population decline and fragmentation (Yao *et al.* 2007). Similarly, rare palm species (e.g. *Beccariophoenix madagascariensis*) from Madagascar retain high genetic diversity despite anthropogenic population decline and fragmentation (Shapcott *et al.* 2007). In contrast, low genetic diversity was observed in a naturally fragmented but rapidly declining palm species (*Livistona carinensis*) that occurs along valley systems in Yemen (Shapcott *et al.* 2009).

The relatively high genetic diversity found in three populations of *M. oppositifolia* indicates that these populations have not been through repeated genetic bottlenecks and random genetic drift. However, a comparison of genetic diversity between seedlings and adult cohorts from Bernica show that rare alleles present in the adult trees are absent in the seedling cohort (data shown in Supporting Information, Table S2). Together with the comparatively low genetic diversity in the smallest population (Mt. Jasmin) this suggests that the populations are showing early signs of genetic erosion. The parental populations which sired the current adult populations of Copolia and Mt. Sebert must have been at least similar in size to the

current Bernica adult population to maintain the observed levels of allelic richness. This suggests that the reduction in population size has occurred within the last generation of adults.

Although the nuclear microsatellite markers used here do not allow us to evaluate overall adaptive variation or local adaptation, our results provide an early warning of the processes (genetic drift and inbreeding) which are likely to lead to a reduction in the adaptive capacity and thus long-term viability of the species (Reed & Frankham 2003). The evaluation of genetic diversity and gene flow using neutral genetic markers combined with pollination experiments enables us to link offspring viability with inter-population gene flow. Recent advances in conservation genomics will enable us to more specifically examine adaptive traits (Ekblom & Galindo 2010; Ouborg *et al.* 2010; Tautz *et al.* 2010) in threatened tree species such as *M. oppositifolia*. Combined with common garden experiments and progeny trials, such information will enable local adaptation and outbreeding depression to be evaluated in relation to genetic rescue and translocation of progeny in these long-lived plant species. However, in the shorter-term this study provides important insights for making immediate conservation decisions necessary to conserve the existing adult populations.

Are remnant populations genetically structured?

Medusagyne oppositifolia is restricted to four isolated inselbergs which are separated from each other by at least 2.4 kilometres. The significant genetic differentiation observed among *M. oppositifolia* populations (expressed by an overall F_{ST} of 0.15, high pairwise F_{ST} values, and high numbers of private alleles in all populations) is consistent with the view that historic exchange of genes between these populations has been limited. Apart from isolation by distance, the topography of Mahé, the phenological asynchronies in flowering within and between populations (which would further reduce the effective population size), and competition for pollinators by other plants (particularly several profusely flowering alien species), might also account for the genetic isolation of *M. oppositifolia* populations.

Reproductive isolation is also evident within the Bernica population as revealed by the genetic sub-structuring. Insect pollination and wind-dispersed seeds are thought to promote relatively long distance gene dispersal (Dick *et al.* 2003; Lander *et al.* 2010), but the strong positive FSGS and the short distance between parent pairs of wild seedlings in the Bernica population suggests that, for *M. oppositifolia* this appears not to be the case. A possible explanation for the clusters is that the topography of inselbergs limits gene flow by pollen and seed. Thus the largest population (Bernica) is mainly distributed on different sides of the inselberg around its domed peak, and seeds and pollen may not be effectively dispersed from

one side to the other. Isolation by local topography might therefore represent a barrier to gene flow leading to genetic clustering within small populations, with implications for increased susceptibility to inbreeding.

To what extent does contemporary realised gene flow link remnant populations?

The parentage analysis of wild seedlings showed that realised gene flow by pollen and seed dispersal is relatively low at Bernica. The furthest distance between reproductive individuals on Bernica is 280 m, and maximum recorded pollen flow was 207 m, though 85% of pollen flow events were less than 100 m. Seed dispersal was similarly limited, with most seeds being dispersed less than 50 m assuming the mother tree is the closer of the two parents to the sampled seedlings. Such limited gene flow, and the observed genetic sub-structuring, suggests that contemporary gene flow between inselbergs is unlikely.

Kinship analysis revealed that relatedness between adult individuals in the three small populations is higher than that on Bernica within the same spatial range (2 to 200 m). In the Mt. Jasmin population the remaining two trees have a very high kinship coefficient ($F = 0.57$), which is much higher than that expected for full sibs ($F = 0.25$). This high kinship coefficient could have resulted from successive matings among related parents, indicating a historically inbred population. Even at Mt. Sebert where trees are relatively distant to each other (separated by as much as 200 m), relatedness is still very high ($F = 0.31$) and mating between these surviving adult trees could result in elevated inbreeding and subsequent inbreeding depression.

Can genetic rescue increase fitness in a small isolated population?

Artificial cross-pollinations between populations of *M. oppositifolia* increased the proportion of viable seeds, and between-population crosses had higher seedling survival (190.7 days ± 18.5 SE) than within-population crosses (155.5 days ± 14.8 SE). The higher proportion of viable seeds and higher seedling survival for between-population crosses, indicate fitness benefits of between-population pollinations. Such benefits are not realised by gene flow among wild populations which appears restricted within inselbergs. Our results indicate that genetic rescue, through artificial cross pollination, may be successful in enhancing the production of viable seed. Doing so might not only promote regeneration, which is currently non-existent in these populations, but might also contribute to the conservation of the alleles unique to these populations. Furthermore, our results also indicate that low gene flow rates and population isolation are severe threats to offspring viability of species with small

population sizes. Thus, the importance of maintenance of continuous habitats and genetic connectivity between populations has to be of highest priority in conservation management plans of rare tree species.

An alternative explanation for lack of seedling recruitment on the smaller populations is pollinator limitation. This is, however, unlikely as we did not detect significant differences in seed set or viable seeds between the control (open pollinated) and within-population crosses. Reproduction is also likely to be limited by non-synchronous flowering, and therefore limited cross-pollen sources, which is more likely in small populations by virtue of fewer available flowering individuals. Nevertheless, no difference in seed set between open-pollinated and artificial between- and within-cross pollinations show that pollination itself is not a limiting factor. Instead, the significantly higher viable seed production resulting from between-population crosses indicates that recruitment failure is more likely due to pollen quality rather than quantity.

Our results demonstrate that genetic rescue through artificial between-population cross pollination of *M. oppositifolia* is possible, and might represent a better option than within-population crosses for the ecological and genetic rescue of the three smaller populations. Genetic rescue must, however, consider that introducing new alleles to populations may undermine evolved local adaptation among recipient populations and might even lead to outbreeding depression expressed at later life history stages (Edmands 1999; Tallmon *et al.* 2004) and genetic swamping (Hufford & Mazer 2003). The inselberg habitats are, however, geologically similar, occur within a 250 m elevational range, and are located within 15 km of each other, and differences in environmental conditions are slight - the likelihood of outbreeding depression through a disruption of local adaptation is therefore probably low, but can't be excluded at this point. Even so, when populations are on the verge of extinction, with only a few extant individuals left, genetic rescue and artificial cross-pollination provide the only remaining option of conserving multiple populations together with the distinct genetic diversity that each remnant population represents.

One caveat that needs to be acknowledged is that seed set, unlike fruit set and viable seed production was not different among the between- and within-population crosses. While the lack of difference could be a genuine result, we must also consider that between-population crosses involved the transportation of flowers from one inselberg to another, possibly resulting in reduced pollen availability for between-population compared to within-population treatments. Further, low overall germination rates suggest that nursery conditions were not ideal. *Medusagyne oppositifolia* is, however, difficult to maintain in nursery

conditions, and it is also possible that germination rates are intrinsically low for other, biological, reasons.

Conservation and management implications

Three of the four populations of *M. oppositifolia* have fewer than 10 individuals and are vulnerable to catastrophic events such as storms, droughts, fire or disease. The continued spread of alien invasive plants, which are beginning to encroach upon inselberg habitats (Kaiser-Bunbury *et al.* 2011), may result in elevated inter-specific competition for pollinators which may further reduce reproductive success (Ghazoul 2004). Seed banking, an option that has been proposed for many endangered plant species, is not a feasible conservation strategy for *M. oppositifolia*, as its seeds are recalcitrant and viability decreases rapidly with the duration of several months of storage (D Matatiken, unpubl. data). Our results show that seed viability, germination success and seedling survival is intrinsically low (partly due to genetic reasons), and any additional intervention (e.g. storage) that further decreases seed viability will be counter-productive to the species' conservation.

We advocate the germination of seed in nurseries to establish populations of seedlings for subsequent restoration efforts. Urgent conservation action to reverse the complete recruitment failure in the three small populations is clearly needed to preserve the unique genetic diversity they contain. Having determined that natural recruitment within small populations is unlikely, in part due to the close relatedness of adult individuals, efforts to rescue these populations are likely to depend on artificial cross pollinations using pollen from Bernica individuals. The observed increased fitness associated with genetic rescue offers the best chance to secure the long-term viability of these populations, and the species as a whole. Without such action, it seems likely that within a few years, or at best a few decades, *M. oppositifolia* will only be found in the single population at Bernica and a substantial portion of this species genetic diversity will have been lost.

Our results demonstrate the potential for genetic rescue to support both *in-situ* and ex-situ conservation of threatened long-lived tree species. Controlled pollination and seedling transplants should be considered as a next step to increase numbers of individuals within the smaller populations, and enable the ex-situ conservation of genetic resources. This study emphasises the need to maintain gene flow between individuals and populations of threatened tree species, especially through pollen dispersal. In other threatened tree species with limited pollen dispersal and high relatedness within remnant populations, whether it be recently fragmented tropical species (Collevatti *et al.* 2010; Collevatti & Hay 2011; Eckert *et al.* 2010)

or chronically fragmented temperate species (Dubreuil *et al.* 2010), genetic rescue might be the only means of preventing population or even species extinction.

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SUPPORTING INFORMATION

Table S1: Summary table of results of pollination experiments. Fruit set, seed set and survival rates are given in mean proportions ± SE. S = Selfed individuals, W = Within population crosses, B = Between population crosses, N = Bagged individuals with no hand pollination. Fruit set is defined as the proportion of flowers that developed into fruits. Viable seed set is defined as the proportion of developed seeds; Seed set as the proportions of maximum potential seeds. Significant differences between treatments B and W are indicated as: * = $p < 0.05$; *** = $p < 0.001$.

	N	S	W	B
# Flowers treated	795	163	143	160
# Developed fruits	92	14	105	136
# Fruits used for seed set calculations	778	156	136	156
# Trees	3	3	3	3
# Bags	19	14	9	14
# Viable seeds	391	18	1780	2296
# All developed seeds	497	20	2488	2524
Max. potential # seeds	31034	6226	5450	6258
# Germinants	0	4	32	49
Fruit Set (%) ***	0.10 (±0.03)	0.16 (±0.07)	0.72 (±0.09)	0.86 (±0.06)
Viable seed set ***	0.07 (±0.01)	0.03 (±0.01)	0.52 (±0.03)	0.77 (±0.03)
Seed set	0.01 (±0.001)	0.003 (±0.002)	0.32 (±0.02)	0.37 (±0.02)
Germination rate (%)	0	0.22	0.02	0.02
Survival rate (days) *	0	101.8 (±60.1)	155.5 (±14.8)	190.7 (±18.5)

Table S2: Allele frequencies lower 0.05 for adults and seedlings at the Bernica population. Marked in bold are alleles that are present in the adults but not in the seedlings.

Locus	Allele	Bernica Adults	Bernica Seedlings
A114	101	0.033	0.051
A114	111	0.033	0.013
B107	147	0.026	0.013
B107	153	0.026	0.038
D6	289	0.013	0.013
D6	**305**	**0.013**	**0.000**
B112	150	0.032	0.013
B112	174	0.026	0.013
B112	**186**	**0.013**	**0.000**
B112	188	0.019	0.026
B112	**190**	**0.006**	**0.000**
A9	**240**	**0.019**	**0.000**
A9	**250**	**0.006**	**0.000**
A9	**270**	**0.006**	**0.000**
B8a	**166**	**0.019**	**0.000**
B8a	**172**	**0.038**	**0.000**
B8a	176	0.026	0.026
B8a	**178**	**0.006**	**0.000**
C12	**206**	**0.019**	**0.000**
A4	239	0.038	0.051
A4	243	0.038	0.026
A4	**247**	**0.006**	**0.000**
A4	251	0.038	0.128
A7	**274**	**0.006**	**0.000**
A7	286	0.019	0.013

(a)
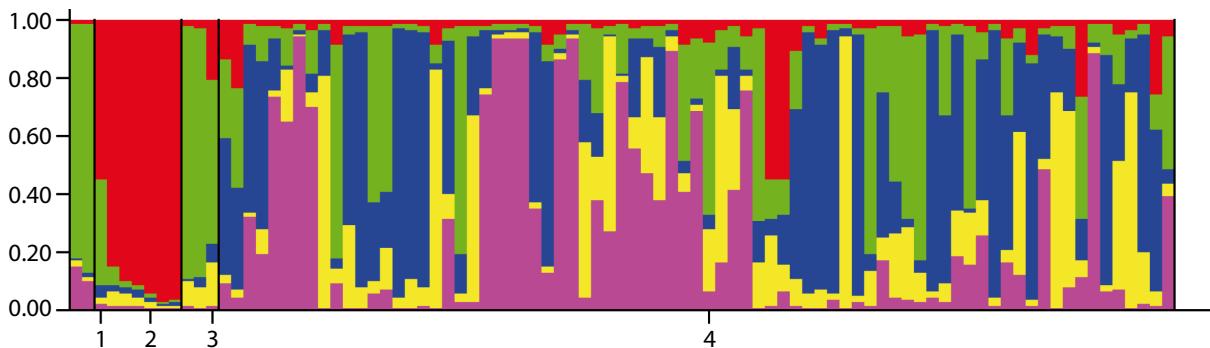
(b)
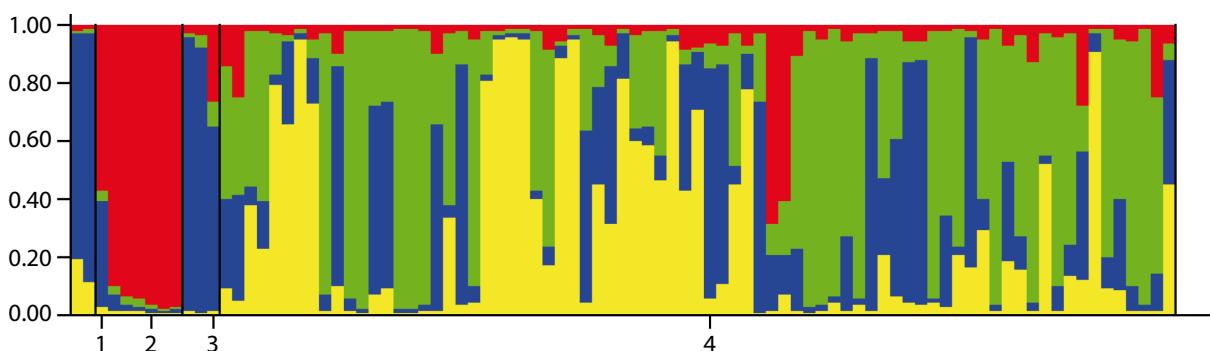
Figure S1: Bayesian structure analysis of *Medusagyne oppositifolia* with the STRUCTURE software. Bars represent individual *Medusagyne oppositifolia* trees with their assignment proportions (y axis) to the different clusters. 1 = Mt. Jasmin, 2 = Mt. Sebert, 3 = Copolia, 4 = Bernica, for population assignment see Fig. 1. Performing the analysis for **(a)** K_5 (5 different grey shades) and **(b)** K_4 (4 different grey shades).

Forest fragmentation genetics in a formerly widespread island endemic tree: *Vateriopsis seychellarum* (Dipterocarpaceae)

with C.J. Kettle, C.N. Kaiser-Bunbury, T. Valentin, J. Mougal and J. Ghazoul
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ABSTRACT

Habitat fragmentation and changed land use have seriously reduced population size in many tropical forest tree species. Formerly widespread species with limited gene flow may be particularly vulnerable to the negative genetic effects of forest fragmentation and small population size. *Vateriopsis seychellarum* (Dipterocarpaceae) is a formerly widespread canopy tree of the Seychelles, but is now reduced to 132 adult individuals distributed in eleven sites. Using ten microsatellite loci, a genetic inventory of all adult trees and a sample of 317 progeny we demonstrate that despite its restricted range, overall genetic diversity was relatively high (H_E: 0.56). The juvenile cohort, however, had significantly lower allelic richness (adults R_S: 3.91; juveniles R_S: 2.83) and observed heterozygosity than adult trees (adults H_O: 0.62; juveniles H_O: 0.48). Rare alleles were fewer and kinship between individuals was stronger in juveniles. Significant fine-scale spatial genetic structure (FSGS) was observed in remnant adults, and parentage analysis indicated that more than 90% of sampled progeny disperse < 25 m and pollen dispersed < 50 m. The molecular data confirmed that two populations were derived entirely from self-fertilised offspring from a single surviving mother tree. These populations produce viable offspring. Despite this extreme genetic bottleneck, self-compatibility may provide *V. seychellarum* with some resistance to the genetic consequences of habitat fragmentation, at least in the short term. We discuss our findings in the context of other rare and threatened dipterocarp species which are vulnerable to miss-management of genetic resources and population fragmentation.

INTRODUCTION

Fragmentation of formerly continuous forest landscapes reduces population size and increases isolation, potentially altering demographic structure, mating systems, genetic diversity, and long-term viability of forest tree species (Bacles *et al.* 2005; Ghazoul 2005; Lowe *et al.* 2005). Following fragmentation trees may become increasingly reproductively isolated, with more frequent inbreeding and loss of rare alleles, negatively affecting seed production and progeny viability (Cascante *et al.* 2002; Ellstrand & Elam 1993; Stacy *et al.* 1996). Such changes in the breeding system reduce effective population size (Loveless & Hamrick 1984; Rossetto *et al.* 2004). Extreme genetic bottlenecks (survival of only few individuals) will result in considerable loss of genetic diversity, requiring several generations for diversity to recover to a new equilibrium (Luikart *et al.* 1998; Nei *et al.* 1975).

Long-distance gene flow can be important in maintaining genetic diversity in fragmented populations by preventing inbreeding and limiting genetic drift (Hartl & Clark 1997). These effects might ensure the viability of populations in the long-term (Kramer *et al.* 2008; Lande 1988, 1995; Petit & Hampe 2006). Additionally, breeding system characteristics such as self-compatibility may contribute to the persistence of populations in isolation (Levin 2010). The absence of long-distance gene flow has already been demonstrated to have potentially negative effects on many tropical forest tree species (Finger *et al.* 2011; Sebbenn *et al.* 2011 and refs there in).

Tropical forests have suffered from habitat fragmentation over the last century, mainly due to forest clearance for agriculture and extensive logging. The Dipterocarpaceae, which are extraordinary in their dominance of lowland forest canopies in Southeast Asia, have been extensively logged and cleared (Sodhi *et al.* 2010). Despite the economic and ecological importance of this timber family, few studies have examined the population genetic and reproductive implications of fragmentation of dipterocarp species (but see Lee *et al.* 2006; Fukue *et al.* 2007). One difficulty has been the challenge of comprehensively mapping the distributions of dipterocarp populations over the large areas necessary to study gene exchange. In this context, *Vateriopsis seychellarum,* the sole representative of the Dipterocarpaceae in the Seychelles, provides an ideal opportunity to study the impacts of fragmentation on a dipterocarp species that has been reduced to eleven discrete populations from a formerly far more widespread distribution.

The rain forest of the Seychelles was extensively exploited for timber and replaced by cinnamon and fruit tree plantations in the 19th century until just a few forest fragments

remained at higher altitudes in 1870 (Baker 1877; Diels 1922). As a result many formerly widespread and abundant species, such as *Campnosperma seychellarum, Northea hornei* and *Vateriopsis seychellarum*, now survive only in small populations scattered across the island (Carlström 1996; Fleischmann 1997). *Vateriopsis seychellarum* was believed to be widespread over the forested lowlands of Mahé (the main island of the Seychelles) up to about 400 m a.s.l. (Diels 1922). As a valuable timber tree it was heavily exploited, and only 132 known adults now survive at 11 sites, two of which are occupied by just single mature individuals. *Vateriopsis seychellarum* is listed as critically endangered by the IUCN Red List (IUCN 2011).

The aim of this study was to determine the extent to which deleterious genetic consequences of small population size, following habitat fragmentation, threaten *V. seychellarum*. Its insect pollinated flowers and gravity-dispersed fruit are similar to many dipterocarps. The study of this species may therefore serve as analogue for a wide range of dipterocarp species elsewhere in South and Southeast Asia. Given the shortage of documented evidence for past population history in the remaining forest patches, we use molecular techniques to evaluate contemporary gene flow, to determine the degree of isolation of current remnant populations and to make inferences on past population history.

We use a genetic inventory of all known adult trees across all populations and a subset of juveniles to test the hypothesis that this potentially formerly widespread species has experienced extreme genetic bottlenecks, and that limited gene flow is insufficient to connect the remnant populations. We ask the questions: i) is there evidence of extreme population bottlenecks in *V. seychellarum*, indicated by a loss of genetic diversity; ii) are remnant populations genetically structured; and iii) is realised contemporary gene flow by both pollen and seed restricted. We address these questions by using ten microsatellite loci to evaluate genetic diversity, inbreeding coefficients and fine-scale spatial genetic structure (FSGS) in adult trees and a parentage assignment of wild juveniles.

Understanding the genetic and ecological consequences of habitat degradation and extreme population bottlenecks has broad relevance to the viability of many tropical tree species. This study is especially relevant for the Dipterocarpaceae as some species are known to have limited pollen and seed dispersal (Kettle *et al.* 2011a; Osada *et al.* 2001; Seidler & Plotkin 2006). Fine-scale spatial genetic structure is also common in the family (Harata *et al.* in press; Kettle *et al.* 2011a; Lee *et al.* 2006). Consequently, a reduction in population size is predicted to create isolated patches of highly related individuals. Habitat restoration is urgently required, not only for *V. seychellarum* but for many native forest tree species of the

Seychelles, and more broadly across tropical Asia. We expect our results to be directly applicable to restoration planning, seed sourcing and conservation management of genetic resources in *V. seychellarum*, as well as other important timber species (particularly dipterocarps) with limited gene dispersal mechanisms and similar functional traits (such as self-compatible mating system).

MATERIALS AND METHODS

Study species and populations

Vateriopsis seychellarum is a unique and basal evolutionary lineage of the Dipterocarpaceae (Friedmann 1994) and the sole representative of the Dipterocarpaceae in the Seychelles. It is endemic to the main island of the Seychelles, Mahé, where only 132 known adults remain spread across eleven sites: Le Niol, Sans Souci, Cap Vert, Tea Plantation, La Misère, Mt. Cotton, Rivière L'Islette, L'Abondance, Brulée, Salazie and André (see Fig. 1). Two populations (Le Niol and André) are purported to be plantations derived from seeds collected from the single isolated Tea Plantation tree (Huber & Ismail 2006; Willy André personal communication). This species was heavily exploited for its timber in the 19th century as the inland forests of the Seychelles were cleared for timber, agriculture and copra production (Baker 1877) and was nearly extinct by the end of the 19th century (Huber & Ismail 2006). The age of the remaining adults is not known and it is difficult to ascertain whether the current adult tree populations pre-date fragmentation. Nevertheless, planted populations with a mean DBH of 7.9 – 14.8 cm are reported to be 30 years old (Willy André personal communication). A DBH increment of 1.0 to 1.5 mm per year has been reported in 10 year old dipterocarp species plots in Thailand (Bunyavejchewin 1999). Thus, assuming the same DBH increment in *V. seychellarum* trees the remaining natural populations (except Rivière L'Islette) are likely to be 100 – 200 years old (mean DBH 20.8 – 29.4 cm, Tea Plantation tree: 63.7 cm), and at least the largest trees probably pre-date fragmentation in the mid 19th century (Table 1). As 30 year old trees produce juveniles, a generation time of at least 30 years seems plausible for this species.

We mapped all known adult individuals using a GPS (Garmin 60CSx). Two of the sites include only a single tree, while three sites each have fewer than 10 individuals. While most populations are reproductive, the trees from Mt. Cotton produced no flowers or fruits for the duration of the study (2008 – 2010), and no juveniles could be found (Table 1). Based on our observations, *V. seychellarum* flowers irregularly and non-synchronously among and within populations. Flowers have a corolla diameter of 3 - 4 cm and are cream colored. The pollinator community is not well known but small beetles were observed on the flowers, and generally beetles are important pollinators for many Seychelles endemics on Mahé (Kaiser-Bunbury *et al.* 2011) and other Seychelles islands (Woodell 1979).

Flowers develop into a (mostly) single-seeded fruit that is spheroid (up to 6 cm in diameter) and wingless. Fruits are gravity dispersed and mostly fall directly below the mother tree. Four *V. seychellarum* populations are located in the vicinity of rivers and ravines, so secondary dispersal by water cannot be excluded, although fruits sink. The seeds are recalcitrant and germinate immediately after fruits are shed.

Table 1: Population information for *Vateriospis seychellarum*. Mean tree hight and total number of juveniles are estimated values; information on mean DBH, elevation and location are measured values, ± Standard Error. Max dist adults = Maximun distance measured between the two most distant trees. LN = Le Niol, SS = Sans Souci, CV = Cap Vert, TP = Tea Plantation, LM = La Misère, MC = Mt. Cotton, RL = Rivière L'Islette, LA = L'Abondance, BR = Brulée, SA = Salazie, AN = André.

Pop ID	#Adults	#Juvenile	Max dist adults(m)	Mean tree hight (m)	Mean tree DBH (cm)	Lat	Long
LN	11	160	44.13	9.05 ($\pm$1.01)	7.87 ($\pm$1.36)	9488104	326160
SS	19	50	109.59	14.31($\pm$0.94)	25.92($\pm$2.64)	9486904	327572
CV	6	200	589.71	15.67 ($\pm$0.33)	20.89 ($\pm$2.82)	9486992	323253
TP	1	40	-	18.00	63.69	9485312	326855
LM	1	28	-	15.00	26.59	9483648	330446
MC	16	0	150.01	16.27 ($\pm$0.55)	29.38 ($\pm$2.45)	9486943	325856
RL	30	27	69.55	9.29 ($\pm$0.70)	11.11 ($\pm$1.65)	9485600	325831
LA	32	400	120.75	11.16 ($\pm$0.78)	22.84 ($\pm$2.09)	9481194	331907
BR	3	50	47.63	no data	no data	9478672	334169
SA	11	600	136.49	12.18 ($\pm$1.43)	21.57 ($\pm$3.25)	9485079	328149
AN	2	8	4.67	10.50 ($\pm$0.50)	14.81 ($\pm$0.48)	9486814	327920

Sampling and genetic analysis

We made a complete genetic inventory of all known remaining adults (132) and a sample of 317 juveniles of *V. seychellarum* across the eleven sites on Mahé (Table 1, Fig. 1). Three of the 132 trees could not be genotyped (two at Sans Souci and one at L'Abondance) due to low DNA quality. We collected a subset of juveniles at all available age and height classes in each population. It is difficult to estimate the age of the collected juveniles as these may be retained for relatively long time periods in the seedling stage until the canopy is opened, allowing them to grow into sapling or pole stage trees. We consider that the combined sample of seedlings, saplings and pole stage trees include a range of age classes from a minimum of one year up to at least ten years. Usually, juveniles were aggregated under the mother tree, often with more than 50 juveniles occurring within ten m of the stem. In such cases we collected leaf samples of a maximum of ten randomly selected juveniles underneath individual mother trees. We repeated this sampling strategy for all trees. In the case of single isolated mother trees we collected 40 juveniles per tree. This sampling strategy was applied over the entire range of each population to ensure a representative sample of the genetic variation in

juveniles. Leaf samples were collected at all sites for adults and juveniles from 2008 to 2010 and stored in silica gel. DNA was extracted using the QIAGEN DNeasy Plant Maxi Kit, following the manufacturer's protocol. PCR reactions were carried out in a thermal cycler (Bio-Rad Dyad Cycler). Microsatellite loci were amplified from c. 10ng diluted DNA in a Mastermix containing 1X PCR buffer (Promega colorless Flexi GoTaq PCR buffer), $MgCl_2$, dNTPs, specific primer pairs and *Taq* polymerase (Promega), for details see Finger *et al.* (2010). We used an ABI3730 for genotyping and genemapper 3.5 software (Applied Biosystems) for fragment analysis. Ten microsatellite loci were analysed (01, 10, 11, 12, 14, 15, 20, 21, 22, 23) and the forward primer of each pair was labelled with an M13-tag at its five ′-end as described by Schuelke (2000).

Statistics

Assessment of genetic diversity

Deviation from Hardy Weinberg Equilibrium (HWE) at each locus was calculated in CERVUS 3.0 (Kalinowski *et al.* 2007). Linkage disequilibrium and inbreeding coefficients (F_{IS}) using 10000 permutations were calculated in Arlequin 3.5 (Excoffier *et al.* 2005). Population genetic parameters including observed and expected heterozygosities (H_O, H_E), number of alleles (N_A), and the number of private alleles (P_A) were generated in GenAlEx (Peakall & Smouse 2006). In addition, allelic richness (R_S) was calculated in FSTAT 2.9.3.2 (Goudet 1995). Because this study includes a complete sample of all known individuals there is no risk of sampling bias (Leberg 2002).

Assessment of genetic structure

Differentiation between populations was assessed using pairwise F_{ST} values calculated using Arlequin 3.5. To test for the presence of geographical groupings of related samples, we applied a Bayesian cluster analysis to all individuals using the software STRUCTURE (Pritchard *et al.* 2000). The batch run function was used to carry out a total of 150 runs, 10 each for one to 15 clusters (K1 to K15). For each run the burn-in and simulation length was 30 000 and 100 000, respectively. We used the refined ad hoc statistic ΔK based on the rate of change in the log probability of data between successive K values (Evanno *et al.* 2005). It was calculated as ΔK = ([mL(K + 1) - 2mL(K) + mL(K - 1)])/sdL(K), where L(K) is the logarithm of the probability that K is the correct number of clusters, m is the mean and sd is the standard deviation.

Assessment of within population spatial genetic structure and relatedness among individuals

Within-population spatial genetic structure was assessed using 10000 permutations in the program SPAGeDI 1.3 (Hardy & Vekemans 2002). This tests the null hypothesis of no correlation between spatial distance and multilocus kinship (F) of individuals. The analysis was conducted over the whole dataset of adults and juveniles. The kinship coefficients were estimated using the allele frequencies over all natural populations (excluding Le Niol and André). Based on the maximum within-population distance of 160 m between individuals the distance classes were chosen as follows: 0-10 m to 120-160 m in total with 10 m intervals from 0-100 m, 20 m intervals from 100-120 m and a 40 m interval from 120-160. A minimum of 35 pairs per distance class was maintained for this analysis.

The *Sp*-statistic as a measure of intensity of fine-scale genetic structure (FSGS) was calculated as $Sp = -b_F/(1-F_{(1)})$ where b_F is the mean regression slope with natural log of distance (ln) distance and $F_{(1)}$ is the mean pairwise kinship coefficient for the smallest distance class (Vekemans & Hardy 2004). The standard deviation is calculated as $(SE\ b_F)/(1-F_{(1)})$ where SE is the standard error (Olivier Hardy personal communication). We also compared the mean within population individual kinship coefficients *F* (Loiselle *et al.* 1995) among all populations and between adults and juveniles in each population.

Estimating realised gene flow using parentage analysis

Using multilocus genotypes (ten loci) of 275 juveniles (excluding the juveniles from known plantations Le Niol and André) we applied a maximum likelihood exclusion analysis in CERVUS 3.0, to assign the two most likely candidate parents (Kalinowski *et al.* 2007; Marshall *et al.* 1998) in all natural populations. Pollen dispersal distance was deduced for each juvenile based on the position of the two most likely parent trees assigned by CERVUS. Simulations of paternity were run using the allele frequencies of all adult reproductive trees and the following settings: 10000 cycles; minimum number of loci typed 5, unknown parents; all surviving adults across the species range were set as candidate parents for juveniles; 1% for proportion of loci mistyped, and 97% for proportion of loci genotyped. The proportion of candidate parents sampled was set at 90%, as we could not be absolutely sure to have found all remaining potential parents and know three trees which we could not genotype and are thus missing in the analysis. Assignment was based upon the 96%, 90% and 80% confidence level of the critical LOD score. Seed dispersal was calculated as the distance between the juvenile and its closest assigned parent trees, as we know that seeds are primarily dispersed by gravity we assume that pollen dispersal is greater than seed dispersal.

RESULTS

Genetic diversity and inbreeding

      1.Species level

The ten loci each yielded between six and 19 alleles, with a total number of 120 alleles over all populations and loci. No linkage disequilibrium was observed for any pair of loci after Bonferroni correction. Further analyses were performed on multi-locus data from all ten microsatellites. After Bonferroni correction, and exclusion of populations having deviations from HWE in this study (Le Niol, L'Abondance, Sans Souci and Salazie), only one locus (23) out of our 10 showed a significant ($p < 0.05$) but low homozygous excess indicating low frequency of null alleles.

      2.Comparison between populations

Genetic diversity indicated by R_S (based on 5 diploid individual) and H_E was highest in population Cap Vert ($R_S = 5.13$; $H_E = 0.73$), lowest values were observed for Le Niol ($R_S = 1.90$; $H_E = 0.44$) and André ($H_E = 0.38$), see Table 1 for more details. The highest number of private alleles was found in Rivière L'Islette (6), Mt. Cotton (8) and L'Abondance (10). Significant F_{IS} values were observed in the adult and juvenile population of Le Niol (0.30, $p < 0.01$; 0.33, $p < 0.001$, respectively) and L'Abondance (0.12, $p < 0.01$; 0.14, $p < 0.001$, respectively). No significant F_{IS} values were detected in any of the other adult populations in which F_{IS} could be calculated (i.e. sites with more than one individual).

      3.Comparison between cohorts

When comparing the mean over populations and loci, a 95% confidence interval showed significantly lower values of genetic diversity in juveniles compared to adults for R_S and H_O. Values for F_{IS}, H_E and N_A were not significantly different between adults and juveniles. In total 46 rare alleles (with a frequency of 0.05 or lower) were found in adults but of these only 23 were in the juvenile cohort. The overall F_{IS} for adults was 0.09 ($p < 0.001$) and 0.07 ($p < 0.01$) for juveniles.

      We also calculated the overall F_{IS}, R_S, H_O, H_E and N_A values excluding the known plantations Le Niol and André. Without these two plantations, comparisons of genetic diversity (R_S, H_O, H_E, N_A) did not change substantially (see Table 2). The overall F_{IS} value was 0.08 ($p < 0.001$) for adults and 0.06 ($p < 0.001$) for juveniles.

A comparison between cohorts among individual populations showed significant differences for R_S in Cap Vert, Tea Plantation, Brulée and André (all $p < 0.05$). No significant differences were found for H_O and H_E. Also no significant differences were found for F_{IS} in any population between adults and juveniles after Bonferroni correction (see Table 1).
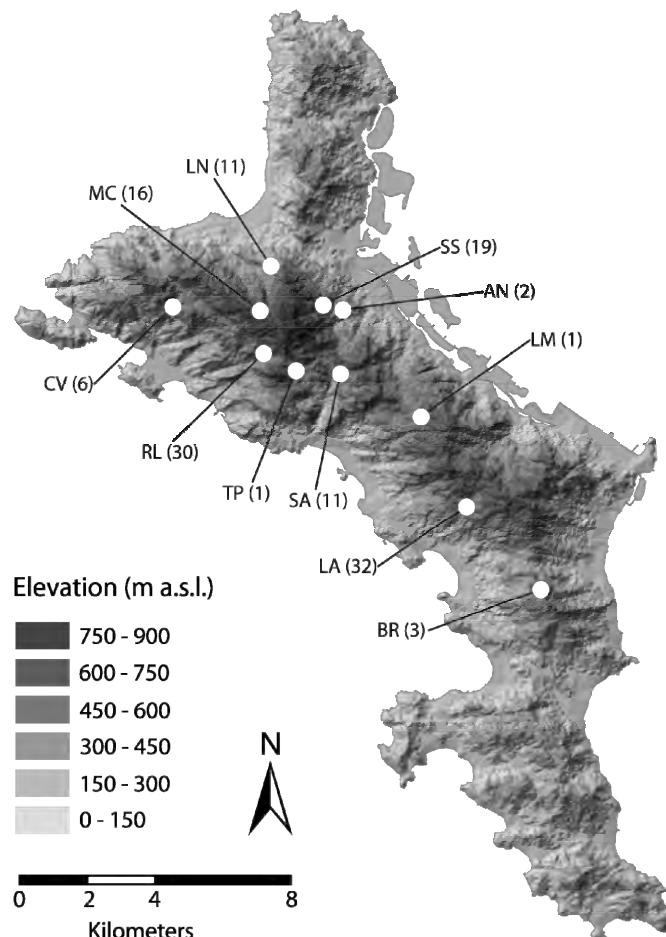
Figure 1: Map of the Seychelles main Island Mahé, with the 11 known *Vateriopsis seychellarum* populations. Numbers of individuals for the different populations are shown in brackets. LN = Le Niol, SS = Sans Souci, CV = Cap Vert, TP = Tea Plantation, LM = La Misère, MC = Mt. Cotton, RL = Rivière L'Islette, LA = L'Abondance, BR = Brulée, SA = Salazie, AN = André.

Genetic differentiation - Adults

The overall F_{ST} value across all adult populations was 0.30 ($p < 0.001$). Excluding populations Le Niol and André (populations confirmed as planted), overall F_{ST} value was 0.20 ($p < 0.001$). Pairwise genetic distances (Nei 1972) of populations in which F_{ST} values could be calculated (with more than one individual) were highest between Le Niol and the rest of the populations. Lowest values were found between Cap Vert and Mt. Cotton and all other adult populations (see Table S1, Supporting Information). Bayesian structure analysis of *V.*

seychellarum individuals discriminated the adult individuals into four and seven clusters. The probability values (mean Ln(Pr) and ΔK) reached their highest peak at K = 4. Additional but smaller peaks were observed at K = 2 and K = 7 (Table S1, Supporting information). Following the protocol of Pritchard *et al.* (2010) we chose the number of clusters that firstly had high probability values and secondly made biological sense, based upon this K = 4 and K = 7 are the most plausible clustering solutions. This analysis places Tea Plantation and the populations Le Niol and André constantly into a single cluster (Fig. 2a and b).

Table 2: Genetic variability of 10 microsatellite loci estimated for all populations of *Vateriopsis seychellarum*. Abbreviations: Ad: Adults; Sg: Seedlings; N_A: Mean number of alleles; H_O: observed heterozygosity; H_E: expected heterozygosity; $R_{S(1,5)}$: Allelic richness, based on one or five diploid individuals; P_A: number of private alleles; F_{IS}: Inbreeding coefficient; ± SE. P values: * = p < 0.05, ** = p < 0.01, *** = p < 0.001. LN = Le Niol, SS = Sans Souci, CV = Cap Vert, TP = Tea Plantation, LM = La Misère, MC = Mt. Cotton, RL = Rivière L'Islette, LA = L'Abondance, BR = Brulée, SA = Salazie, AN = André.

POP ID	n Ad	n Sg	N_A Ad	N_A Sg	H_O Ad	H_O Sg	H_E Ad	H_E Sg	$R_{S(1)}$ Ad	$R_{S(1)}$ Sg	$R_{S(5)}$ Ad	$R_{S(5)}$ Sg	P_A Ad	F_{IS} Ad	F_{IS} Sg
LN	11	34	1.9 (±0.10)	1.9 (±0.10)	0.35 (±0.07)	0.27 (±0.05)	0.44 (±0.05)	0.33 (±0.05)	1.46 (±0.05)	1.34 (±0.05)	1.90 (±0.10)	1.82 (±0.10)	0	0.30*	0.33***
SS	17	21	4.0 (±0.42)	3.6 (±0.50)	0.47 (±0.08)	0.40 (±0.08)	0.55 (±0.06)	0.48 (±0.07)	1.57 (±0.06)	1.49 (±0.07)	3.16 (±0.30)	2.75 (±0.30)	3	0.05ns	0.11ns
CV	6	40	5.5 (±0.45)	5.6 (±0.58)	0.76 (±0.08)	0.60 (±0.08)	0.73 (±0.04)	0.71 (±0.05)	1.79 (±0.04)	1.72 (±0.05)	5.13 (±0.38)	4.19 (±0.34)	3	0.02ns	0.11**
TP	1	36	2.0 (±0.00)	1.9 (±0.10)	1.00 (±0.03)	0.55 (±0.05)	0.50 (±0.00)	0.45 (±0.05)	2.00 (±0.00)	1.45 (±0.05)	-	1.90 (±0.10)	0	0.00ns	-0.23ns
LM	1	28	1.5 (±0.17)	1.5 (±0.17)	0.50 (±0.00)	0.23 (±0.08)	0.25 (±0.08)	0.24 (±0.08)	1.50 (±0.17)	1.25 (±0.08)	-	1.50 (±0.17)	0	0.00ns	-0.03ns
MC	16	0	6.7 (±0.80)	-	0.65 (±0.05)	-	0.70 (±0.04)	-	1.72 (±0.04)	-	4.48 (±0.41)	-	8	0.05ns	-
RL	30	17	5.6 (±0.69)	4.0 (±0.49)	0.57 (±0.06)	0.58 (±0.07)	0.61 (±0.05)	0.56 (±0.07)	1.62 (±0.05)	1.58 (±0.06)	3.61 (±0.35)	3.11 (±0.34)	6	0.07ns	-0.12ns
LA	31	66	5.5 (±0.34)	4.9 (±0.48)	0.57 (±0.07)	0.52 (±0.07)	0.65 (±0.06)	0.63 (±0.07)	1.67 (±0.06)	1.64 (±0.07)	3.76 (±0.31)	3.61 (±0.32)	10	0.12**	0.14***
BR	3	36	2.7 (±0.26)	3.1 (±0.31)	0.57 (±0.12)	0.49 (±0.09)	0.49 (±0.07)	0.49 (±0.07)	1.59 (±0.08)	1.50 (±0.07)	-	2.57 (±0.22)	3	-0.08n	-0.04ns
SA	11	30	4.3 (±0.50)	4.0 (±0.39)	0.50 (±0.05)	0.46 (±0.03)	0.58 (±0.04)	0.59 (±0.03)	1.60 (±0.04)	1.60 (±0.03)	3.32 (±0.27)	2.96 (±0.18)	2	0.13ns	0.21***
AN	2	8	1.9 (±0.10)	1.9 (±0.10)	0.40 (±0.10)	0.44 (±0.08)	0.38 (±0.05)	0.34 (±0.05)	1.50 (±0.06)	1.37 (±0.06)	-	1.85 (±0.10)	0	0.13ns	-0.29ns
Mean	129	317	3.76 (±0.21)	3.24 (±0.17)	0.58 (±0.03)	0.45 (±0.02)	0.53 (±0.02)	0.48 (±0.02)	1.64 (±0.03)	1.49 (±0.02)	3.62 (±0.16)	2.61 (±0.11)	-	-	-
Mean No plantations	116	275	4.17 (±0.23)	3.58 (±0.20)	0.62 (±0.03)	0.48 (±0.03)	0.56 (±0.02)	0.52 (±0.03)	1.67 (±0.03)	1.53 (±0.03)	3.91 (±0.16)	2.83 (±0.13)	-	-	-

(a)

(b)
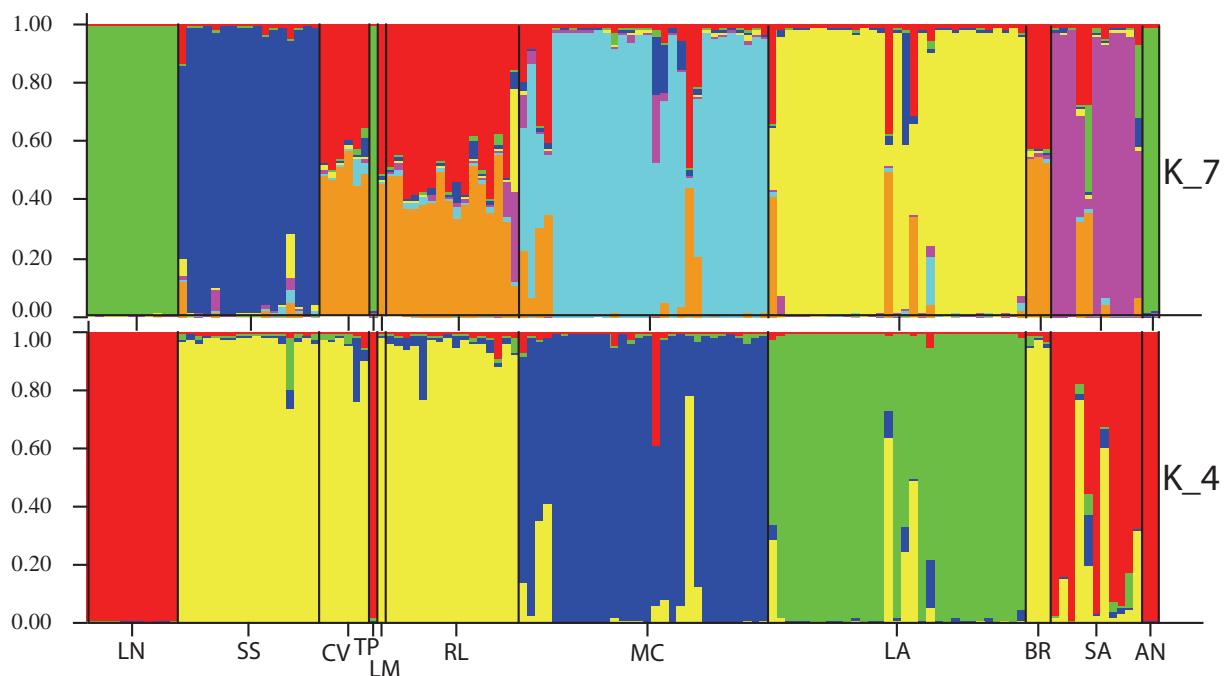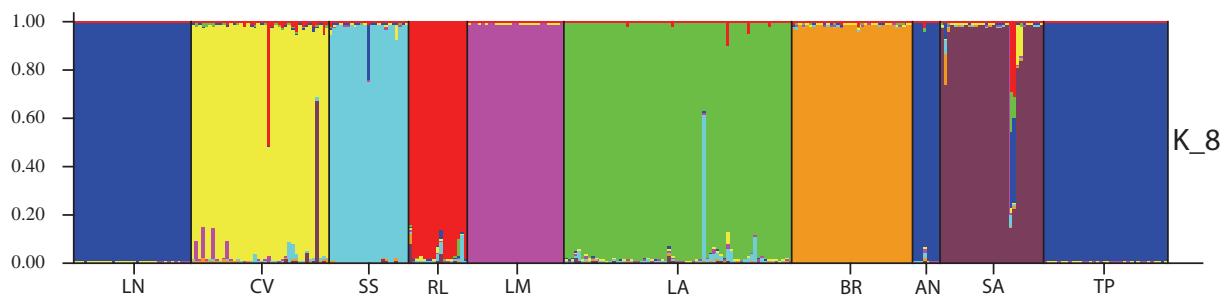
Figure 2: Bayesian structure analysis of *Vateriopsis seychellarum* with the STRUCTURE software (Pritchard et al. 2000). Bars represent individual *Vateriopsis seychellarum* individuals with their assignment proportions (y axis) to the different clusters. Performing the analysis for (a) adults for K_4 and K_7 (4 and 7 different grey shades), representing 4 and 7 genetic clusters and (b) K_8 (8 different grey shades), for a clustering solution of 8 genetic clusters for seedlings.

Genetic differentiation - Seedlings

The overall F_{ST} value for juveniles was 0.42 (p < 0.001), excluding populations Le Niol and André F_{ST} = 0.38 (p < 0.001). Pairwise genetic distances (Nei 1972) in all populations was greater in juveniles than in adults (Table S1, Supporting Information). The Bayesian structure analysis of *V. seychellarum* juveniles discriminated the juvenile individuals into eight clusters. The probability values (mean Ln(Pr) and ΔK) reached their highest peak at K = 2. Nevertheless, the biologically more relevant solution was the second highest peak at K = 8 (Table S1, Supporting Information and Fig. 2b).

Within Population fine-scale spatial genetic structure and kinship

Significant fine-scale spatial genetic structure (FSGS) was detected across all populations, in distance classes up to 160m for adult (Fig. 3a) and juvenile cohorts (Fig. 3b), as indicated by significant regression of kinship against spatial distance, adults $Sp = 0.05 \pm 0.004$ SE and juveniles $Sp = 0.09 \pm 0.007$ SE.

The lowest mean individual kinship coefficient (F) was observed in Cap Vert adults: $F = 0.06$ (± 0.03 SE), highest values in La Misère juveniles: $F = 0.70$ (± 0.004 SE). The highest kinship coefficients between individuals of different populations were recorded for individuals of the Tea Plantation, Le Niol and André (Tea Plantation – Le Niol: 0.43 ± 0.01 SE; Tea Plantation – André: 0.38 ± 0.01 SE; André – Le Niol: 0.44 ± 0.04 SE). The species level mean individual kinship coefficient was greater in juveniles compared to adults (Juveniles $F = 0.29 \pm 0.002$ SE; Adults $F = 0.19 \pm 0.004$ SE, respectively), see Fig. S1 in Supporting Information.

(a)   **(b)**
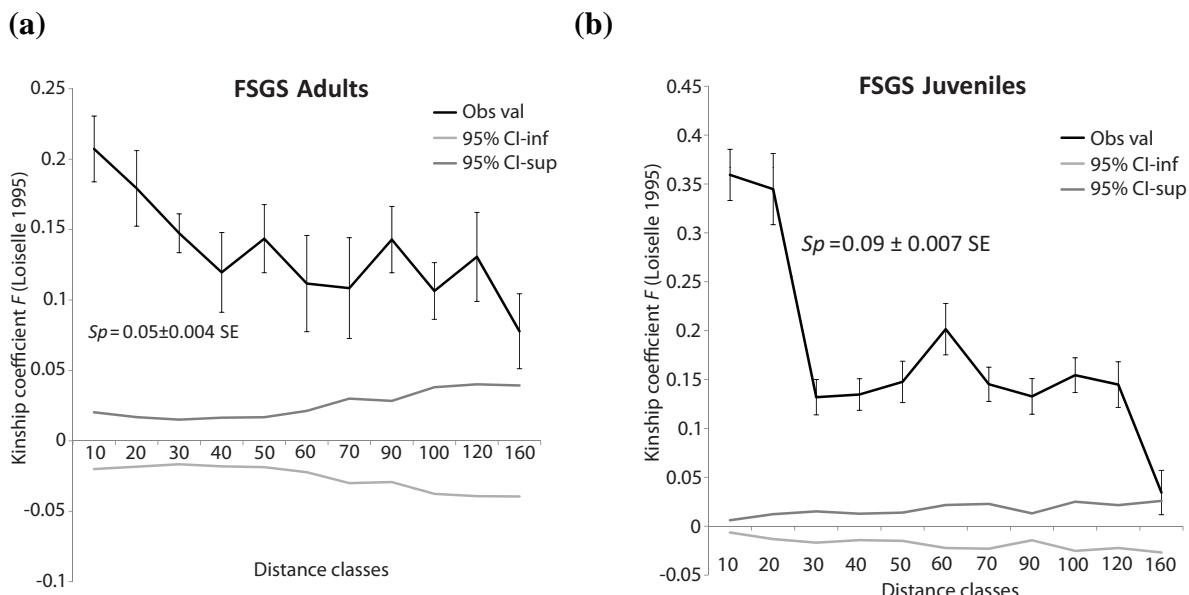
Figure 3: Fine-scale spatial genetic structure (FSGS) of *Vateriopsis seychellarum* adults (a) and seedlings (b). Correlogram of average kinship coefficient (F) of adult individuals over all populations for 12 distance classes. The solid line represents the average F values. The dotted lines represent the 95% (two-tailed) CI of the average F distribution calculated from 10000 permutations of spatial distance among pairs of adults.

Contemporary Pollen and Seed dispersal and mating system

Using parentage assignment we were able to assign 79% (216) of our juveniles (n = 275) with 90% confidence, and 60% of all juveniles with 96% confidence. On a more relaxed 80% confidence level we were able to assign 99% of the juveniles, we present here the more conservative 90% confidence level. Realised pollen dispersal ranged between 0 m (selfed) to 4.7 km in one case, with 94% of all juveniles resulting from mating events of less than 50

metres. Assuming that the nearest parent was the maternal tree, 90% of assigned realised seed dispersal events were less than 25 m, only 10% of seed dispersal was found between 25 and 100 m, with a maximum seed dispersal distance of 67.3 m (Fig. 4).

Selfing rates (number of cases where parentage analysis determined the father and mother as the same tree) are relatively high in most populations but considerably higher in Salazie (80%), Cap Vert (39%) and Sans Souci (38%). Lower values are observed in L'Abondance (18%) and Brulée (17%) and in Rivière L'Islette (10%). Individual isolated trees (La Misère, Tea Plantation) had exclusively selfed juveniles (Table 3). The overall selfing rate was 53%.

In the populations Cap Vert and Sans Souci three assigned fathers are found to be from outside the populations (1.4% events in a total of 216 assigned juveniles). In Sans Souci the father from two juveniles was identified as one tree at Mt. Cotton (Distance Sans Souci to Mt. Cotton = 1.7 km) and in Cap Vert one juveniles was assigned to a father from the population Salazie (Distance Cap Vert to Salazie = 4.8 km).
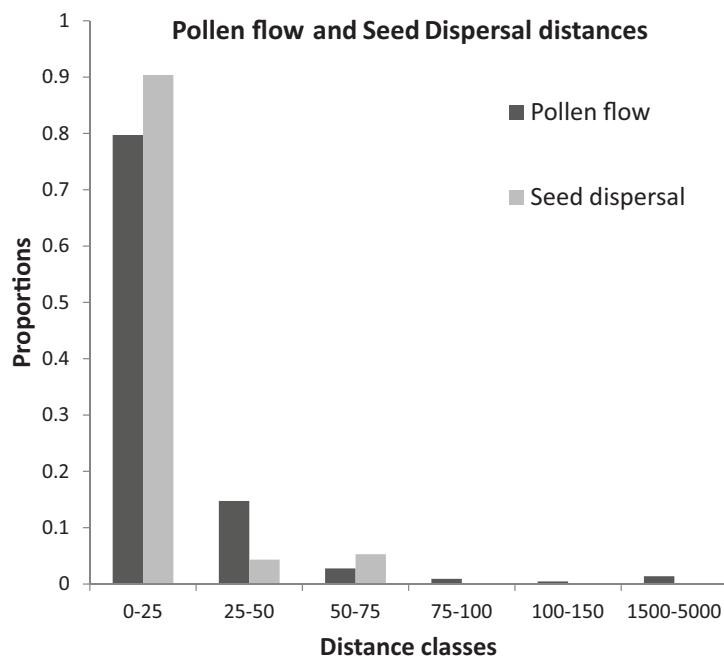
Figure 4: Frequency distributions of realised pollen and seed dispersal distances in *Vateriopsis seychellarum* populations, calculated from n = 275 seedlings and parent pairs.

Table 3: Results from the parentage analysis for 74% seedlings (n = 217) based on 90% assignments confidence showing the number of contributing trees to seedling production and selfing rate in the different populations. The numbers of trees that contributed to more than 20% to seedling production are given in Freq > 20%. SS = Sans Souci, CV = Cap Vert, TP = Tea Plantation, LM = La Misère, RL = Rivière L'Islette, LA = L'Abondance, BR = Brulée, SA = Salazie, MC = Mt. Cotton.

Population	#Contributing Adults	#Assigned Seedlings	Freq > 20%	Prop. selfed seedlings
CV	7	36	3	0.39
SS	7	13	2	0.38
RL	9	10	1	0.10
LA	27	40	0	0.18
BR	3	28	2	0.17
SA	6	26	1	0.80
TP	1	36	1	1.00
LM	1	28	1	1.00

DISCUSSION

Vateriopsis seychellarum is a critically endangered species, found in small numbers in only a few isolated populations in the Seychelles. Its landscape context is, on a small scale, typical of many dipterocarp species in Southeast Asia, the main centre of dipterocarp species diversity, where logging and agricultural expansion has fragmented lowland forest habitats. Our study of *V. seychellarum* provides insights into opportunities for ecological and genetic restoration efforts, and explores how the patterns of contemporary gene flow and mating system characteristics influence the viability of *V. seychellarum* in particular and other dipterocarps more generally.

Evidence for extreme genetic bottlenecks

The genetic diversity in V. seychellarum adults is comparable among populations with the exception of two plantation populations (Le Niol and André) and the single tree at La Misère where genetic diversity was lower. A parentage analysis of the adult trees from Le Niol and André, using all other adult trees as potential parents, demonstrated that these trees are selfed progeny derived from the single isolated tree at Tea Plantation. This tree is the largest known remaining V. seychellarum individual (DBH: 63.7 cm, Height: 18 m) and is easily accessible for collecting seed.

The small population at Cap Vert, despite comprising only six individuals, retains one of the highest values of genetic diversity (allelic richness) and underlines the value of some very small populations for conservation of genetic variation (Schneller & Holderegger 1996). Allelic richness, observed heterozygosity and number of alleles decreased from adult to juvenile stages, and the kinship coefficient between individuals increased, possibly caused by limited pollen and seed dispersal and frequent self-fertilisation. We interpret the absence of 50% of rare alleles in the V. seychellarum juvenile cohort as an early sign of genetic erosion following habitat fragmentation (Kettle et al. 2007). This difference in levels of genetic diversity could also be a consequence of differences in effective breeding population sizes, for example, a mass-fruiting event where all remaining adults contribute to juvenile production could greatly increase the observed diversity in a single cohort. A number of Asian dipterocarp species show annual flowering, but there is no evidence of superannual mass flowering for V. seychellarum on the Seychelles. The Mt. Cotton population, despite having relatively high genetic diversity, currently lacks any natural regeneration, and during three years of field visits no flowers, fruits or juveniles were observed. Numerous ecological

constraints, such as habitat quality, mycorrhiza availability (Tedersoo 2007), herbivory, or pollinator limitation might conceivably limit recruitment success in this population, although there is no evidence to implicate any one of these factors specifically.

Genetic structure, seed and pollen dispersal

Vateriopsis seychellarum adults have strong genetic differentiation, revealed as high pairwise F_{ST} values, private alleles, and a clear genetic clustering. The fine scale spatial genetic structure analysis (FSGS) over all adults within populations demonstrates a close relatedness of individuals up to 150 m. This implies that despite a formerly more widespread distribution, gene dispersal has been somewhat restricted. The most plausible explanation for this is limited pollen and seed dispersal. The wingless fruit of *V. seychellarum* provides no obvious means for dispersal beyond the shadow of the mother tree's canopy. Pollen flow is likely to be more extensive, but the pollinators, although poorly known, are likely to be small beetles which are not thought to cover large distances. Restricted gene flow has been noted for several dipterocarp species elsewhere, suggesting that the genetically structured populations found in *V. seychellarum* are not uncommon for this family. In Malaysian Borneo, *Shorea xanthopylla* and *Parashorea tomentella* show significant FSGS partly due to limited seed and pollen dispersal (Kettle *et al.* 2011b). Dipterocarp species with highly mobile insect pollinators appear to have weaker FSGS compared with species with smaller pollinators (Harata *et al.* in press; Kettle *et al.* 2011a). The mountainous topography of Mahé might further impede gene flow in *V. seychellarum*. The wind-pollinated *Shorea robusta* in hilly regions in Nepal, showed higher levels of genetic differentiation among populations, naturally disjunct along river valleys, compared with larger continuous populations (Pandey & Geburek 2010). These examples indicate that habitat fragmentation may have similar genetic consequences for many dipterocarp species of different geographical regions as gene dispersal modes are often comparable.

Genetic differentiation among populations of *V. seychellarum* is stronger in juvenile cohorts (higher pairwise F_{ST}), which could be interpreted as increased genetic isolation in currently fragmented populations. This observation is supported by a stronger clustering solution in the Bayesian analysis for the juveniles (eight genetic clusters compared to four and seven genetic clusters in adults). Furthermore, the intensity of FSGS is stronger in juveniles, as indicated by the higher *Sp* value (0.09 in juveniles and 0.05 in adults). These results might also be a consequence of sampling juveniles which were produced over comparatively few (possibly up to ten) years. Nevertheless, as these juveniles represent the main contribution to

future generations of this species the increased differentiation as indicated by the clustering analysis does suggest greater isolation. The parentage analysis of 275 juveniles demonstrated that contemporary seed dispersal is limited, and 94% of juveniles genotyped were less than 25 m from their assigned mother, with a maximum observed seed dispersal distance of 67 m. Limited seed dispersal in *V. seychellarum* is consistent with its gravity mode of seed dispersal, a common characteristic of dipterocarps (Seidler & Plotkin 2006; Suzuki & Ashton 1996).

Pollen dispersal distances were low in *V. seychellarum* (90% less than 50 m), and most assigned fathers were within-populations. We observed three (1.4% of all observations) apparent long-distance pollen dispersal events, with pollen donors identified as Mt. Cotton and Salazie individuals, for one juvenile at Cap Vert (Distance Cap Vert to Salazie = 4.8 km) and two at Sans Souci (Distance Sans Souci to Mt. Cotton = 1.7 km). It is possible that small pollinators (possibly small beetles) could have crossed to relatively distant populations but this seems unlikely on account of both distance and topography. Pollen dispersal of over 500m has been observed in other dipterocarp species (Kenta *et al.* 2004), but an alternative explanation for apparent long-distance pollen dispersal events in *V. seychellarum* could be erroneous assignment resulting from failure to sample the true fathers in Sans Souci and Cap Vert: two adults from Sans Souci could not be genotyped and there is the possibility that we may not have found all adults at Cap Vert. It may also be possible that the potential parents have been logged and may thus no longer exist. No pollen donors from Mt. Cotton or Salazie were observed at closer sites and the Mt. Cotton population showed no sign of recruitment or flowering, further supporting low probability of long-distance pollen flow. When excluding Mt. Cotton and Salazie from the analysis all juveniles were assigned to parents within the same population and juveniles assigned to the adult at Mt. Cotton had allele mismatches indicating they are probably not the real parents. Although we cannot completely exclude the potential for rare long-distance pollen flow in *V. seychellarum*, such long distance dispersal has not been sufficient to prevent genetic structuring over relatively narrow spatial scales (< 5 km), we conclude that it must be very rare, if it occurs at all.

Mating system and its implications for population viability

A high degree of selfing was apparent in some populations of *V. seychellarum* (Salazie, 80%, Sans Souci 38% and Cap Vert 39%), and all juveniles sampled from the single isolated individuals (Tea Plantation, La Misère) were selfed. Studies in other dipterocarps have shown a range of mating systems from complete outcrossing to apomixis (Bawa 1998). In *Shorea*

acuminata which is predominantly visited by thrips, single pollen grain genotyping collected from insects demonstrated high levels of self-pollination through geitonogamy (Kondo *et al.* 2011). In predominately outcrossing dipterocarps, selfing rates tend to increase with reduced population density and fewer flowering conspecifics (Fukue *et al.* 2007; Nagamitsu *et al.* 2001; Naito *et al.* 2008; Tani *et al.* 2009). Selfing might, however, have benefits in terms of predator avoidance (Ghazoul & Satake 2009), reducing maladapted genes, or assuring reproduction (Levin 2010). It is noteworthy that in a comparison of two dipterocarp species with contrasting mating systems, the outcrossing *Shorea leprosula* exhibited a greater loss of genetic diversity due to logging than the self-compatible *Shorea ovalis* (Ng *et al.* 2009). Nevertheless, selfed progeny tend to have higher rates of abortion or mortality at early ontogenic stages as seen in representatives of three dipterocarp genera: *Dipterocarpus obtusifolius*, *Shorea siamensis* and *Dryobalanops lanceolata* (Ghazoul 1997; Ghazoul *et al.* 1998; Momose *et al.* 1996). Self-fertilization can also lead to genetic problems associated with increased homozygosity and inbreeding depression, as well as reduced effective population sizes and genetic diversity (Hamrick & Godt 1996; Ingvarsson 2002; Schoen & Brown 1991). In other dipterocarp species such as *Neobalanocarpus heimii* the seed mass and germination success of selfed seeds is lower than that of outcrossed seeds (Naito *et al.* 2005). At this stage it is difficult to predict whether inbred and selfed populations of *V. seychellarum* will be able to maintain their reproductive output and performance over multiple generations, but a precautionary approach to population restoration efforts avoiding inbreeding through careful selection of seed sources may be advisable.

Implications for conservation and management

We detected extreme genetic bottlenecks in the two planted populations of *V. seychellarum* (Le Niol and André). All other populations (with more than one individual) retain high genetic diversity and low kinship coefficients, consistent with the idea that these are remnants of formerly larger 'natural' populations. Low selfing rates (L'Abondance and Rivière L'Islette) and high numbers of contributing parents to juvenile production in L'Abondance could indicate less disturbed habitats but could also mirror the higher numbers of individuals in these populations. Mt. Cotton, having high genetic diversity and low pairwise F_{ST} values compared to other populations supports the view that this is a refugial population. Based on these findings we make a number of management recommendations.

Like all dipterocarp species, *V. seychellarum* seeds are recalcitrant and cannot easily be stored in conventional seed banks. *Ex-situ* conservation will thus only be achieved through

nursery grown seedling banks. Ideally about 20 seeds from each individual tree should be collected from populations where inbreeding is known to be low in adults and juveniles, such as Mt. Cotton, Rivière L'Islette, Sans Souci and Brulée to ensure sampling the whole range of adult genetic variety. *Ex-situ* collections have been able to capture 94% of the whole species genetic diversity in a rare palm (*Leucothrinax morrisii* Arecaceae) within 58 individuals (Namoff *et al.* 2010) demonstrating that even a relatively modest number of individuals (100-200) can help conserve genetic diversity *ex-situ*. Similarly, in the endangered endemic dipterocarp *Vatica guangxiensis* in China, 28 *ex-situ* individuals conserved 88.31% of the neutral genetic variation of natural populations (Li *et al.* 2002). Larger scale ex-*situ* seedling banks (several 1000 juveniles) could provide germplasm for habitat restoration and material for transplant experiments into their original populations. Such population augmentation should ensure that seeds are collected from donor sites which are not only diverse and outbred, but share similar site conditions, to minimise the likelihood of disrupting any local adaptation. In addition, common garden experiments using progeny from controlled pollination experiments could be used to test whether inbreeding or outbreeding depression occur in these populations. We expect outbreeding depression to be unlikely due to the similarity of habitats and limited potential for local adaptation.

New populations should also be established at strategic locations. Such locations should consider the ecological context of the relatively short gene dispersal distances (mostly less than 50 m) and should thus be located close to and between remaining populations to enhance connectivity of existing populations. Clearly, management efforts will only be successful if large natural populations such as L'Abondance, Rivière L'Islette, Sans Souci and Mt. Cotton are protected.

Trees from both plantations (Le Niol and André) produce progeny that were assigned as within-population matings and these juveniles and saplings showed no obvious detrimental inbreeding effects. Even so, self-compatible species often reveal the effects of inbreeding depression at late life stages (Husband & Schemske 1996; Thiele *et al.* 2010) and the long term growth and survival of *V. seychellarum* progeny warrant further investigation. Repeated bouts of inbreeding within these plantations are likely to further erode the genetic base of these plantations, decreasing their potential to adapt to novel pests and pathogens which are issues of special concern for island populations (Burdon 2001; Namkoong 1991; Spielman *et al.* 2004). Collecting seeds from these two sites in the future is therefore unlikely to contribute much to preserving the species-wide genetic diversity. Efforts should be invested in collecting seed from a variety of population sources, including relict trees, to ensure the maintenance of

species-wide genetic diversity in restoration efforts. Such a strategy has been shown to be potentially successful for restoration in the Australian *Eucalyptus leucoxylon* (Ottewell *et al.* 2009). However, due to the reduced genetic diversity found in seed collections from some single isolated trees in *V. seychellarum* (Tea Plantation tree), our study indicates that this should be conducted with caution, especially in self-compatible species.

Our results suggest that limited gene flow in *V. seychellarum* may lead to long-term population isolation. Many other dipterocarp species have also been observed to have relatively limited pollen and seed dispersal (Ghazoul *et al.* 1998; Kettle *et al.* 2011b; Osada *et al.* 2001; Seidler & Plotkin 2006). This has implications for a large number of rare and threatened dipterocarp species which continue to experience habitat degradation and fragmentation. Estimates of habitat loss in Sabah (Malaysia) show that 32 of 33 dipterocarp species are threatened, particularly species with narrow distributions (Maycock *et al.* 2012). Ng *et al.* (2009) demonstrate that even a single logging event can cause genetic erosion in an outcrossing dipterocarp (*Shorea leprosula*) by loss of rare alleles. Another dipterocarp, *Shorea lumutensis*, lost 80% of its population size due to logging in the Peninsular of Malaysia (Lee *et al.* 2006). Similar to *V. seychellarum*, *S. lumutensis* survives in few populations with few mature individuals (about 500) but retains a large amount of genetic variation in adults that probably pre-date habitat fragmentation. Our study suggests that species like *S. lumutensis* may be particularly vulnerable to population isolation due to significant spatial genetic structure and relatively low pollen flow rates (175m). Other tropical tree species with limited dispersal potential may show similar trends, for example the response of *Copaifera langsdorffii* (Fabaceae) in Brazil to habitat fragmentation (Sebbenn *et al.* 2011).

Less than half of the original forests of Southeast Asia remain (Sodhi *et al.* 2004) and Southeast Asian forest restoration is now seen to be an essential tool for dipterocarp (and other forest tree species) conservation and recovery (Kettle 2010). This study advances our understanding of how patterns of gene flow and existing genetic diversity relate to population recruitment and viability and will lead to better informed conservation and restoration.

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SUPPORTING INFORMATION

Table S1: Pairwise F_{ST} values for *Vateriopsis seychellarum* adults and seedlings after Bonferroni correction. Significant results are marked as bold numbers. Above the diagonal are values for seedlings and below those for adults. Abbreviations: Ad: Adults; Sg: Seedlings. LN = Le Niol, SS = Sans Souci, CV = Cap Vert, TP = Tea Plantation, LM = La Misère, MC = Mt. Cotton, RL = Rivière L'Islette, LA = L'Abondance, BR = Brulée, SA = Salazie, AN = André.

Ad	Sg	LN	SS	CV	TP	LM	MC	RL	LA	BR	SA	AN
LN		-	**0.54**	**0.38**	**0.10**	**0.66**	NA	**0.46**	**0.44**	**0.53**	**0.44**	**0.29**
SS		**0.39**	-	**0.20**	**0.45**	**0.52**	NA	**0.25**	**0.18**	**0.33**	**0.32**	**0.46**
CV		0.30	0.13	-	**0.31**	**0.35**	NA	**0.16**	**0.16**	**0.23**	**0.19**	**0.30**
TP		NA	NA	NA	-	**0.66**	NA	**0.35**	**0.38**	**0.45**	**0.35**	0.09
LM		NA	NA	NA	NA	-	NA	**0.52**	**0.40**	**0.50**	**0.51**	0.68
MC		**0.32**	**0.15**	**0.05**	NA	NA	-	NA	NA	NA	NA	NA
RL		**0.32**	**0.23**	**0.13**	NA	NA	**0.11**	-	**0.21**	**0.29**	**0.27**	0.36
LA		**0.34**	**0.21**	**0.10**	NA	NA	**0.13**	**0.18**	-	**0.22**	**0.27**	**0.36**
BR		0.40	0.27	0.11	NA	NA	0.11	**0.22**	**0.15**	-	**0.32**	**0.45**
SA		**0.33**	**0.27**	0.17	NA	NA	**0.16**	**0.25**	**0.25**	0.24	-	0.34
AN		-0.01	0.31	0.17	NA	NA	0.22	0.26	0.26	0.29	0.26	-

Table S2: ΔK values calculated from the mean Ln(Pr) values obtained from the STRUCTURE analysis for *Vateriopsis seychellarum* adults and seedlings.

	Adults			Seedlings	
K	mean Ln(Pr) ± SD	ΔK		mean Ln(Pr) ± SD	ΔK
1	-4669.29 ± 0.46	-		-11354.64 ± 0.83	-
2	-4254.73 ± 12.66	5.14		-9473.23 ± 35.79	23.47
3	-3905.32 ± 44.15	2.26		-8431.66 ± 51.85	5.21
4	-3655.65 ± 12.52	8.51		-7660.37 ± 57.41	3.12
5	-3512.50 ± 23.67	1.99		-7068.38 ± 93.96	1.51
6	-3416.84 ± 103.28	0.91		-6618.39 ± 66.59	1.74
7	-3295.44 ± 18.86	2.97		-6284.24 ± 87.79	0.75
8	-3469.25 ± 59.54	1.83		-6016.24 ± 22.85	5.13
9	-3414.30 ± 43.70	1.87		-5865.54 ± 59.16	0.24
10	-3441.04 ± 65.94	0.34		-5729.49 ± 59.06	0.92
11	-3445.60 ± 74.49	0.78		-5648.01 ± 82.86	0.28
12	-3508.34 ± 76.59	1.12		-5543.23 ± 58.81	0.97-
13	-3485.09 ± 92.15	1.03		-5495.50 ± 42.44	0.22
14	-3556.36 ± 100.39	0.14		-5457.47 ± 107.93	0.55
15	-3613.32 ± 115.11	-		-5360.52 ± 74.04	
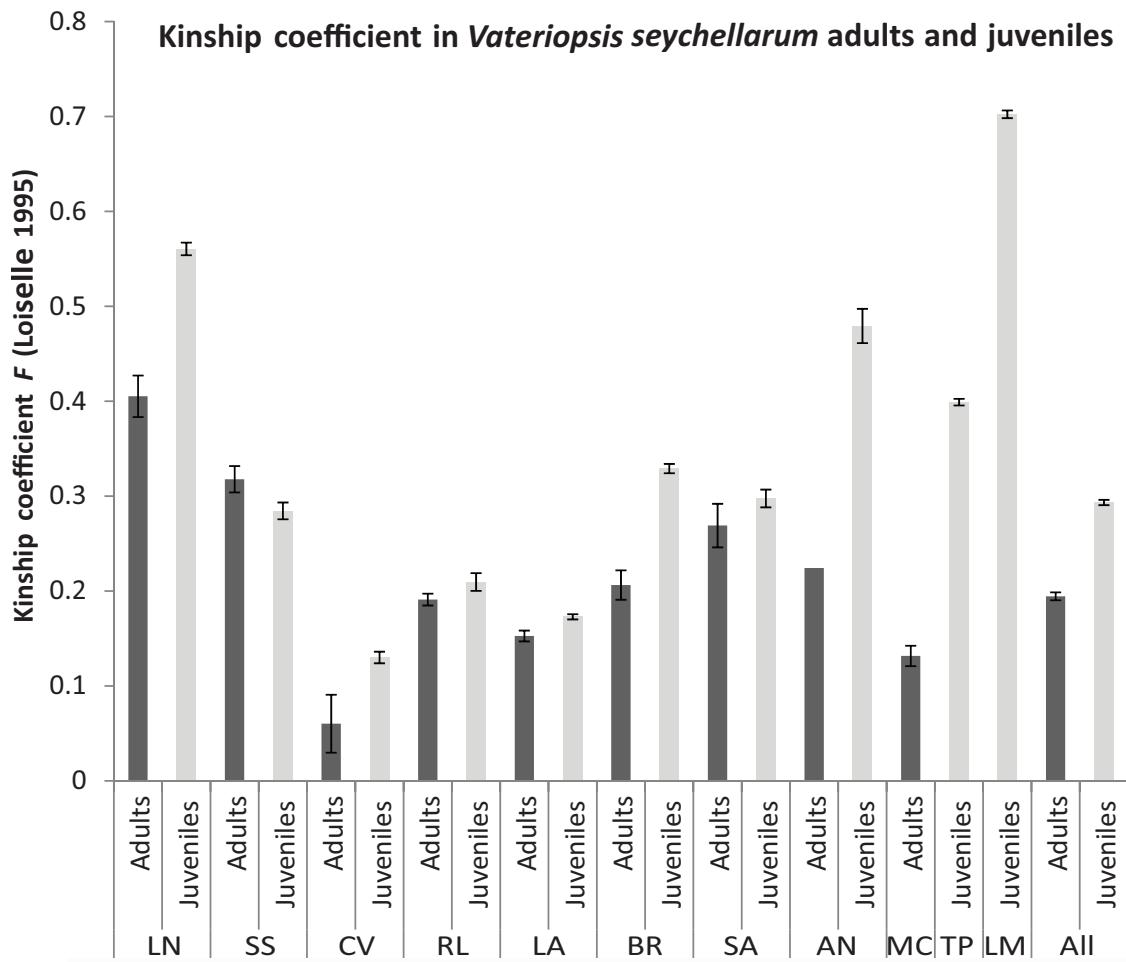
Figure S1: Average kinship coefficient (*F*) of adult and seedling individuals of *Vateriopsis seychellarum* in the different populations and over all populations. LN = Le Niol, SS = Sans Souci, CV = Cap Vert, TP = Tea Plantation, LM = La Misère, MC = Mt. Cotton, RL = Rivière L'Islette, LA = L'Abondance, BR = Brulée, SA = Salazie, AN = André.

Genetic connectivity of the moth pollinated tree *Glionnetia sericea* (Rubiaceae) in a highly fragmented habitat

with C.J. Kettle, C.N. Kaiser-Bunbury, T. Valentin and J. Ghazoul
publication in preparation

ABSTRACT

Long distance gene flow is thought to be one prerequisite for the survival of plant species in fragmented environments. Human influences have lead to severe habitat fragmentation in the Seychelles islands, with many species surviving in small and isolated populations. The endemic and endangered tree *Glionnetia sericea* is restricted to habitats at altitudes between 400m and 900m in the Seychelles which have been considerably fragmented over the last 200 years. This study explores the genetic and ecological consequences of habitat fragmentation in this species by analysing patterns of genetic diversity in a sample of adults, seedlings and seeds, and by using controlled pollination experiments. Our results show no decrease in genetic diversity and no increase in genetic structuring from adult to seedling cohorts. Despite significant levels of inbreeding in some populations, inbreeding is not elevated from adult to seedling cohorts, nor is a loss of rare alleles in seedlings observed. A Bayesian structure analysis and parentage analysis indicate extensive historical and contemporary gene flow among remnant populations. Pollination experiments and a parentage analysis show that *Glionnetia sericea* is self-compatible. Artificial pollination experiments compared to open pollinated treatments provided no evidence for pollen limitation in isolated fragmented sites. The highly mobile and specialized pollinators (*Agrius convolvuli* and *Cenophodes tamsi*) may enable extensive gene flow thus mitigating the potential negative ecological and genetic effects of habitat fragmentation in this species. We conclude that contemporary gene flow is sufficient to maintain genetic connectivity in this rare and restricted endemic of the Seychelles.

INTRODUCTION

In fragmented landscapes, be they naturally fragmented or fragmented due to anthropogenic degradation, the degree of genetic exchange between fragments may be important to ensure a species' survival. Negative genetic effects, such as inbreeding depression and loss of genetic diversity have been observed to follow population isolation and subsequent restricted gene flow (Van Geert *et al.* 2008). Species with the ability for long-distance gene flow may consequently be less prone to population isolation following recent habitat fragmentation compared to species with limited gene dispersal (Ahmed *et al.* 2009; Dick 2001).

Wind pollinated tree species are thought to be less vulnerable to the effects of habitat fragmentation as pollen can be transported over long distances (Buschbom *et al.* 2011; Robledo-Arnuncio 2011). Similarly, mobile pollinators have been shown to transport pollen over extensive distances (in the range of 10's of kilometres), thus ensuring the genetic connectivity between populations that are geographically disjunct (Dick 2001; Lander *et al.* 2010; Sork & Smouse 2006). Even single isolated trees within degraded landscapes can act as pollen donors to distant forest stands demonstrating the potential for insect pollinated tree species to maintain gene exchange across fragmented landscapes (Lander *et al.* 2010; Ottewell *et al.* 2009; White *et al.* 2002). Previous studies have shown that habitat fragmentation negatively affects pollination success and reproductive output of both generalist and specialist pollinated plant species to a similar proportion (Aizen *et al.* 2002; Ashworth *et al.* 2004). Tree species with specialised pollinators might though be less vulnerable to pollinator competition by introduced invasive plant species compared to generalist pollinated plants because of the tight coupling of the mutualistic relationship between plant and pollinator (Ashworth *et al.* 2004; Bond 1994). Such factors may be especially important in island systems prone to introduction of invasive plant species (Kaiser-Bunbury *et al.* 2011; Kueffer *et al.* 2010).

Glionnetia sericea is an endangered (IUCN 2011) endemic tree of the Seychelles archipelago with a specialised pollinator mutualism. Pollination is by two hawk moth species (*Agrius convolvuli* and *Cenophodes tamsi*) which have long probosces to reach the nectar and can potentially fly long distances (Haber & Frankie 1989; Janzen 1994). *Glionnetia sericea* is naturally restricted to habitats within altitudes of about 400m – 900m. These hilltop mist forest habitats on the island of Mahé, the main Island of the Seychelles, have most probably been naturally fragmented since several thousand years. The current populations of *G. sericea* have been reduced in size due to extensive deforestation during the 20th century (Diels 1992)

and subsequent invasion of this habitat by introduced invasive species such as *Cinnamomum verum*, *Psidium littorale*, *Adenanthera pavonina* and *Tabebuia pallida* (Fleischman 1997).

Patches of *G. sericea* vary from extremely small (1-12 individuals) on granitic outcrops (inselbergs) to relatively large and more continuous (about 100 individuals) in mist forests. Increase in habitat degradation (mainly in mist forest areas) and numbers of invasive species may threaten the long-term survival of this species (Daehler *et al.* 2004) and the inselbergs provide important refugia for *G. sericea* due to their harsh environmental conditions often not suitable to invasives (Fleischmann 1997a). In the long-term the species is likely to mainly survive in these patchy granitic outcrop refugia, so it's important to know if a) the species can maintain its connectivity between populations, b) if pollinators can move between populations and c) whether such pollinator movement will be ecologically frequent enough to sustain seed production.

The aim of this study is to understand the variation in mating system in a species which has predicted long distance dispersal and is naturally fragmented. We evaluate historical and contemporary gene flow among remnant populations of *G. sericea* to explore the idea that this species will survive in inselberg refugia following habitat fragmentation. In this context we address the following questions: (i) Are remnant adults or progeny of *G. sericea* genetically structured? (ii) To what extent does contemporary gene flow connect populations? (iii) To what extent are *G. sericea* pollinators and subsequently pollination success affected by fragmentation? We address these questions using a combination of genetic analysis of adults, seedlings and seeds in six populations, using a set of ten nuclear microsatellites. A pollinator exclusion experiment was used to explore the potential effects of pollen limitation. Additionally we use artificial pollination experiments between a single isolated small population (total: 12 individuals) and a larger less isolated population (> 60 individuals) to explore the potential for a fitness advantage from long distance pollen dispersal. The results from this study are relevant for the conservation and restoration of island species in small populations and provide insights into the importance of long-distance pollen dispersal by insect pollinators in recently fragmented landscapes. This species' naturally patchy population structure provides a useful study system for investigations of the genetic consequences of habitat fragmentation in a species which is predicted to have extensive pollen dispersal.

<div align="center">

MATERIALS AND METHODS

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Study species and populations

The rare tree *Glionnetia sericea* (Rubiaceae) is endemic to the Seychelles archipelago. It is found on two islands, Mahé and Silhouette, and the total population size is estimated less than 2500 individuals (Mahé and Silhouette) by the IUCN, where it is also classified as endangered (IUCN red list 2011). Based upon our own observations we estimate less than 1000 individuals survive on Mahé (Figure 1). This estimate is based on the ten known discrete sites each which have a maximum of approximately 100 individuals. *Glionnetia sericea* is distributed in mist forests at higher altitudes between 400m and 900m and is representative of a plant community occurring in virgin forest remnants. The current distribution includes some relatively large and continuous populations, consisting of up to 100 individuals, and some smaller more isolated populations typically found on granitic outcrops (inselbergs).. Nevertheless, inselberg populations have usually lower numbers of individuals (1-12, which on larger inselbergs reach up to > 60 individuals) due to the lack of soil on the rocky outcrops. Mist forest populations are usually larger (about 100 individuals) and more continuous.

The species is pollinated by two hawk moths species *Agrius convolvuli* and *Cenophodes tamsi* (Kaiser-Bunbury *et al.* 2011) and seeds are dispersed by gravity and possibly also by wind. These traits are commensurate with the potential for long distance gene flow and may render the species less vulnerable to the genetic consequences of habitat fragmentation. We sampled leaf material for genetic analysis from adults, seedlings and seeds of six sites (five for seed collections), four inselberg habitats (Mt. Sebert, Copolia1 + 2, Morne Blanc) and two mist forest sites (Mt. Jasmin, Gongo Rouge), see Table 1.
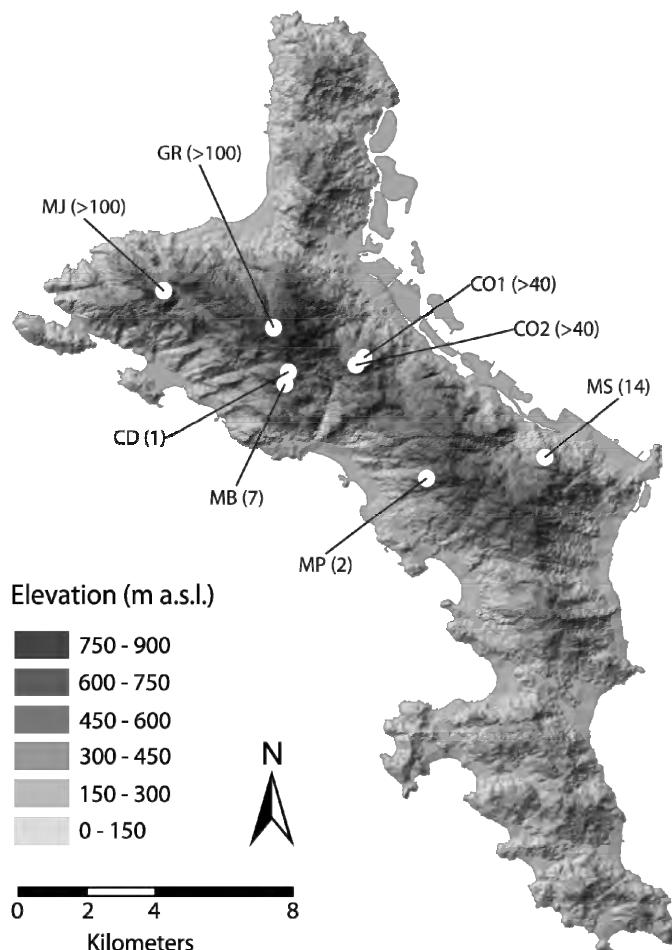
Figure 1: Map of the Seychelles main Island Mahé, with the known *Glionnetia sericea* populations. Numbers of estimated individuals are marked in brackets. MS = Mt. Sebert, MJ = Mt. Jasmin, GR = Gongo Rouge, CO1 = Copolia1, CO2 = Copolia2, MB = Morne Blanc.

Sampling and genetic analysis

In 2009 we collected leaf material for DNA extraction from a sample of 206 *Glionnetia sericea* adults and 196 seedlings at six sites and 620 seeds (from 24 mother trees) at five sites (due to difficult tree accessibility we were not able to collect seeds from Morne Blanc). Leaf material was immediately dried and stored in silica gel. DNA was extracted from the leaves using the QIAGEN DNeasy 96 Plant Kit, following the manufacturer's protocol. All samples were screened at a total of ten nuclear microsatellite loci, details of which are described in Finger *et al.* (2011). Fragment analysis was conducted using an ABI3730 sequencer and genotyped using Genemapper 3.5 software (Applied Biosystems). No significant linkage disequilibrium was observed for any pair of loci after bonferroni correction. Therefore, further analyses were performed on multi-locus data from all ten microsatellites.

Assessment of genetic diversity and inbreeding

Presence of null alleles (Selkoe & Toonen 2006) was tested using the program MICRO-CHECKER (Van Oosterhout *et al.* 2004). Number of alleles (N_A), number of effective alleles (N_E), allele frequencies and the number of private alleles (P_A) were calculated using GenAlEx 6 (Peakall & Smouse 2006). Allelic richness (R_S) was calculated using FSTAT 2.9.3.2 (Goudet 1995). Tests of linkage disequilibrium, observed and expected heterozygosities (H_O, H_E) and inbreeding coefficients (F_{IS}) using 10,000 permutations, were calculated with Arlequin 3.5 (Excoffier et al. 2005).

Assessment of genetic structure over the species range

Overall F_{ST} values were calculated with Arlequin 3.5. Differentiation between populations was assessed using pairwise F_{ST} values calculated using FSTAT. To test for the presence of geographical groupings of related samples, we applied a Bayesian cluster analysis to all individuals using the software STRUCTURE (Pritchard *et al.* 2000). The batch run function was used to carry out a total of 100 runs, ten each for one to ten clusters (K1 to K10). For each run the burn-in and simulation length was 10,000 and 30,000, respectively. Since the log probability values for the different K values have been shown to be of little reliability in other cases, the more refined ad hoc statistic ΔK based on the rate of change in the log probability of data between successive K values (Evanno *et al.* 2005)was used. It is calculated as ΔK = ([mL(K + 1) - 2mL(K) + mL(K - 1)])/sdL(K), where L(K) is the logarithm of the probability that K is the correct number of clusters, m is the mean and sd is the standard deviation.

Assessment of within population spatial genetic structure and relatedness among individuals

Fine scale spatial genetic structure was assessed using 10,000 permutations using SPAGEDI 1.3 (Hardy & Vekemans 2002). This tests the null hypothesis of no correlation between spatial distance and multilocus kinship (F) of individuals. The analysis was conducted over the large populations (Mt. Jasmin, Copolia1, Copolia2 and Gongo Rouge) of adults and also juveniles. To visualize results of the FSGS, *F* values were assigned over a set of ten distance classes and plotted against the distance using all individuals as a single sample.

The *Sp*-statistic as a measure of intensity of fine-scale genetic structure (FSGS) was calculated as *Sp*= -b_F/ (1-$F_{(1)}$) where b_F is the mean regression slope with natural log of distance (ln) distance and $F_{(1)}$ is the mean pairwise kinship coefficient for the smallest distance class (Vekemans & Hardy 2004). The standard deviation is calculated as (SE b_F)/ (1-$F_{(1)}$) where SE is the standard error (Hardy *pers comm.*).

Estimating historical gene dispersal from FSGS

Based upon the assumption that the observed fine-scale spatial genetic structure (FSGS) represents an equilibrium isolation-by-distance pattern, it is possible to estimate historical gene dispersal for adult plants using spatial coordinates and genotype data of sampled adults (Hardy *et al.* 2006). Wrights neighbourhood size $Nb \approx 4\pi D_e$ was estimated as $= - (1 - F_N)/b_k$ (Vekemans & Hardy 2004), where b_k is the regression slope within a distance class of $\sigma_g < d_{ij} < 20\sigma_g$. This estimation of Nb is dependent on the effective density D_e (Hardy et al. 2006). As σ_g is unknown, an iterative approach is applied to estimate jointly Nb and σ^2_g knowing D_e, and was calculated in SPAGEDI 1.3. In woody species, $D/4$ has been considered as an appropriate estimate of effective tree population density (Hardy *et al.* 2006), where D is the census population density. D was estimated as 1000 individuals. The extent of occurrence (EOO) and area of occurrence (AOO) are both estimated to be at most 33.5 km² on Mahé (IUCN 2012). This equals 29.9 individuals per km^2 and 0.299 individuals per ha and D_e ($D/4$) was 0.07 individuals per ha. As an alternative we also performed calculations based on the D_e obtained by POLDISP.

Estimating realised gene flow using parentage analysis

Using multilocus genotypes (ten loci) of 196 seedlings we applied a maximum likelihood exclusion analysis in CERVUS 3.0, to assign the two most likely candidate parents (Kalinowski *et al.* 2007; Marshall *et al.* 1998). Pollen dispersal distance was deduced for each seedling based on the position of the two most likely parent trees assigned by CERVUS. Simulations of paternity were run using the allele frequencies of all adult reproductive trees and the following settings: 10000 cycles; minimum number of loci typed 5, unknown parents; all surviving adults across the species range were set as candidate parents for seedlings; 1% for proportion of loci mistyped, and 0.94% for proportion of loci genotyped. The proportion of candidate parents sampled was set at 80%, as we know we did not sample all remaining potential parents but tried to sample most individuals within our study populations. Assignment was based upon the 70% and 60% confidence level of the critical LOD score as overall assignment rates were rather low (see results). Seed dispersal was calculated as the distance between the seedlings to their closest assigned parent trees as we know that seeds are primarily dispersed by gravity.

The TWOGENER analysis was performed using the software package POLDISP (Robledo-Arnuncio *et al.* 2007) for 24 progeny arrays (mother trees) of 620 progeny (seeds) from five populations as an indirect estimate of contemporary pollen flow. The mean effective

pollen dispersal distance (δ) was calculated assuming a normal distribution mode. The effective number of pollen donors was calculated as $Nep = 1/(2*\Phi ft)$, according to Smouse et al. (2001), where Φft is the differentiation in pollen gene pool. We used the value of D_e (effective tree density) derived from the TWOGENER analysis. The effective pollination neighborhood area (Aep) was calculated as Nep/D_e.

Experimental pollinator exclusion

To test for the potential effects of pollinator limitation, the actual effectiveness of pollen dispersal by large and mobile pollinators of *G. sericea*, in small and distal but also in large and central populations we conducted the following crosses in 2009 to test for potential pollen limitation. For 16 trees at Copolia and 7 trees on Mt. Sebert, inflorescences, each with about 30 flowers at bud stage, were enclosed within Delnet pollination bags. In total 25 bags were used on Mt. Sebert, and 35 bags on Copolia. Within each bag the following treatments were applied, at Mt. Sebert: 1. Selfing, using pollen sourced from flowers of the same tree; 2. Within-population crosses using mixed pollen sourced from four or five other trees at Mt. Sebert; 3. Between-population crosses using a random mix of pollen donors (four or five trees) at Mt. Jasmin; 4. Between-population crosses using a random mix of pollen donors (four or five trees) at Gongo Rouge and 5. Unmanipulated (non-pollinated) flowers as complete pollen exclusion. On Copolia the same treatments were applied except for treatment 4. As a control we determined seed set of unmanipulated flowers outside pollination bags and therefore openly accessible to pollinators. Stigma viability was tested by the presence of peroxidase using a Peroxtesmo Ko test (Dafni & Maués 1998). One 15 x 15 mm Peroxtesmo Ko paper (Machery-Nagel, Germany) was soaked in 1ml distilled water and a droplet of this solution applied directly onto the stigmas on flowers of varying ages to be use of the period of stigma viability. Pollen was transferred by use of brush to viable stigmas of flowers. Flowers were individually labelled to distinguish between treatments. In total, on Mt. Sebert 87 flowers were pollinated for between-population with Mt. Jasmin and 41 for between-population with Gongo Rouge, 86 within-population crosses and 82 selfed treatment, 547 were not pollinated and 87 fruits from 3 trees were collected for the controls. For Mt. Sebert 102 flowers were pollinated for between-population, 80 within-population crosses and 95 selfed treatment, 866 were not pollinated and 231 fruits from 9 trees were collected for the controls. The pollination bags were retained until the fruits were ripe for collection. Mature seeds proved to be either viable (developed and full) or non-viable (developed but empty). To determine fitness values for each pollination treatment we recorded fruit set (developed fruits

as a proportion of treated flowers) and seedset (viable seeds as a proportion of all developed seeds).

Data analysis

Statistical analyses of the pollinator exclusion experiment were carried out in R, version 2.10.1 (R Development Core Team 2010). We used generalized linear mixed-effects models (GLMM) with a binomial error distribution to analyse fruit and seed set. GLMM can account for the nested experimental design, and we included bags nested in trees as a random effect in the model (Bolker *et al.* 2009). We applied the lmer function from the lme4 package (Bates 2005). Due to the different number of flowers and fruits per tree and treatment the data for seed set were unbalanced. Our analysis is robust for unbalanced data (Bolker *et al.* 2009) by using the "cbind" function which calculates fruit and seed set weighted by sample sizes. We ran two sets of models: a main effect model with Treatment as fixed effect to determine the level of selfing, and a full model with Treatment (only within- and between-population crosses) and Tree ID as fixed effects to look at the effects of between population crosses. Model selection was based on the Akaike Information Criterion (AIC), and lowest AIC values defined minimum adequate models. After running the analysis we decided to remove the seeds from the selfed and the non-pollination treatments as this increased the power of our model and we were mainly interested in the comparison of within- and between-population crosses.

If possible ripe and full fruits were collected for the controls. Often the ripe fruits were already opened and had released some or even most of their seeds, so that the number of seeds from all collected fruits was added per tree and taken as one sample for each tree. Thus, only 19 samples could be collected as a control group for Copolia and 9 for Mt. Sebert. We only sampled developed seeds for the control group and so we compared seed set values of the control group with only developed seeds of the other treatments. To test for significant differences the 95% confidence interval was calculated for the mean seed set values.

RESULTS

Genetic diversity and inbreeding of adult and seedling cohorts

At the species level the ten loci yielded between three and 15 alleles, with a total number of 83 alleles. A comparison of genetic diversity over all loci and populations is given in Table 1. Significant inbreeding (F_{IS}) was detected over all adults (0.11, p < 0.001). H_E values ranged from 0.39 (± 0.10 SE) in Morne Blanc to 0.58 (± 0.05 SE) in Copolia2. Allelic richness, based on five diploid individuals ranged from 2.63 (± 0.50 SE) in Morne Blanc to 3.20 (± 0.50 SE) in Mt. Jasmin. Gongo Rouge contained the highest numbers of private alleles (six) jointly with Mt. Jasmin (five). Significant inbreeding coefficient were obtained for populations Mt. Jasmin (0.14, p = 0.003), Copolia1 (0.18, p = 0.002) and Copolia2 (0.19, p = 0.02), see Table 1.

Within the seedling cohorts overall F_{IS} values were 0.12 (p < 0.001) and seed cohorts 0.12 (p < 0.001). H_E values ranged similarly to the adults cohort from 0.40 (± 0.09 SE) in Morne Blanc to 0.58 (± 0.05 SE) in Copolia2. Allelic richness, based on five diploid individuals ranged from 2.47 (± 0.36 SE) in Morne Blanc to 3.21 (± 0.50 SE) in Mt. Jasmin. Significant inbreeding coefficient were obtained for populations Mt. Jasmin (0.17, p = 0.003), Copolia1 (0.15, p = 0.02) and Copolia2 (0.09, p = 0.03), see Table 1.

Genetic differentiation

The overall F_{ST} value for adults was 0.11 (p < 0001), 0.08 (p < 0.001) for seedlings and 0.12 (p < 0.001) for the seeds. Mean pairwise genetic distances (Nei 1978) were significantly different but relatively low among all populations (Table 2). The geographically most distal populations; Mt. Jasmin and Copolia1 (0.05, p < 0.05; 8km) obtained similar values as the geographically close sites; Copolia1 and Copolia2 (0.03, ns p > 0.05; 1 km). Mt. Sebert, a geographically distal and isolated population had highest pairwise genetic distances. Similarly Morne Blanc which is geographically close to other populations also showed high parwise F_{ST} values. The STRUCTURE analysis identified three and seven distinct genetic clusters (K3, K7) as the most likely (and biologically relevant) solutions (ΔK for K3 = 32.12; for K7 = 6.24), where both K3 and K7 indicated a delineation of the Copolia1 and Copolia2 populations from all other individuals (see Fig. 2).

Table 1: Genetic variability of 10 microsatellite loci estimated for all populations of *Medusagyne oppositifolia*. Abbreviations: Ad: Adults; Sg: Seedlings; Sd: Seeds; n: number of genotyped individuals; N_A: Mean number of alleles; N_E: effective number of alleles; H_O: observed heterozygosity; H_E: expected heterozygosity; R_S: Allelic richness, based on five diploid individuals; P_A: total number of private alleles (mean frequency); F_{IS}: Inbreeding coefficient; ± SE. * = p < 0.05, ** = p < 0.01, *** = p < 0.001. MS = Mt. Sebert, MJ = Mt. Jasmin, GR = Gongo Rouge, CO1 = Copolia1, CO2 = Copolia2, MB = Morne Blanc.

POP ID	n		N_A		H_O		H_E		$R_{S(5)}$		R_A		P_A	F_{IS}		
	Ad	Sg	Ad	Sg	Ad	Sg	Ad	Sg	Ad	Sg	Ad	Sg	Ad	Ad	Sg	Sd
MS	12	20	3.80 (±0.71)	4.50 (±0.90)	0.47 (±0.09)	0.34 (±0.07)	0.48 (±0.09)	0.46 (±0.10)	3.10 (±0.50)	3.15 (±0.55)	6	14	2	0.08	0.13	0.02
MJ	69	43	5.60 (±0.99)	4.90 (±0.85)	0.44 (±0.08)	0.47 (±0.08)	0.52 (±0.09)	0.51 (±0.09)	3.21 (±0.51)	3.21 (±0.50)	21	14	5	0.15***	0.15**	0.10**
CO1	28	31	4.70 (±0.76)	4.50 (±0.69)	0.44 (±0.08)	0.47 (±0.07)	0.48 (±0.09)	0.49 (±0.07)	3.04 (±0.43)	2.97 (±0.34)	14	15	2	0.14*	0.12*	0.17***
CO2	34	48	4.10 (±0.71)	4.40 (±0.64)	0.55 (±0.06)	0.50 (±0.03)	0.57 (±0.05)	0.58 (±0.05)	3.09 (±0.35)	3.13 (±0.31)	8	10	1	0.12*	0.11**	0.15*
GR	56	24	5.10 (±1.11)	3.90 (±0.64)	0.39 (±0.08)	0.37 (±0.07)	0.47 (±0.10)	0.47 (±0.09)	3.06 (±0.50)	2.90 (±0.42)	19	7	6	0.05	0.20**	0.17***
MB	7	30	2.80 (±0.53)	3.80 (±0.66)	0.43 (±0.11)	0.39 (±0.08)	0.39 (±0.10)	0.40 (±0.09)	2.63 (±0.50)	2.47 (±0.36)	3	13	2	-0.31	-0.04	na

Table 2: Pairwise F_{ST} values between adult and seedling populations, * = p < 0.05 with adjusted nominal level (0.0008) for multiple comparisons. MS = Mt. Sebert, MJ = Mt. Jasmin, GR = Gongo Rouge, CO1 = Copolia1, CO2 = Copolia2, MB = Morne Blanc.

Sg\Ad	MS	MJ	CO1	CO2	GR	MB
MS	0	0.13*	0.10*	0.18*	0.08*	0.12*
MJ	0.07	0	0.04*	0.10*	0.06*	0.06
CO1	0.07	0.06*	0	0.08*	0.04*	0.08*
CO2	0.07	0.06*	0.04*	0	0.14*	0.16*
GR	0.06	0.06*	0.04*	0.07*	0	0.05*
MB	0.12	0.09*	0.15*	0.13*	0.14*	0

(a)

(b)
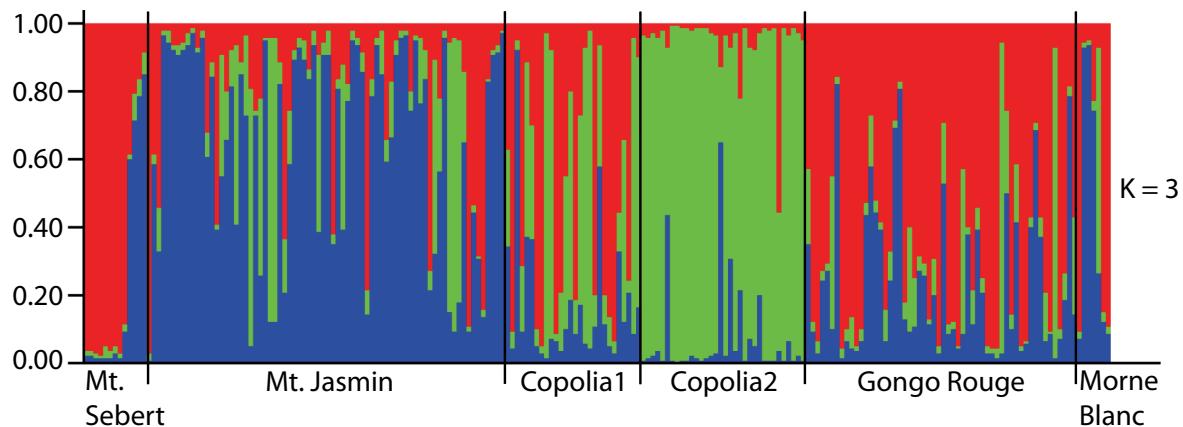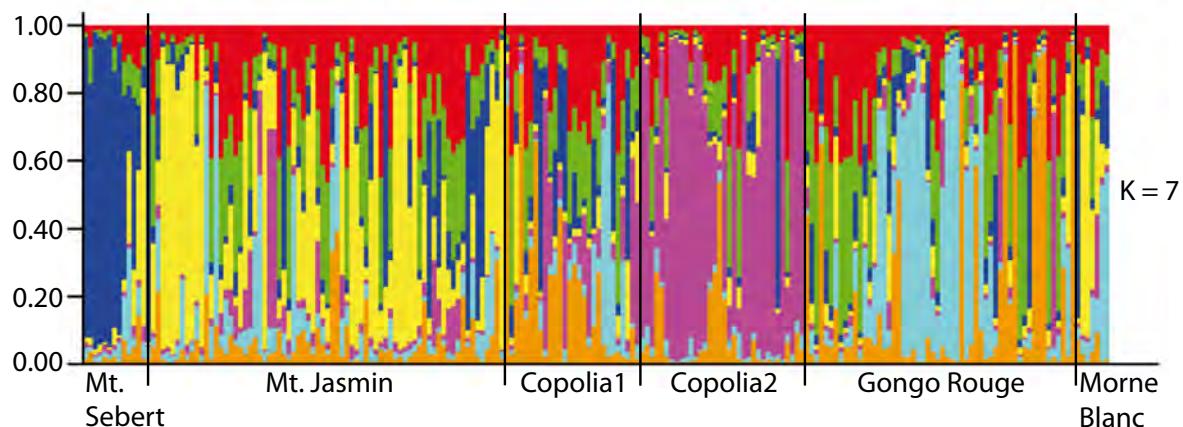
Figure 2: Bayesian structure analysis of *Glionnetia sericea* with the STRUCTURE software (Pritchard et al. 2000). Bars represent individual *Glionnetia sericea* adult individuals with their assignment proportions (y axis) to the different clusters. Performing the analysis for (a) K_3 (three different grey shades), representing three genetic clusters and (b) K_7 (seven different grey shades), for a clustering solution of seven genetic clusters.

Within population fine-scale spatial genetic structure and kinship

Significant fine-scale spatial genetic structure (FSGS) was detected in adult trees over all populations, as indicated by significant regression of kinship against spatial distance. Kinship coefficients (*F*) are significantly different from zero within pairs of trees up to 80 m apart (p < 0.001) (Fig. 3). For most individual populations we did not have enough pairs to conduct a FSGS, only three populations had enough pairs (Mt. Jasmin, Gongo Rouge, Copolia 1 and 2) but these were not distributed in the same distance classes which is why we did not compare FSGS for individual populations.
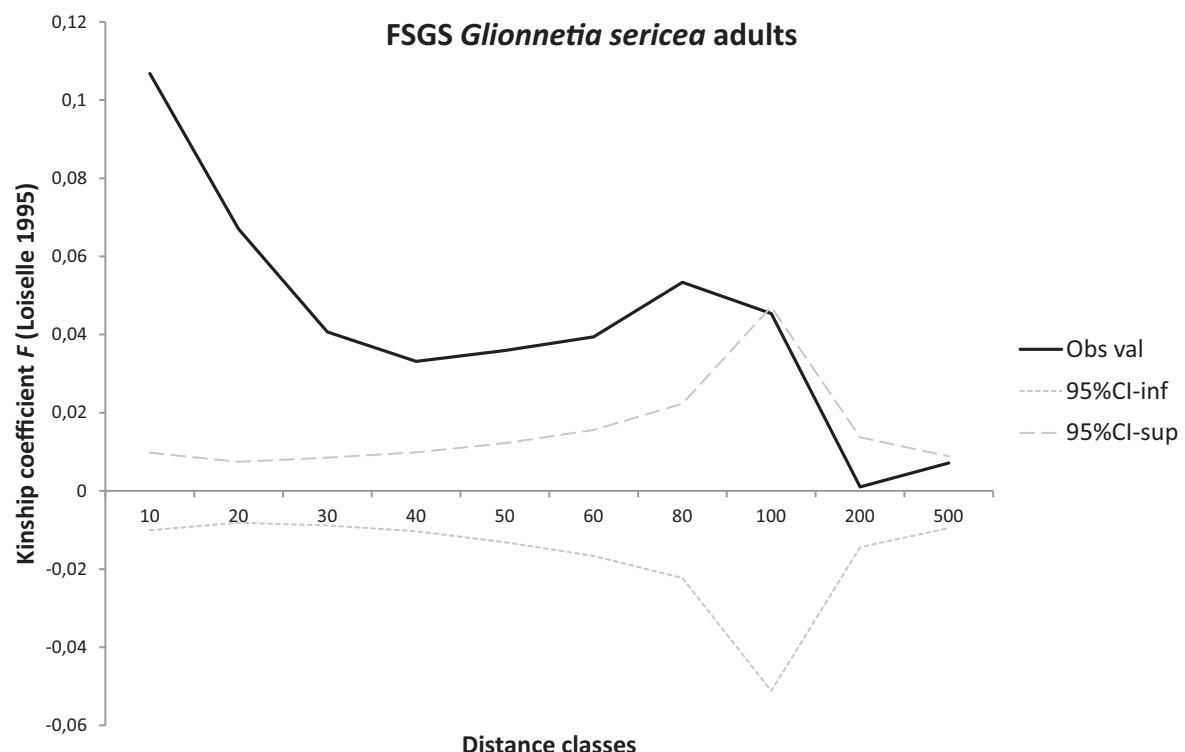
Figure 3: Correlogram of average kinship coefficient (*F*) of adult individuals of *Glionnetia sericea* at the individual populations. The solid line represents the average *F* values. The dotted lines represent the 95% (two-tailed) CI of the average F distribution calculated from 10000 permutations of spatial distance among pairs of adults.

Estimation of historic gene dispersal

Our estimates of historical gene dispersal distance over the 33.5 km^2 extent of occurrence (EOO) on Mahé, based on restricted b_k (0-10m), could not produce σ_g and *Nb* for a D_e 0.29 individuals per ha as these did not converge. With the D_e obtained from POLDISP (see next paragraph) we obtained a $\sigma_g = 143.56$ m and a neighbourhood size *Nb* = 78.81 m for 206 individuals (assuming D_e = D/4). σ_g and *Nb* values did not converge for individual populations.

The TWOGENER analysis revealed a tree density of 0.99 trees per ha, the number of effective fathers per mother tree was *Nep* = 2.92 and the effective pollination neighborhood area was *Aep* = 3.01 ha. The average pollen dispersal distance (δ) was 9.97m.

Contemporary gene flow and mating system

Using parentage assignment we were able to assign only 17% (33) of our seedlings (n = 196) with 70% confidence, and 28% (54) of all seedlings with 60% confidence. We present here the results for the more relaxed 60% confidence level. Between-population comparisons are difficult due to the low number of seedlings that could be assigned. Overall, of the 54 assigned seedlings 39% mating events resulted from matings within populations and 61% from gene flow between populations. Selfing rates (number of cases where parentage analysis determined the father and mother as the same tree) over all populations was 9% (5 seedlings).

A paternity analysis conducted for the seeds, given known mothers, could assign 72% (447) seeds with 70% confidence and 90% (558) seeds with 60% confidence. We present here the results for the more relaxed 60% confidence level. Selfing rates for the different populations ranged between 10% and 41% with an overall selfing rate of 19%. Of the assigned progeny, 30% (169 seeds) were from between-population mating events and 70% (389) from within-population crosses (Table 3).

Table 3: Gene flow and selfing rates for *Glionnetia sericea* populations obtained from a parentage (seedlings) and paternity analysis (seeds). MS = Mt. Sebert, MJ = Mt. Jasmin, GR = Gongo Rouge, CO1 = Copolia1, CO2 = Copolia2, MB = Morne Blanc.

Population	n	# Fathers from within population		# Fathers from outside populations		Selfed	
	Seeds	Number	Proportion	Number	Proportion	Number	Proportion
MS	172	112	0.651	60	0.349	20	0.116
MJ	78	55	0.705	23	0.295	8	0.103
CO1	199	139	0.698	60	0.302	35	0.176
CO2	18	12	0.667	6	0.333	3	0.167
GR	91	71	0.780	20	0.220	37	0.407
All	**558**	**389**	**0.697**	**169**	**0.303**	**103**	**0.185**
	Seedlings						
MS	1	0	0	1	1	0	0
MJ	12	8	0.667	4	0.333	1	0.083
CO1	9	0	0	9	1	0	0
CO2	13	7	0.538	6	0.462	0	0
GR	7	3	0.429	4	0.571	1	0.143
MB	12	3	0.25	9	0.750	3	0.25
All	**54**	**21**	**0.389**	**33**	**0.611**	**5**	**0.093**

Between population pollination crossing experiment

Fruit and seed set Copolia

Fruit set was not significantly different among between-population (0.31 ± 0.06 SE) and within-population crosses (0.52 ± 0.05 SE; z = -1.857, p = 0.06). Fruit set for selfed and no-pollination treatments were 0.46 ± 0.07 SE and 0.09 ± 0.02 SE, respectively. Highest fruit set was obtained for the open controls (0.84 ± 0.02 SE).

Seed set (as a proportion of developed seeds) was not significantly different among between-population (0.20 ± 0.04 SE) and within-population treatments (0.26 ± 0.04 SE; z = 0.54; p = 0.59). There was no significant tree interaction effect with treatment. The mean viable seed of the open control group was not significantly different than that from the other treatments (Controls: 0.63, 95% CI 0.57 – 0.69; between-population: 0.70, 95% CI 0.62 – 0.77; within-population: 0.63, 95% CI 0.56 – 0.70). Viable seed set for selfed was high (0.28 ± 0.04) and low for pollen exclusion (0.01 ± 0.00 SE), see Table 3a and b.

Fruit and seed set Mt. Sebert

Fruit set was not significantly different among between-population Mt. Jasmin or Gongo Rouge (0.63 ± 0.09 SE and 0.65 ± 0.11 SE, respectively) and within-population crosses (0.60 ± 0.07 SE; z = 0.50, p = 0.62 and z = -0.89, p = 0.38, respectively). Fruit set for selfed and no-pollination treatments were 0.44 ± 0.07 SE and 0.18 ± 0.03 SE, respectively. Highest fruit set was obtained for the open controls (0.76 ± 0.03 SE).

Seed set (as a proportion of developed seeds) was not significantly different among between-population (Mt. Jasmin) and (Gongo Rouge) (0.36 ± 0.04 SE and 0.35 ± 0.06 SE, respectively) and within-population treatments (0.38 ± 0.04 SE; z = 0.50, p = 0.62 and z = -0.89, p = 0.38, respectively). There was no significant tree interaction effect with treatment. The mean viable seed of the control group was not significantly different than that from the other treatments (Controls: 0.56, 95% CI 0.45 – 0.66; between-population (Mt. Jasmin): 0.66, 95% CI 0.61 – 0.70; between-population (Gongo Rouge): 0.58, 95% CI 0.51 – 0.66; within-population: 0.62, 95% CI 0.56 – 0.67) Viable seed set for selfed was high (0.28 ± 0.04) and low for no-pollination (0.01 ± 0.00 SE), see Table 3a and b.

Table 4a: Summary table of results of pollination experiments. Fruit set and seed set are given in mean proportions ± SE. SE = Selfed individuals, WI = Within population crosses, BW = Between population crosses with individuals from Mt. Jasmin, BW2 = Between population crosses with individuals from Gongo Rouge, NO = Bagged individuals with no hand pollination, OP = Controls. Fruit set is defined as the proportion of flowers that developed into fruits. Seed set is defined as the proportion of developed seeds. MS = Mt. Sebert, CO = Copolia.

Pops		Treatments					
	All fruits	SE	WI	BW	BW2	NO	OP
MS	Mean Seed set	0.23±0.04	0.38±0.04	0.36±0.04	0.35±0.06	0.05±0.01	-
	#Pollinations	78	80	81	37	517	-
	#Used fruits	75	71	78	33	495	-
	Fruit set	0.44±0.07	0.60±0.07	0.63±0.09	0.65±0.11	0.18±0.03	0.76±0.03
	# Fruits	82	86	87	41	547	87
	# Trees	6	7	7	5	7	3
	# Bags	21	22	18	13	25	-
CO	Mean Seed set	0.28±0.04	0.26±0.04	0.20±0.04	ND	0.01±0.00	-
	#Pollinations	89	81	96	ND	797	-
	#Used fruits	84	75	89	ND	745	-
	Fruit set	0.46±0.07	0.52±0.05	0.31±0.06	ND	0.09±0.02	0.84±0.02
	# Fruits	95	80	102	ND	866	231
	# Trees	14	14	14	ND	16	9
	# Bags	28	23	27	ND	35	-
	Only full fruits	SE	WI	BW	BW2	NO	OP
MS	Mean Seed set	0.58±0.04	0.62±0.03	0.66±0.02	0.58±0.04	0.66±0.03	0.56±0.05
	Used fruits	30	43	42	20	36	9
	-95% CI	0.50	0.56	0.61	0.51	0.61	0.45
	+95% CI	0.66	0.67	0.70	0.66	0.72	0.66
CO	Mean Seed set	0.63±0.03	0.63±0.04	0.70±0.04	ND	0.65±0.06	0.63±0.04
	Used fruits	36	31	26	ND	15	19
	-95% CI	0.58	0.56	0.62	ND	0.53	0.57
	+95% CI	0.68	0.70	0.77	ND	0.76	0.69

Table 4b: Significant differences in fruit and seed set for the different treatments. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

	SE	WI	BW	BW2	NO	OP
MS\CO				Fruit set		
SE	-	ns	ns	ND	***	***
WI	*	-	ns	ND	***	***
BW	ns	ns	-	ND	***	***
BW2	**	ns	ns	-	ND	ND
NO	***	***	***	***	-	***
OP	**	ns	ns	ns	***	-
MS\CO				Seed set		
SE	-	ns	ns	ND	**	-
WI	**	-	ns	ND	***	-
BW	*	ns	-	ND	ns	-
BW2	ns	ns	ns	-	ND	-
NO	ns	ns	ns	*	-	-

DISCUSSION

Genetic diversity and inbreeding in fragmented environment

The relatively high genetic diversity found in *Glionnetia sericea* in all populations is similar in small and large populations indicating a stable population structure in adult trees. The only population with a lower genetic diversity is Morne Blanc which comprises only nine individuals (of which seven have been genotyped) and which also represent trees with the smallest DBH compared to the other populations. Thus, this population might be relatively young, possibly a founder population. Over all populations, genetic diversity does not differ between adults and seedlings and no loss of rare alleles could be detected which would indicate genetic erosion from adult to seedling cohorts.

Significant levels of inbreeding were present in adults and seedlings of three populations, Mt. Jasmin, Copolia1 and Copolia2. Furthermore, in Gongo Rouge we detected a significant inbreeding coefficient in seedlings only, which may hint towards a change in contemporary gene flow and negative genetic impact for the seedlings in this population. Indeed, in Gongo Rouge we also found highest selfing rates in the analysed seeds and high selfing rates in the seedlings compared to the other populations. Angeloni *et al.* (2011) found that significant levels of inbreeding are not unusual for species that persist in small populations and which have purged deleterious alleles, but whether purging is also valid for *G. sericea* cannot be answered from our data.

Our parentage and paternity analysis assigning seedlings and seeds, respectively, indicated that selfing rates were lower and outcrossing rates higher from seed to seedling stages in all populations. Of the 558 seeds that could be assigned to fathers (albeit with limited confidence), 103 (19%) were found to be selfed seeds, yet of the 54 seedlings that could be assigned to parent pairs only 9% could be assigned as selfed progeny. This finding may indicate a selection process against selfed seeds during the germination process. We should point out though that the overall assignment rates were very low for seedlings (17%) and so these estimates are relatively low compared to the total number of genotyped progeny. It is apparent that our sampling was insufficient to determine the majority of the mating events. An alternative explanation for these low assignment rates could also be that true paternal assignment is difficult due to the low genetic differentiation and high genetic similarity between *G. sericea* individuals and populations.

Interestingly, the smallest populations (Mt. Sebert and Morne Blanc), are the only two populations without significant inbreeding values in seedlings and/or adults, thus small population sizes do not seem to be positively associated with inbreeding in *G. sericea*. It might be that inbred individuals are especially disadvantaged in these harsh conditions, inselberg habitats being generally drier and hotter, and providing little soil accumulation compared to the surrounding habitats (Fleischmann 1997b; Hunter 2002; Porembski *et al.* 1994; Szarzynski 2000).

Population connectivity and the role of plant pollinator interactions

The Bayesian structure analysis clearly demonstrates a low genetic structuring which is supported by low but significant overall F_{ST} and pairwise F_{ST} values. Populations that are geographically most distal (e.g. Mt. Sebert and Mt. Jasmin) have similar pairwise F_{ST} values to the more proximal sites (e.g. Copolia2 and Gongo Rouge). Interestingly, all populations exhibit some private alleles suggesting some degree of genetic differentiation, at least in the sampled individuals. We cannot exclude though that these private alleles may as well be found in unsampled individuals as we were not able to do a complete genetic inventory of *G. sericea* individuals on Mahé. Nevertheless, the low genetic structuring over the whole island range indicates relatively high levels of historical gene flow. The pairwise F_{ST} values do not increase in the seedling cohorts, supporting the view that contemporary gene flow is not restricted.

Historical gene dispersal distances determined from FSGS were found to be 143.56 m which is relatively short considering the detected low genetic differentiation. The TWOGENER analysis similarly detected low average pollen dispersal distances of 9.97 m. Both of these analyses are sensitive to the effective tree density used. We cannot exclude the possibility that our estimation of 0.30 individuals per ha was too low and may have biased the analysis, since *G. Sericea* trees are often highly clumped in populations. Nevertheless, the calculated tree density independently calculated by POLDISP based on GPS coordinates and adult genotypes was comparable, at 0.99 trees per ha. Due to the clumped dispersal of *G. sericea* these low obtained historical gene dispersal and contemporary pollen dispersal distances may also indicate that (besides long distance gene flow) pollen is also distributed effectively between neighbouring trees and that the vast majority of gene flow is over relatively short distance. It seems though that occasional long distance gene flow events are sufficient to prevent genetic differentiation over evolutionary time scales.

Our parentage and paternity analysis indicate that long distance gene flow occurs in *G. sericea*. Of the 558 assigned seeds 389 (70%) resulted from within population and 169 (30%) from between population pollen flow. For the 54 assigned seedlings 21 (39%) resulted from matings within population and 33 (61%) from matings between populations. This hints towards extensive gene flow with outcrossing progeny performing better at later life stages. But as mentioned above these results should be treated with some caution because of the low overall confidence levels in the assignment. The more robust TWOGENER analysis revealed a relatively low effective number of 2.92 pollen donors per mother tree. This suggests that despite low numbers of fathers per mother tree pollen distribution in general is very good within and between populations.

The positive fine-scale spatial genetic structuring (FSGS) observed in *G. sericea* adults indicates limited historical gene flow at least at the fine-scale (< 100 m), given the significant relatedness between individuals up to 80m. This partly stands in contrast to our findings demonstrating long-distance gene dispersal, but is also congruent with the low historical gene dispersal mentioned above. Such a significant FSGS could indicate limited seed dispersal abilities, while pollen flow is of long distance. This hypothesis would explain the fact that overall differentiation is low, implying that seed dispersal ability of the species is less than pollen dispersal. Highly mobile pollinators such as hawk moths may be able to fly distances of up to 10km and thereby transporting pollen between the most distant sites in occasional long distance dispersal events which would be sufficient to prevent genetic differentiation (Janzen 1994, Haber & Frankie 1989).

Is pollen dispersal limited in small and distant populations?

Historical and contemporary gene flow by pollen seems to have connected populations over the whole of Mahé, despite human induced habitat fragmentation. Whether this is also the case for small (12 individuals) and isolated populations is not clear. We conducted artificial pollination experiments at two different sites, a small population (Mt. Sebert) and a larger population (> 60 individuals) at Copolia. We could not detect any pollen limitation in both populations as seed and fruit set were similar for open treatments and within- and between-population treatments. This result implies that non-manipulated open pollinated flowers develop similarly into fruits and seeds compared to flowers that have been supplied with pollen by hand pollination. Further, the small and isolated population, Mt. Sebert, had similar seed and fruit set values compared to Copolia. If pollen quantity can be associated with the

amount of seed and fruit set in *G. sericea* this would imply similar numbers of pollination events in both populations.

For both populations we could confirm self-compatibility even though for Mt. Sebert seed set and fruit set of selfed seeds were significantly lower compared to the other treatments. Contrarily, selfed flowers on Copolia had similar seed and fruit set compared the within- and between-population crosses. Neither fruit set nor seed set differed significantly between the treatments within-population and between-population at the two sites, which may demonstrate that pollen quality does not differ between populations. Non pollinated flowers within bags (pollen exclusion) did produce fruits and seeds at both sites but at much lower frequencies showing that apogamy is possible but reproductive output and potentially seed viability is low. This also implies that *G. sericea* may be vulnerable to the decline in pollinator abundance, which is important information for conservation strategies aiming to preserve the species viability in the long-term.

We have to account for several caveats. Fruit set was higher for the open controls in both populations indicating that the bags did have a negative effect on the flowers. As seed set was similar between the controls and the different hand pollination treatments we consider it unlikely that the bagging had an overall effect on seed set. The number of controls was rather low and thus the comparison might be biased. The between-population crosses involved the transportation of pollen from one inselberg to another with pollen stored in open tubes over night before being applied to flowers the following day. This may have resulted in reduced pollen viability for between-population compared to within-population treatments. However, if hawkmoths do transport pollen between sites as indicated by our genetic analysis, pollen viability is presumably sufficiently long (one or two days) to allow natural transport to distal sites. We were not able to germinate any of our seedlings due to unsuitable nursery conditions. Thus we cannot say whether there may be differences in seed viability for the different treatments. In field pollination experiments, unintended pollen transfer may also result in some degree of contamination within our pollination bags which could explain similar responses for the within-, between-population crosses and the controls.

Our study on *G. sericea* from the Seychelles has shown that highly mobile pollinators (hawkmoths) can transport pollen effectively over relatively long distances and that potentially wind dispersed seeds contribute to long-distance gene exchange between populations. (This may be especially important for long-distance gene flow when seed dispersal is low.) We could also show that pollinator limitation due to habitat degradation or disruption of the plant-pollinator specialization may decrease the species reproductive output

and thus long-term population viability. So far, we could not detect pollen limitation even in very distant populations, demonstrating a certain resistance to habitat fragmentation and/ or the invasiveness of alien species of these hawkmoths species in the Seychelles. Maintenance of the plant-pollinator interaction highlights the needs to not only consider single species but rather to protect whole ecosystems and communities.

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Vulnerability of tree species to the genetic consequences of habitat fragmentation: the importance of divergent population histories

with C.J. Kettle and J. Ghazoul
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ABSTRACT

Human induced rapid forest fragmentation is especially pronounced in tropical regions where forests are increasingly over exploited for their valuable timber species and converted to alternative land uses. This creates small and isolated populations for many tree species. We hypothesise that species with historically or naturally patchy distributions may be adapted to persist in small population sizes and thus less vulnerable to negative effects of habitat fragmentation compared to species that were historically more widespread and only recently fragmented. This hypothesis is tested by comparing empirical studies of three endemic tree species from the Seychelles with contrasting population histories and supplemented with findings from a thorough literature review. Our results from 70 studies suggest that the 49 formerly widespread species are indeed more prone to negative genetic and ecological consequences (54%) of habitat fragmentation compared to historically fragmented species (29%). Furthermore, negative effects to habitat fragmentation were associated with short distance gene flow in formerly widespread species. These finding are supported by our empirical work in the three Seychelles tree species. The formerly widespread species, *Vateriopsis seychellarum* (Dipterocarpaceae) showed a significant loss of genetic diversity and increased genetic differentiation from adult to seedling stage. The historically patchily distributed species *Glionnetia sericea* (Rubiaceae) showed no significant negative response to habitat fragmentation and long-distance pollen flow. The historically fragmented species *Medusagyne oppositifolia* (Ochnaceae) showed no negative response at least in its largest population (78 individuals). Our findings indicate that population history is an important factor shaping trees' vulnerability to habitat fragmentation. These positive effects of long

distance gene flow, underline the need to adopt a process based rather than species based approach to conservation, especially for taxa with complex mutualism.

INTRODUCTION

Habitat fragmentation can have strong genetic and ecological consequences for plant species. Modifications of pollinator behavior through changed landscapes may lead to disrupted reproduction, elevated inbreeding, loss of genetic diversity and genetic bottlenecks (Bacles *et al.* 2005; Cascante *et al.* 2002; Ghazoul 2005). The magnitude of these consequences may depend on multiple factors including: population history, mating system, ability to purge deleterious alleles, pollen flow and seed dispersal ability. For example, inbreeding depression can be low in small and long-isolated populations that have been purged of deleterious alleles (Byers & Waller 1999). In contrast species that have experienced recent rapid reduction in population size may be especially vulnerable to inbreeding depression (Keller & Waller 2002; Lande & Schemske 1985). Species that have survived in naturally small and isolated populations over long time periods may therefore be less vulnerable to recent habitat fragmentation compared to species with naturally more continuous populations.

The theoretical expectations that inbreeding depression is not merely linked to population size has, in part, been supported by a recent meta-analysis which examined the relationship between population size, life history and inbreeding depression in 107 plant species (Angeloni *et al.* 2011). This study concluded that inbreeding depression is common across plant species regardless of their rarity, and the magnitude of inbreeding depression tends to decrease as population size decreases (Angeloni et al. 2011). Their results are congruent with the idea that deleterious alleles have been purged in small populations with higher levels of background inbreeding. Nevertheless, population size has been found to have an important role in maintaining genetic diversity which is predicted to be important for species fitness (Frankham 2005; O'Grady *et al.* 2004) and supported by several reviews of empirical and experimental studies across plant and animal taxa (Kolb 2008; Leimu *et al.* 2006; Reed 2005; Reed & Frankham 2003).

The genetic consequences of habitat fragmentation centre on the relationships between population size, gene flow, genetic diversity and fitness. However, relationship between these factors is complicated by population history, especially in long-lives trees. A meta-analysis by Honnay & Jacquement (2007), found that common plant species were often more susceptible to the loss of genetic diversity through habitat fragmentation than rare plant species. None of these previous meta-analyses have differentiated between historically or naturally fragmented species and species that are rare for other reasons (e.g. recent habitat fragmentation). It is possible that contradictory results, such as differing responses of small populations to levels

of genetic diversity and inbreeding depression, might be caused by different population histories among these species. Human activities such as intensive logging, agricultural expansion, habitat clearance, fire, and road and urban development cause rapid habitat fragmentation of tree populations, especially in the tropics (Vitousek *et al.* 1997; Wright & Muller-Landau 2006). Thus, disentangling the effects of population histories from population size and life history traits is important but also challenging, due to the numerous compounding factors among species.

Tree species characterised by long generation times, high fecundity and extensive gene flow (Petit & Hampe 2006) are predicted to be least vulnerable to fragmentation (Kramer *et al.* 2008). Gene flow, in fragmented landscapes, is an especially important factor because long-distance gene flow through pollen or seed dispersal can maintain effective population sizes and ensure connectivity of recently fragmented populations (Ahmed *et al.* 2009; Bacles & Jump 2011; Dick 2001). For example, small populations and individual trees remain connected in the insect-pollinated rare tree *Gomortega keule* from Central Chile despite habitat fragmentation (Lander *et al.* 2010). Indeed, Kamm *et al.* (2009) showed that open landscapes even promote gene flow in the rare insect-pollinated tree *Sorbus domestica*. In contrast, the wind pollinated New Caledonian conifer tree *Araucaria nemorosa,* shows early signs of genetic erosion most likely as a consequence of limited gene dispersal and elevated inbreeding in a highly fragmented landscape (Kettle *et al.* 2007).

The aim of this study is to advance our understanding of the importance of population history on tree species vulnerability to habitat fragmentation. We hypothesise that species with historically patchy distributions are less vulnerable to habitat fragmentation compared to species that were historically more widespread and only recently reduced to small population sizes. We further hypothesise that successful long-distance gene flow by pollen or seed will counteract negative consequences of habitat fragmentation. Thus, formerly widespread species should be less prone to negative genetic effects following habitat fragmentation when the species is capable of long-distance gene flow across fragmented landscapes.

We adopted a two pronged approach to address our aims. We conduct an extensive literature review of the empirical research on fragmentation of tree species and placed these in the context of their different population histories by focusing mainly on genetic effects of habitat fragmentation (loss of genetic diversity, rare alleles, increased levels of inbreeding, genetic differentiation) and also on reproductive output, such as fruit set, seed set and seedling survival or fitness. Second, we combine our own findings from empirical research where we compare three insect pollinated Seychelles island endemic tree species with limited seed

dispersal, but which have differing population histories. *Medusagyne oppositifolia* has always had a historically patchy distribution restricted to inselberg habitats, *Glionnetia sericea* was formerly more widespread but still restricted to habitats between 400 and 900 m, and *Vateriopsis seychellarum* is considered a formerly widespread canopy tree (Finger *et al.* 2011; Finger *et al.* in prep.; Finger *et al.* submitted). All three species suffered to some extent from habitat fragmentation within the last 150 years and nowadays occur in only a few small remaining populations (*M. oppositifolia* four populations, 78 individuals; *V. seychellarum* eleven populations, 132 individuals; *G. sericea* about ten populations, about 1000 individuals). Our aim was to determine to what extent these species are differentially vulnerable to the negative genetic and reproductive consequences of fragmentation, and whether they can maintain genetic diversity and reproductive output. Specifically we ask the following questions: i) what are the consequences of recent habitat fragmentation on the genetic diversity and differentiation of the three species, ii) is limited gene dispersal affecting genetic diversity, inbreeding and reproductive output in fragmented habitats?

To answer these questions we use molecular genetic information of adults and seedlings and seeds of the three study species to determine changes in genetic diversity, structure and gene flow. We compare the extent of historic and contemporary gene flow and remaining genetic diversity in adults and seedlings. As we have molecular and ecological data covering the entire species range for all three species we can explore the role of population histories on population persistence in a fragmented environment. Tree species seem to be more vulnerable to inbreeding depression compared to shrubs and herbaceous species (Angeloni et al. 2011) and thus the understanding of processes following habitat fragmentation and deciphering the role of demographic history will help to develop appropriate conservation management plans to protect the many species that are currently being threatened by habitat fragmentation (Oldfield *et al.* 1998). This is particularly important for island systems which tend to have high endemism and relatively small and isolated populations (Kier *et al.* 2009).

MATERIALS AND METHODS

A thorough literature review was conducted on studies of genetic consequences of fragmentation where it was possible to determine the historical distribution of species and history of fragmentation. We limited our review to genetic (microsatellite marker, allozymes/isozymes or AFLPs) and ecological studies of tree species and their responses to habitat fragmentation and/or small population size. We focused on variables that are likely to influence species persistence in fragmentation: 1) genetic diversity (allelic richness, expected heterozygosity, loss of rare alleles), 2) inbreeding coefficients, 3) genetic differentiation and 4) reproductive output/fitness in fragmented versus non fragmented environments. Further, we also recorded, if available, information on gene dispersal distances and species mating systems. To identify positive correlations between genetic diversity and within species population size we a) recorded loss of genetic diversity in seedling cohorts or b) in case only adults were examined recorded responses of fragmented versus non fragmented habitats or c) for historically fragmented or rare species that pre-date fragmentation looked at general levels of genetic diversity to see whether such continuous fragmentation effected genetic diversity.

We differentiated between formerly widespread (FW) and historically fragmented (HF) species i.e. species which have highly specializations niches which are patchily distributed To assess whether our findings were significantly different between negative and neutral responses within FW and HF we applied a χ^2 test in R, version 2.10.1 (R Development Core Team 2010). For comparisons between FW and HF we applied a 2 sample binomial test for comparison of proportions in R.

Empirical studies in three island endemic trees

In previous studies complete genetic inventories have been made for adults of *M. oppositifolia* and *V. seychellarum*, a subset of their seedlings, and a further subset of adults and seedlings of *G. sericea* (Finger *et al.* 2011, Finger *et al.* submitted, Finger *et al.* in prep.). We use these data for the species specific comparisons of historical distributions and its influence on species persistence in small populations following habitat fragmentation.

Study species and populations

Medusagyne oppositifolia (Ochnaceae) and *Vateriopsis seychellarum* (Dipterocarpaceae) are endemics to Mahé the main island of the Seychelles, *Glionnetia sericea* (Rubiaceae) is found

on Mahé and Silhouette. Soon after the arrival of first settlers the forests of Mahé in the late 18th century, the forests of the island were exploited for timber and cleared for agriculture and plantations. By the late 19th century few patches of native forests remained at higher altitudes (Diels 1922). Many native plants were thus greatly reduced in size and area. The introduction of alien species has also had considerable impact on forest composition over the last 150 years, often further restricting native species to relatively inhospitable habitats such as inselbergs (granitic outcrops) or steep gulley and ravines (Fleischmann 1997).

Medusagyne oppositifolia has likely always been restricted to inselberg habitats and has thus always had a naturally patchy distribution, but frequent fires and an increase of invasive species on inselbergs has further reduced the species distribution so that today only 90 adult trees remain in four isolated populations. Seventy-eight of the remaining *M. oppositifolia* adults are found in one population, the only population where recruitment is apparent.

In contrast *V. seychellarum* is thought to have had a historically more widespread distribution. As an important timber species it has been massively exploited, today only 132 adult trees survive in eleven populations of which all (except one) are recruiting. *Glionnetia sericea* is assumed to represent an intermediate species being restricted to habitats between 400 – 900 m altitude but which has also been reduced in population size. It is found on inselberg and mist forest habitats in large (>100) and small (<15) populations all of which are recruiting.

All three species are insect pollinated, *M. oppositifolia* pollinated by native and invasive bee species, *V. seychellarum* by small beetles and *G. sericea* by hawkmoths. The three species have limited seed dispersal, *V. seychellarum* and *G. sericea* are self-compatible whereas *M. oppositifolia* is mainly outcrossing (Finger *et al.* 2011, Finger *et al.* submitted, Finger *et al*. in prep.).

RESULTS

Literature review

A total of 70 genetic and ecological studies on tropical and temperate trees were analysed, including a total of 66 tree species. Within these only 16 studies analysed historically fragmented (HF) species, 49 formerly widespread (FW) species and five studies could not be clearly classified (Table 1). Negative and/or no responses were recorded for each study divided into four categories: genetic diversity, inbreeding coefficient, genetic differentiation and reproductive output. Thus every study could have a maximum of four responses. For FW species we found a total of 91 responses within these four categories. Of these 49 (54%) were negative responses to habitat fragmentation but these were not significantly higher compared to no responses (p = 0.46). HF species had a total of 41 responses in the four categories of which a significantly lower amount 12 (29%, p = 0.008) were negative responses (Table 2). Overall FW species had a significantly higher proportion of negative responses compared to HF species (p = 0.015). Negative effects for FW species were mainly found to be elevated genetic differentiation (nine (67%) negative responses) and lowered reproductive output (12 (67%) negative responses) even though these values were not significantly different to neutral responses (Table 2).

FW species that showed no or low effects to habitat fragmentation were observed to have high amounts of long-distance contemporary gene flow (distances of more than 500 m): within 42 neutral responses we had information on gene flow from 10 studies, with seven (70%) of these being of long distance. Of the FW species which did exhibit negative genetic response to habitat fragmentation (49 responses) 11 studies included information on gene flow and out of these seven (64%) were of short dispersal. This trend was not detected in the naturally fragmented species. Within the 29 responses with no effects to habitat fragmentation seven studies had information on gene flow. Here, five (71%) were short distance gene flow.

Empirical studies in three island endemics

Genetic diversity (N_A, H_O, H_E and R_S) did not differ significantly from adult to seedling or seed cohort for *M. oppositifolia* and *G. sericea*. Seedlings of *M. oppositifolia* were only found in one large population, comparing genetic diversity of adults and seedlings of the same populations did not result in significant differences. Inbreeding coefficient was not significant for adults or seedlings of this *M. oppositifolia* population. For *G. sericea* the F_{IS} values were significant in adult and seedlings, but did not differ significantly between cohorts. Significant

differences in genetic diversity were found in *Vateriopsis seychellarum* for H_O and R_S. No significant differences in F_{IS} values could be detected between adults and seedlings but rare alleles are lost in seedling cohorts (Table 3).

For *M. oppositifolia* F_{ST} values could only be calculated for adults. No differences in F_{ST} values could be detected in *G. sericea* but for *V. seychellarum* stronger differentiation was found in seedlings (Table 3).

Table 1: Literature review including genetic (Microsatellite = MS, AFLPs, Allozymes = Allo and Isozymes = Isoz) and ecological studies which analyze effects of habitat fragmentation on tree species. Abbreviations: Ad = Adults, Prog = Progeny, FW = Formerly widespread, HF = Historically fragmented, Out = Outcrossing, SI = Self-incompatible, SC = Self-compatible, na = no analysis done, ? = no clear or lacking information, Y = yes, N = no. Responses to habitat fragmentation were classified as: 1) the decrease of genetic diversity (Allelic richness, expected heterozygosity, loss of rare alleles), 2) increase of inbreeding, 3) increase in genetic differentiation and 4) decrease in reproductive output or fitness traits (seed set, fruit set, seed weight, germination rate, seedling survival, herbivory) of adults or progeny following fragmentation.

No	Species	Family	Study area	Life stage analysed	Type of rarity	Time in fragmentation	Mating system	Gene flow	Marker system	Decr. genetic div.	Incr. inbr.	Incr. genetic diff.	Decr. reprod. output/ fitness	Comments	Reference
1	*Acacia caven*	Fabaceae	Argentinia	Prog	FW	30a	SI	?	No	na	na	na	N		Ashworth & Marti 2011
2	*Acer saccharum*	Aceraceae	USA	Ad	FW	since 20th century	SC	?	Allo	Y	?	Y	na		Baucom et al. 2005
3	*Anacardium excelsum*	Anacardiaceae	Costa Rica	Prog	FW	?	Mainly SI	short?	No	na	na	na	Y		Ghazoul & McLeish 2001
4	*Araucaria angustifolia*	Araucareaceae	Brazil	Ad, Prog	FW	Since 1900	?	?	MS (8)	Y	Y	Y	na	recruitment problems	Bittencourt & Sebbenn 2009
5	*Araucaria nemorosa*	Araucareaceae	New Caledonia	Ad, Prog	FW	150a	?	?	MS (7)	Y	Y	N	na	recruitment problems	Kettle et al. 2007
6	*Aristotelia chilensis*	Elaeoearpaceae	Chile	Ad, Prog	FW	?	?	long?	No	na	na	na	N	Fruity seeds dispersed by birds	Valdivia & Simonetti 2006
7	*Astronium graveolens*	Anacardiaceae	Brazil	Ad, Prog	FW	since 18th century	?	?	Allo	N	N	N	na		Schwarcz et al. 2010
8	*Beccariophoenix madagascariensis*	Arecaceae	Madagascar	Ad	?	?	?	?	MS (2)	Normal	High	High	na		Shapcott et al. 2007
9	*Bursera simaruba*	Burseraceae	Puerto Rico	Ad, Prog	FW	Since 16th to 19th century		long (up to 600m)	Allo	Y	Y?	?	Y	Effects strongest in fragment < 4 trees	Dunphy & Hamrick 2007
10	*Carapa guianensis*	Meliaceae	Costa Rica	Ad, Prog	?	10a	?	short	MS (3)	Y?	?	?	na		Dayanandan et al. 1999
11	*Cedrus brevifolia*	Pinaceae	Cyprus	Ad	HF	Continuous and anthropogenic	Out	Wind pollinated	MS (6)	High	Low	Low	na	Restricted to small region in Cyprus Compared fragments	Eliades et al. 2011
12	*Celtis ehrenbergiana*	Celtidaceae	Argentinia	Prog	FW	30a	SC	?	No	na	na	na	N	and continuous forest	Ashworth 2011
13	*Copaifera langsdorfii*	Caesalpinioideae	Brazil	Ad, Prog Prog	FW	60-80a	Mixed	short (<100m)	MS (8)	Y	Y	?	na	Few sapling but abundant seedlings, only seed set related to	Sebbenn et al. 2011
14	*Craigia yunnanensis*	Tiliaceae	China		FW	?	Mixed	?	No	na	na	na	Y?		Gao et al. 2010

No.	Species	Family	Country		FW	Time	Mating system	Pollen dispersal distance	Marker					Comments	Reference
15	*Crataegus monogyna*	Rosaceae	Spain	Prog	FW?	?	?	?	No	na	na	na	Y	population size Effects due to decrease in fruit dispersers (Birds) rather than fragment size	Herrera & Garcia 2010
16	*Dalbergia monticola*	Fabaceae	East Madagascar	Ad	FW	>100a	Sexual	?	MS (8)	Y	Y	?	na		Andrianoelina et al. 2009
17	*Dicorynia guianensis*	Fabaceae	French Guiana	Ad, Prog	FW?	Exp plots	Out	long	MS (6)	Normal	Low	?	na		Latouche-Halle et al. 2003, 2004
18	*Dinizia excelsa*	Fabaceae	Brazil	Ad, Prog	FW	15–20a	Out	long (up to 1509m)	MS (5)	?	?	?	na	slight increase in selfing	Dick et al 2003
19	*Dipteryx panamensis*	Fabaceae	Costa Rica	Ad, Prog	FW	60a	Out, SC	long (up to 2.3km)	MS (9)	Y	?	Y	na	Increasing pollen flow distances in smaller fragments	Hanson et al. 2008
20	*Duckeodendron cestroides*	Solanaceae	Brazil	Ad, Prog	FW	30a	?	?	No	na	na	na	Y		Cramer et al. 2007
21	*Elaeocarpus grandis*	Elaeocarpaceae	North Australia	Ad, Prog	FW	100a	?	?	MS (5)	N	Y	?	na		Rossetto et al. 2004
22	*Entandrophragma cylindricum*	Meliaceae	Cameroon	Ad, Prog	FW	2a, 30a	Out	long (up to 2095m)	MS (4)	N	N	?	na		Lourmas et al. 2007
23	*Eucalyptus benthamii*	Myrtaceae	Australia	Ad, Prog	FW?	160a	Mixed	short	MS (7)	N	Y	?	Y	highest genetic diversity in small populations	Butcher et al. 2005
24	*Eucalyptus globulus*	Myrtaceae	Tasmania, Australia	Prog	FW	Since 19th century	Mixed	short (<200m)	MS (6)	N	Y	?	Y	Decreased outcrossing rate and enhanced correlated paternity in fragments	Mimura et al. 2009
25	*Eucalyptus wandoo*	Myrtaceae	Australia	Prog	FW	?	Mixed	long (>1km)	MS (6)	?	?	?	na	Maintainance of long-distance pollen flow, similar to historical pollen flow	Byrne et al. 2008
26	*Eurycorymbus*	Sapindaceae	China	Ad, Prog	FW	Several 100a	Out	long	MS (6)	N	N	?	na		Wang et al.

	cavaleriei														2010
27	*Fagus sylvatica*	Fagaceae	Catalonia	Ad	FW	>600a	Out	? (>2000m)	MS (6)	Y	Y	Y	na	Higher seedling and adult but not sapling mortality in fragments	Jump & Penuelas 2006
28	*Fagus sylvatica*	Fagaceae	Catalonia	Ad	FW	>600a	Out	?	No	na	na	na	Y/N		Barbeta *et al.* 2011
29	*Ficus arpazusa*	Moraceae	Brazil	Ad, Prog	FW?	?	Out	long (>5.6km)	Allo	N	N	?	na		Nazareno & de Carvalho 2009
30	*Glionnetia sericea*	Rubiaceae	Seychelles	Ad, Prog	HF	150a	SC	long	MS (10)	N	N	N	na		Finger *et al.* in prep.
31	*Guaiacum sanctum*	Zygophyllaceae	Costa Rica	Ad, Prog	FW	Since 16th Century, Recently	Mixed	long (> 4km)	Allo	Normal	?	?	na	Effects due to decrease in fruit	Fuchs & Hamrick 2011
32	*Ilex aquifolium*	Aquifoliaceae	Spain	Prog	FW?	?	?	?	No	na	na	na	Y	dispersers (Birds) rather than fragment size	Herrera & Garcia 2010
33	*Juniperus communis*	Cupressaceae	Ireland	Ad	FW?	Since 1960	Out	?	MS (3)	Normal	Low	?	na	Low recruitemt rates	Provan *et al.* 2008
34	*Lepidorrhachis mooreana*	Arecaceae	Lord Howe Island	Ad	HF	Continuous	?	?	MS (7)	N	N	N	na	Lack of recruitment due to Rattus rattus, in absence of predator	Shapcott *et al.* 2011
35	*Livistona carinensis*	Arecaceae	Djibouti	Ad, Prog	HF	rapid decline	?	?	MS (9)	Low	High	High	na	recruitment problems	Shapcott *et al.* 2009
36	*Macadamia integrifolia*	Proteaceae	Australia	Ad, Prog	FW?	?	Partially SI	?	No	na	na	na	N	Increased reproductive output in fragments, potentially due to more light	Neal *et al.* 2010
37	*Macadamia tetraphylla*	Proteaceae	Australia	Ad, Prog	FW	200a	Partially SI	?	Isoz	?	?	?	Y	Pollen transfer of 2km enhance seed set and seed weight but not germination	Pisanu *et al.* 2009

#	Species	Family	Location	Stage	Habitat	Time	Breeding	Distance	Marker					Notes (rate or genetic diversity)	Reference
38	*Magnolia obovata*	Magnoliaceae	Japan	Ad, Prog	FW	?	?	?	MS (11)	Y	Y	?	N		Isagi et al. 2007
39	*Manilkara huberi*	Sapotaceae	Brazil	Ad, Prog	FW	?	Mainly SI	short	MS (7)	N	?	?	na		Azevedo et al. 2007
40	*Medusagyne oppositifolia*	Ochnaceae	Seychelles	Ad, Prog	HF	Continuous	Out	short (<200m)	MS (10)	N	N	N	na	Lack of recruitment in small populations	Finger et al. 2011
41	*Metrodorea nigra*	Rutaceae	Brazil	Ad, Prog	FW	since 18th century	Out	?	Allo	N	N	N	na		Schwarcz et al. 2010
42	*Metrosideron boninensis*	Myrtaceae	Bonin Islands	Ad	HF?	120a	?	?	MS (8)	Normal	?	High	na		Kaneko et al. 2008
43	*Metrosideron boninensis*	Myrtaceae	Bonin Islands	Ad	HF?	120a	?	short	MS (8), Allo	Normal	?	High	na		Kaneko et al. 2008
44	*Metrosideros bartlettii*	Myrtaceae	New Zealand	Ad	HF	Continuous	SC	?	AFLP	Low	?	High	na	only 31 trees in 3 pops, recruitment problems	Drummond et al. 2000
45	*Myrciaria floribunda*	Myrtaceae	Brazil	young Ad	FW	since 1910	?	?	Allo	Y	N	Y	na	Most results non significant Lower seed production in	Franceschinelli et al. 2007
46	*Nothofagus glauca*	Nothofagaceae	Chile	Prog	FW	last decades	?	?	No	na	na	na	Y	small fragments due to predation	Burgos et al. 2008
47	*Picea koyamae*	Pinaceae	Japan	Ad	HF relict	Continuous	?	?	MS (5)	Higher genetic diversity and lower inbreeding in continuous populations					Katsuki et al. 2011
48	*Pinus elliottii var. Densa*	Pinaceae	South Florida	Ad	FW?	>100a	Out	?	MS (6)	N	N	N	na		Williams et al. 2007
49	*Pinus sylvestris*	Pinaceae	Italian Alps and Apennines	Ad	HF?	Continuous	?	?	MS (3)	High	Low	High	na		Scalfi et al. 2009
50	*Podocarpus elatus*	Podocarpaceae	Australia	Ad, Prog	HF?	>200a?	Sexual	?	MS (6)	N	N	N	na		Mellick et al. 2011
51	*Prunus africana*	Rosaceae	Western Kenia	Ad, Prog	FW	80-100a	Out	?	MS (6)	Y	Y	N	na		Farwig et al. 2008
52	*Quercus crispula*	Fagaceae	Japan	Ad, Prog	FW?	?	Out	?	MS (6)	N	N	?	na		Ohsawa et al. 2006
53	*Quercus ilex*	Fagaceae	Central Spain	Ad, Prog	FW/HF	Continuous since 3500BC	?	?	MS (9)	N	Y	N	na		Ortego et al. 2010
54	*Quercus macrocarpa*	Fagaceae	Illinois	Ad, Prog	HF	several 1000a?	?	short	MS (5)	High	Low	?	na		Craft & Ashley 2007
55	*Quercus suber*	Fagaceae	Minorca	Ad	HF	Continuous since 9500BC	?	?	MS (9)	Normal	Low	Low	na		Lorenzo et al. 2009

No	Species	Family	Location											Comment	Reference
56	*Schinus fasciculatus*	Anacardiaceae	Argentinia	Prog	FW	30a	SC	?	No	na	na	na	N		Ashworth & Marti 2011
57	*Shorea leprosula*	Dipterocapaceae	Malaysia	Ad, Prog	FW	1955	Out	?	MS (7)	Y	N	?	na		Ng *et al.* 2009
58	*Shorea ovalis*	Dipterocapaceae	Malaysia	Ad, Prog	FW	1955	SC	?	MS (7)	N	N	?	na		Ng *et al.* 2009
59	*Shorea robusta*	Dipterocapaceae	Nepal	Ad, Prog	FW	?	SC	long	MS (4)	N	N	?	na		Pandey & Geburek 2010
60	*Shorea siamensis*	Dipterocapaceae	Thailand	Prog	FW	20a	Mainly SI	short?	No	na	na	na	Y		Ghazoul & McLeish 2001
61	*Sorbus torminalis*	Rosaceae	Islands in Baltic Sea	Ad, Prog	HF?	Continuous	SI?	?	MS (5)	Normal	He excess	High	na		Rasmussen & Kollmann 2008
62	*Sorbus torminalis*	Rosaceae	Switzerland	Ad, Prog	HF?	last century	?	short (max 119m)	MS (5)	?	N	?	Y	Comparison of only one small isolated versus 1 continuous population	Hoebee *et al.* 2007
63	*Swietenia humilis*	Meliaceae	Southern Honduras	Ad	FW	40	SI	?	MS (10)	Y	N	?	?		White *et al.* 1999, 2002
64	*Swietenia macrophylla*	Meliaceae	Mexico	Ad, Prog	FW	?	?	?	MS (7)	N	?	?	?		Novick *et al.* 2003
65	*Swietenia macrophylla*	Meliaceae	Costa Rica	Ad, Prog	FW?	100a	?	?	MS (5)	N	?	?	?		Cespedes *et al.* 2003 Aldrich & Hamrick 1998; Aldrich *et al.* 1998
66	*Symphonia globulifera*	Clusiaceae	Costa Rica	Ad, Prog	FW	10-30a	?	?	MS (3)	?	Y	Y	?		Aldrich *et al.* 1998
67	*Taxus baccata*	Taxaceae	Catalonia	Ad	FW/HF	Continuous	Out	?	MS (7)	Normal	Low-High	High	?		Dubreuil *et al.* 2010
68	*Torreya taxifolia*	Taxaceae	Florida	Ad	FW	Since 19th century	?	?	Allo	Very low		Low	Deseases	Only small trees remain after fungal desease, close to extinction	Schwartz 1993
69	*Vateriopsis seychellarum*	Dipterocapaceae	Seychelles	Ad, Prog	FW	150a	SC	short (<100m)	MS (10)	Y	Y	Y	?		Finger *et al.* submitted
70	*Vouacapoua americana*	Caesalpiniaceae	French Guiana	Ad	HF?	Continuous	?	short	MS (9)	Normal	Low	Low	?		Dutech *et al.* 2002

Table 2: Results from the literature review. FW = Formerly widespread; HF = Historically fragmented. Number of cases for which no or negative genetic or ecological effects of habitat fragmentation have been found with proportions in brackets. Significant differences are marked in bold.

Type of rarity	Genetic diversity			Inbreeding			Genetic differentiation			Recruitment			Total		
	#No response	#Neg. response	p	#No response	#Neg. response	p	#No response	#Neg. response	p	#No response	#Neg. response	p	#No response	#Neg. response	p
FW	17 (0.53)	15 (0.47)	0.724	15 (0.54)	13 (0.46)	0.706	4 (0.31)	9 (0.69)	0.166	6 (0.33)	12 (0.67)	0.157	42 (0.46)	49 (0.54)	0.463
HF	12 (0.80)	3 (0.20)	**0.020**	10 (0.83)	2 (0.17)	**0.021**	7 (0.54)	6 (0.46)	0.782	0	1 (1.00)	-	29 (0.71)	12 (0.29)	**0.008**
p		0.149			0.154			0.427			-			**0.015**	

Table 3: Summary table of genetic variability of 10 microsatellite loci estimated over the species range of *Medusagyne oppositifolia*, *Glionnetia sericea* and *Vateriopsis seychellarum* (modified from Finger *et al.* 2011, Finger *et al.* submitted, Finger *et al.* in prep.). Abbreviations: n: number of genotyped individuals, in brackets the number of mother trees; N_A: Mean number of alleles; N_E: effective number of alleles; H_O: observed heterozygosity; H_E: expected heterozygosity; R_S: Allelic richness, based on two, five and three diploid individuals; R_A: total number of rare alleles with a frequency < 0.05 (mean frequency); F_{IS}: Inbreeding coefficient); F_{ST} = Genetic differentiation; ($\pm$ SE). * = p < 0.05, ** = p < 0.01, *** = p < 0.001.

SPECIES	n	#Pops	N_A	N_E	H_O	H_E	R_S	R_A	F_{IS}	F_{ST}
M. oppositifolia							$R_{S(2)}$			
Adults	91	4	3.48 (±0.35)	2.40 (±0.17)	0.57 (±0.05)	0.50 (±0.04)	2.21 (±0.10)	19	0.00ns	0.16***
Seedlings	39	1	5.00 (±0.76)	3.06 (±0.42)	0.66 (±0.05)	0.62 (±0.05)	2.39 (±0.15)	12	-0.09ns	-
G. Sericea							$R_{S(5)}$			
Adults	206	6	4.35 (±0.34)	2.66 (±0.20)	0.45 (±0.03)	0.49 (±0.04)	3.02 (±0.18)	68	0.11***	0.12***
Seedlings	196	6	4.33 (±0.29)	2.57 (±0.18)	0.42 (±0.03)	0.48 (±0.03)	2.97 (±0.17)	72	0.12***	0.08***
V. seychellarum							$R_{S(3)}$			
Adults	125	11	4.53 (±0.24)	2.98 (±0.15)	0.56 (±0.03)	0.59 (±0.02)	2.93 (±0.10)	47	0.09***	0.23***
Seedlings	260	9	3.44 (±0.19)	2.44 (±0.14)	0.45 (±0.03)	0.49 (±0.03)	2.38 (±0.09)	34	0.11***	0.36***

DISCUSSION

Our combined literature review and empirical research on a set of three highly fragmented island endemic tree species indicate that formerly widespread species which have been reduced to small isolated populations seem to be more vulnerable to negative genetic effects following habitat fragmented compared to historically fragmented species. Below a critical threshold population size (which may differ for every species), historically fragmented species are similarly prone to negative effects following population isolation. In highly fragmented small populations self-compatible mating can provide a 'safety net' to a species' survival in the short term. Long-distance pollen dispersal by mobile pollinators seems important for connecting populations of a rare tree species even in fragmented habitats and highlights the need to protect not only single species but the complex ecosystem.

Consequences of population history on the persistence of trees in disturbed habitats
Plant species associated with patchy habitats (e.g. inselbergs, mountain tops, swamps) have, consequently, a naturally patchy distribution. The persistence of such species in patchy and isolated habitats might be facilitated by traits that promote long distance gene exchange (through seed or pollen dispersal), or by purging of deleterious alleles (Ellstrand & Elam 1993; Lande & Schemske 1985). Such species might therefore be expected to be less vulnerable to continuing fragmentation as compared to species for which fragmentation is a recent phenomenon and which therefore might lack traits or characteristics that would buffer them from fragmentation effects. In a review of 26 plants, species that had long been patchily distributed were less likely to suffer loss of genetic diversity with continuing population isolation than species that were formerly more or less continuously distributed, though this difference was not statistically significant (Brigham 2003). It could also be shown that three of four formerly widespread species had greater pollen limitation compared to 6 of 13 historically fragmented species, but whether this was caused by a reduction of the number of pollinator species or by less frequent pollinator flower visitations was not explained in that study.

Our review of 70 tree species indicated that historically patchily distributed species respond less negatively to fragmentation compared to formerly widespread species that have been faced with recent habitat fragmentation (Table 2). Our conclusion is based on four criteria: aspects of genetic diversity (allelic richness, expected heterozygosity, loss of rare

alleles), inbreeding coefficient, genetic differentiation and reproductive output (seed set, fruit set, seedling survival/fitness) factors which may or may not be synergistic in their interactions. Within 49 studies of formerly widespread species we found 91 responses within these four categories of which 49 (54%) were negative responses to habitat fragmentation. For example *Araucaria angustifolia* (Araucariaceae) from Brazil has experienced habitat fragmentation since the 19th century and shows strong negative effects, such as a reduced genetic diversity, increased levels of inbreeding and genetic differentiation in progenies (Bittencourt & Sebbenn 2009). Similarly, *Copaifera langsdorfii* (Caesalpinioideae) showed negative genetic responses in progeny after 60 to 80 years of fragmentation. Other studies of formerly widespread species showed no or few effects to recent fragmentation. *Macadamia integrifolia* (Proteaceae) from Australia, for example, had higher reproductive output in fragments, potentially due to more light availability (Neal *et al.* 2010). In Japan *Magnolia obovata* (Magnoliaceae), also a formerly widespread species, had reduced genetic diversity yet no negative effects on reproductive output (Isagi *et al.* 2007). These different responses to recent habitat fragmentation demonstrate that about half (54%) of the formerly widespread species are negatively affected in terms of genetics or reproductive output, implying that historical distributions may not be the only explanatory variable but that gene flow rates may be similarly important, as discussed in the next section. Other factors such as the ability to purge deleterious alleles or to produce viable selfed progeny may also play an important role and could not be followed up in this study.

For the 16 historically fragmented species we had overall 41 responses within our four categories of which only 12 (29%) could be interpreted as negative responses to habitat fragmentation. Of these *Cedrus brevifolia* (Pinaceae), for example, which is naturally restricted to a small region in Cyprus but has been further reduced due to anthropogenic causes, retained high genetic diversity, and had low inbreeding and genetic differentiation (Eliades *et al.* 2011). Similarly, *Lepidorrhachis mooreana* (Arecaceae) a rare palm species restricted to one population in mist forests from Lord Howe Island shows no negative genetic response to fragmentation (Shapcott *et al.* 2011). In Australia *Podocarpus elatus* (Podocarpaceae) a historically fragmented riparian species which has been further fragmented over 200 years, shows no negative genetic effects (Mellick *et al.* 2011). Contrarily, *Metrosideros bartlettii* (Myrtaceae), with only 31 surviving individuals in three remnant native forest patches in the far north of New Zealand, shows recruitment problems, low genetic diversity and high genetic differentiation (Drummond *et al.* 2000). Persistence in small population sizes and resistance to genetic degradation thus appears greater for species

that have long been fragmented, rare or isolated, compared to species that have recently been reduced to small populations.

These trends are partly mirrored in our empirical study of three Seychelles endemic tree species. The historically widespread *Vateriopsis seychellarum* shows considerable reduction in allelic richness, observed heterozygosity and loss of rare alleles from adult to seedling cohorts and an increased genetic differentiation due to low historical and contemporary gene flow (Finger *et al.* submitted). Contrarily, the species with the historically and apparently naturally patchy distribution, *Medusagyne oppositifolia*, retained high genetic diversity in seedlings of the recruiting population of 78 adult individuals and similar patterns of historical and contemporary gene flow (Finger *et al.* 2011). *Glionnetia sericea*, which also has a historically patchy distribution but, in contrast to *M. oppositifolia*, has relatively large populations with tens to hundreds of individuals, shows no sign of loss of genetic diversity or increased genetic differentiation from adult to seedling cohorts, and there is recruitment in all populations, despite significant inbreeding values in adults and seedlings. Population sizes of *G. sericea* may still be large enough to retain genetic diversity in each of its populations regardless of natural distributions and fragmentation history.

While data from our empirical studies on the Seychelles are limited to three species, the results nevertheless fit the pattern that long isolated species display less genetic degradation than formerly widespread but now fragmented species. Our hypothesis predicts that species that have undergone recent and rapid fragmentation would express greatest loss of genetic diversity and higher genetic differentiation among populations, and that this would be reflected in differences in genetic diversity between generations as well as low reproductive output. *Vateriopsis seychellarum*, however, is recruiting seedlings and saplings in all but one population due to a self-compatible mating system, and there is no evidence for inbreeding depression in the current populations. *Medusagyne oppositifolia*, on the other hand, has good recruitment in the single large population, but no recruitment in three other populations as the species is mainly outcrossing. Self-compatible species might thus have an important trait allowing population persistence following fragmentation (Levin 2010; Ng *et al.* 2009) but whether they will suffer from inbreeding depression at a later development stage or generations is difficult to predict (Husband & Schemske 1996; Ingvarsson 2002).

Limited gene dispersal and its consequences for fragmented species
In insect pollinated species long distance gene flow is influenced by the capacity of the pollinators to fly long distances, especially when plant species have limited seed dispersal.

Species with the potential for long distance gene flow by pollen should be least affected by habitat fragmentation. Indeed, this hypothesis is upheld by our review of the literature when only evaluating the 26 studies that had information on gene flow rates (by pollen and/or seed). Our data showed that 70 % of formerly widespread species showed negative responses to habitat fragmentation when these species did not have effective mechanisms for long distance gene flow. Most (67%) formerly widespread species that had no negative responses to habitat fragmentation had mechanisms that promoted long distance gene flow either by pollen or seed (pollen or seed dispersal distances of more than 500 m). For example in an ecological study *Aristotelia chilensis* (Elaeoearpaceae) a formerly widespread tree from Chile showed no negative reproductive output following habitat fragmentation due to its bird dispersed seeds in four neighbouring forest fragments separated by 1 - 4 km (Valdivia & Simonetti 2007). *Dicorynia guianensis* (Fabaceae) from French Guiana, an insect pollinated species with gravity/wind dispersed seeds also has long distance pollen flow (62% pollen coming from outside 9 ha plots) and showed no decreased genetic diversity or increased inbreeding in experimental plots (Latouche-Halle *et al.* 2003, 2004). Similarly, no negative effects of habitat fragmentation were observed for the formerly widespread, insect pollinated and wind dispersed *Entandrophragma cylindricum* (Meliaceae), having pollen flow rates of up to 2 km (Lourmas *et al.* 2007). Such a pattern was not observed for historically fragmented species 71% of which had limited gene dispersal and no negative effects of habitat fragmentation.

Of the three species studied in the Seychelles, *G. sericea* was the only species for which we could demonstrate gene flow between populations that are separated by up to 8 km. This is likely to be due to its large hawkmoth pollinator that can potentially fly across such distances. We expect that smaller pollinators, such as the bees that pollinate *M. oppositifolia*, are unlikely to be able to transfer pollen between populations that are separated by more than a kilometre, but are effective as agents of pollen transfer within single populations, as is the case for *Medusagyne oppositifolia* in which there is weak fine scale spatial genetic structure in the single large population of 78 mature trees (Finger *et al.* 2011). If the pollinators of *V. seychellarum* are indeed small beetles, as we suspect, they are also not likely to be capable of pollen transfer across the distances of more than a kilometre between neighbouring *V. seychellarum* populations. In a continuous habitat these small pollinators may have distributed enough pollen to prevent fixation of alleles, but in the current fragmented landscape genetic exchange between populations seems unlikely, as demonstrated in the strong genetic differentiation and fine scale genetic structure of the species (Finger *et al.* submitted).

Our results and the associated review of the literature provides some evidence that formerly widespread species might be more vulnerable to genetic changes and reproductive problems associated with population isolation following recent habitat fragmentation as compared to species that have long been distributed in small and isolated populations on account of natural distribution of their habitat. Certain traits, such as self-compatibility, large pollinators capable of moving pollen over long distances, or different seed dispersal mechanisms, might shape the nature of the response to habitat fragmentation. Even so, the vulnerability of species to the genetic and ecological consequences of habitat loss and fragmentation might also be a function of their historical distributions. Although our conclusions remain somewhat equivocal, not least due to the limited number of studies, many of which were not designed to address this particular question, there are indications that plant species occurring within natural fragmented distributions might be better adapted to tolerate continuing habitat fragmentation relative to species that occur in more continuous populations.

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General conclusion

PERSISTENCE OF ISLAND TREE SPECIES IN FRAGMENTED LANDSCAPES

The high number of rare and endemic plant species on oceanic islands across the world has led to a high level of biodiversity on island habitats (Kreft *et al.* 2008; Myers *et al.* 2000) representing many unique evolutionary lineages. These islands have gone through and still experience severe human induced habitat fragmentation (e.g. Seychelles, Mauritius, Madagascar, New Zealand, Galapagos, Hawaii) and it is difficult to predict its potential negative genetic and ecological consequences for island flora and fauna. Some species may be less affected or even resilient to fragmentation but it is more likely that most will be negatively affected (Frankham 2001). On the ancient continental islands of the Seychelles habitat fragmentation and degradation occurred over the past 200 years, further enforced by the introduction of several invasive plant species (Hazell *et al.* 2008; Kirk & Racey 1992; Küffer *et al.* 2003). Land clearance occurred over the whole range of the main island, Mahé, leaving only few patches of virgin forest at higher altitudes in the late 19th century. As a consequence most species from Mahé have lost a substantial proportion of their natural habitats.

For this thesis I chose three tree species with contrasting historical distributions, *Medusagyne oppositifolia* with a historically patchy distribution, *Vateriopsis seychellarum*, a formerly widespread canopy tree, and *Glionnetia sericea* as an intermediate species, all endemic to the Seychelles. These species occupy three main habitat types, all of which are found on other tropical islands. *Medusagyne oppositifolia* occurs only on inselbergs (granitic outcrops), *V. seychellarum* in lowland forest and *G. sericea* on inselbergs and mist forests. The aim of this thesis was to explore the immediate genetic effects of habitat loss on these three island endemics by analysing adult individuals which are expected to pre-date habitat fragmentation and comparing that to their progeny which post-date fragmentation events.

I argue that species which occur naturally in fragmented, small and isolated populations (like *M. oppositifolia* and *G. sericea*) are less vulnerable to the negative genetic consequences of fragmentation compared to species which are historically widespread but have recently become highly fragmented. The effects of habitat fragmentation may be particularly acute in formerly widespread species. The results from the empirical data and a review of literature indicate that indeed, formerly widespread species show a higher proportion of negative genetic or ecological responses to habitat fragmentation compared to species which were historically more patchily distributed. As we did not focus our literature review on island species this result also shows that the general effects of habitat fragmentation seem to be similar in continental regions.

The mating system of a species can, at least in the short term, buffer it against the negative effects of population fragmentation and isolation, for example by enabling the production of viable seeds by self-fertilisation. This could be an advantage to island species in general as this enables the foundation of new populations on islands from only a single individual. However, self-fertilization can lead to increased homozygosity and inbreeding depression (Hamrick & Godt 1996; Ingvarsson 2002) which may lead to the conclusion that outcrossing species may have more viable populations in long-term. The obligate outcrossing species, *M. oppositifolia,* was indeed able to maintain a high genetic diversity in its large population. Yet, outcrossing in combination with rapid and extreme population fragmentation has lead to recruitment failure in populations with few remaining individuals. Interestingly, Angeloni *et al.* (2011) suggest that the mating system of a plant species does, in general, not influence the magnitude of inbreeding depression as it is found equally in both self-compatible and self-incompatible species. Thus, resilience to negative effects of landscapes modifications may not be necessarily linked to species' mating systems.

Numerous tree species world-wide have now been driven to extremely low population sizes (Oldfield *et al.* 1998). Some authors have promoted a minimum viable population size (from an ecological and genetic perspective) to assure long-term viability of populations (reviewed by Gilligan *et al.* 1997; Lynch *et al.* 1995; Reed & Bryant 2000). For many island endemic trees attempting to apply such rules may be especially challenging. For example, the so-called 50-500 rule was introduced in Franklin (1980) suggesting that short term effective population sizes should not go less than 50 and long-term effective population sizes should not go below 500. This rule is based upon experience of animal breeding and the observed effects of inbreeding depression in small populations. Firstly it is notoriously difficult to ascertain the effective population size and secondly the ratio of census size to effective

population size can range over several scales of magnitude. Certainly, the census population sizes of many rare and fragmented species from the Seychelles are unlikely to correspond to effective population sizes of this order. It seems that for island habitats species are also able to survive in smaller numbers and retain high genetic diversity as seen for *M. oppositifolia* in its largest population containing only 78 individuals and also populations of *G. sericea* (1-100 individuals per population).

Cleary, from these results we can say that any one single trait (such as species' population histories or mating system) is not going to be a sole determinant for island tree species' persistence or extinction in small populations. It is much more likely that a combination of life history traits and adaptation to small population sizes will confer some resilience of a species to fragmentation.

GENETIC RESCUE AND LONG-DISTANCE GENE FLOW: A VALUE FOR RARE TREE SPECIES?

Habitat fragmentation is a global problem as landscapes become increasingly modified by humans, partly driven by forest clearances, intensive agriculture or urbanisation. Some habitats are also naturally fragmented due to differing biotic and abiotic factors such as small islands, inselbergs, mountain tops or swamps. For tree species, being reduced to small populations is only one aspect of habitat fragmentation. Other aspects, such as population isolation and changed abiotic environmental characteristics are also important consequences of fragmentation. These can all affect plant recruitment success by involving changes in mating patterns (e.g. pollen flow) among populations (Fahrig 2003; Larsen *et al.* 2005; Laurance & Curran 2008; Steffan-Dewenter & Tscharntke 2000). For the conservation of species it is crucial to assess whether such fragmented habitats and changes in mutualistic and antagonistic plant–animal interactions may also affect offspring fitness (e.g. Aizen & Feinsinger 1994; Tscharntke & Brandl 2004; Valladares *et al.* 2006).

Rare plant species are vulnerable to genetic erosion and inbreeding associated with small population size and isolation due to increasing habitat fragmentation, although the degree to which these problems undermine population viability are still contentious (e.g., Reed 2005 and citations therein; Angeloni *et al.* 2011; Le Cadre *et al.* 2008; Leimu *et al.* 2006). We explored genetic and reproductive processes to evaluate whether recruitment

failure in the small populations of *Medusagyne oppositifolia* is linked to genetic problems associated with fragmentation, and whether genetic rescue can mitigate such problems. Inter-population pollination crosses from the large donor population to a small recipient population resulted in higher reproductive success relative to within-population crosses demonstrating the potential for genetic and ecological rescue to support conservation of plant species with limited gene flow.

Moreover, species which are able to transport pollen over long distances due to highly mobile pollinators, such as *Glionnetia sericea,* demonstrate the potential for the maintenance of viable populations in what appear to be fragmented landscapes. This emphasises the need to maintain plant pollinator interactions, and to ensure that conservation efforts focus at the ecosystems level not solely at the species level. For species with limited gene dispersal, such as *Vateriopsis seychellarum,* genetic exchange between populations is far more problematic. In a formerly continuous habitat, short distance gene exchange (pollen flow < 50 m, seed dispersal < 25 m) may have been sufficient to prevent fixation of alleles but a fragmented environment leads to complete population isolation in this species. Therefore it could be shown that genetic rescue is an appropriate conservation strategy for rare tree species surviving in small and isolated populations and should be considered in combination with habitat conservation and restoration.

OUTLOOK

In this thesis I have tried to disentangle mechanistic components of fragmentation which may have considerable application to ecology, conservation, evolution and natural resource management. This thesis has endeavored to examine fragmentation from a relatively narrow perspective focusing on three extremely rare island tree species on one island. Such a focus is important in the short-term to develop conservation strategies and population management practices, which is critical for maintaining viable populations for species at the brink of extinction. Nevertheless, such an approach cannot completely unravel the plethora of complex ways in which fragmentation influences species and habitats.

This is how the thesis has touched on interesting scientific questions which I was not able to fully address but warrant more attention in further projects. Remnant tree populations surviving in habitat fragments are confronted with a highly modified biotic and abiotic environment with novel ecological boundaries (Ewers & Didham 2006) which could not be

explored explicitly in this thesis. More information on possible changes in important abiotic conditions such as air and soil temperature, soil ph, relative humidity and light incidence, possibly caused by changes in plant communities (invasive species), would fully demonstrate the relative contribution of genetic problems to recruitment failure in *M. oppositifolia*.

Furthermore, even though our nursery experiments showed that *M. oppositifolia* seedlings performed better when crossed between populations it would be important to explore whether this can be confirmed in the field. Transplant experiments would not only enhance numbers of individuals in the field but also provide information on the importance of genetic and ecological requirements of the species. In that context it would be important to explore suitable habitats for the establishment of new populations. Complete surveys of *V. seychellarum* and *G. sericea* seedlings and saplings would also help to explore in detail the current population demographic structure of these species and may uncover recruitment problems, e.g. due to lacking size classes, which have not been detected during this work.

Vateriopsis seychellarum specifically has left some open questions. The extremely inbred planted populations, which already survive in the F2 generation, provide the opportunity to examine the long-term effects of inbreeding and self-compatibility. The results from the genetic recue experiments of *M. oppositifolia* suggest that similar benefits might be gained in *V. seychellarum*, but experimental crosses and progeny trials will be necessary to determine if out-crossed progeny out-perform selfed progeny or whether local adaptation may counteract positive effects of crossings.

Many rare species can be found in tropical and subtropical rainforests and typically appear more frequently than common species (Hubbell & Foster 1986; Hubbell *et al.* 2008; Murray & Lepschi 2004; Tracey 1981). Hubbell *et al.* (2008) estimated a total of 11210 tree species in the Brazilian Amazon Basin. Of these an estimation of 3248 species were common (> 1 million individuals) and 5308 species rare (< 10000 individuals). In a fragmented forest, larger patches contain both more species, and more rare tree species. Thus, to maintain species diversity in tropical rainforests it is important to maintain large forest patches but also the number of rare species (Hill & Curran 2003). This emphasises the need for research to focus not only on rare species (as has been done in this thesis) but also currently widespread species, which may be reduced to small populations in the process of current habitat fragmentation.

PRACTICAL CONSERVATION RECOMMENDATIONS

With this thesis I have focused on potential negative genetic effects of habitat fragmentation of three rare island endemics from the Seychelles to understand processes underlying genetic erosion in trees with contrasting historical distributions. One major aim was to inform conservation and management strategies which will directly benefit the three species.

- The small populations of *Medusagyne oppositifolia* survive in only two, three and seven individuals which lack recruitment. As we could show that the lack of recruitment is not caused by pollen limitation (at least in Mt. Sebert) it is very likely that the close relatedness between remaining trees prevent successful seed germination and seedling establishment. Our cross-pollination treatments have resulted in enhanced progeny fitness and therefore it is recommended to repeat these cross-pollinations for all small populations (taking pollen from Bernica population) and grow as many resulting seeds as possible *ex-situ*.

- Following up, germinated seedlings should be grown to several cm until roots are well developed and then planted into the field in the small populations to enhance numbers of individuals. As germination rates are low and seedling mortality is high it is advisable to germinate several thousands of seeds for these trials.

- Similarly seeds should be collected from ideally all *Vateriopsis seychellarum* populations, but as a priority from populations Mt. Cotton (if possible), Rivière L'Islette, Sans Souci and Brulée. A collection of about 20 seeds per tree and their germination in a nursery *ex-situ* is recommended for potential plantations of new populations.

- As gene dispersal is low for *V. seychellarum* it would be important to establish new populations in the vicinity (< 1km) of existing populations to ensure gene exchange between future populations.

- Protection of natural habitats is the prerequisite for the maintenance of viable populations for the three species. This is important to prevent further reductions in population sizes but also to keep habitats for potential forest restoration.

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Development of thirteen polymorphic microsatellite markers for the Seychelles endangered and endemic jellyfish tree *Medusagyne oppositifolia* (Medusagynaceae)

with J. Ghazoul, M. Todd, C.N. Kaiser-Bunbury and C.J. Kettle

ABSTRACT

The jellyfish tree *Medusagyne oppositifolia* is a flagship species of the Seychelles, being not only extremely rare and critically endangered, but also representing a monospecific endemic family, Medusagynaceae. The species survives in four populations on the Island of Mahé, where the total number of reproductive adults is 89. Natural regeneration is only known for the largest of these populations. Understanding the mechanisms driving the lack of natural regeneration in this species has great conservation relevance. We developed thirteen polymorphic microsatellite loci for this species to enable studies of historic and contemporary gene flow. The number of alleles per locus ranged from 2-15 (mean of 6.62 per locus) with an average polymorphic information content of 0.54 across loci. Expected heterozygosity ranged from 0.12-0.66 with only two of the 13 loci showing deviation from Hardy-Weinberg expectation. The markers will help to provide a better understanding of the significance of historic distributions, gene flow and recent anthropogenic habitat degradation for the in-situ and ex-situ conservation of this flagship tree species.

PRIMER NOTE

Medusagyne oppositifolia Baker (Medusagynaceae) is one of the rarest and most threatened tree species in the world. Medusagynaceae is a monospecific family endemic to the Seychelles. Its evolutionary history remains somewhat enigmatic (Fay *et al.* 1997)(Fay et al. 1997). It is commonly known as the jellyfish tree, the name deriving from the seed capsule which resembles an inverted medusa. As an endemic and unique species it has become an emblematic flagship species of the Seychelles.

The species is distributed on granitic outcrops (inselbergs) in four relatively isolated populations on the main island of Mahé. A total number of 89 adult trees survive with 77 individuals occurring in a single population. The species is thought to have a naturally fragmented population structure as it has never been recorded on forested slopes surrounding inselbergs. Its wind dispersed seeds and pollen could make it less sensitive to the deleterious genetic consequences of habitat fragmentation, traits commonly associated with long distance gene flow. Owing to an absence of historical records the extent of decline of *M. oppositifolia* in recent decades is uncertain, but a decline is clear for three of the four populations which now contain only 2-7 mature individuals with no sign of seedlings or saplings. Viable recruitment is only found in the largest population.

Our work aims to investigate the constraints to natural regeneration in this flagship species through a better understanding of the reproductive ecology and specifically, historic genetic differentiation, contemporary gene flow by pollen dispersal, and genetic diversity among remaining populations.

To this end, we describe the characterization of 13 microsatellite markers for *M. oppositifolia*. Enriched libraries were made by Genetic Identification Services (http://www.genetic-id-services.com) from size selected (300-750bp) genomic DNA ligated into the *Hin*d III site of a pUC19 plasmid and enriched using magnetic bead capture (CPG, Inc., Lincoln Park, New Jersey) with biotin-labelled CA(15), Biotin-GA(15), Biotin-AAC(12) and Biotin-TAGA(8). In total, 102 different microsatellite-containing clones were identified from the two di- nucleotide and two tri-nucleotide repeat libraries. The libraries all contain between 10000 and 15000 recombinant cells. Plasmids from 96 positive clones were sequenced and primers designed for 86 microsatellite-containing clones using DesignerPCR version 1.03 (Research Genetics, Inc), of which 24 were tested for polymorphism. Of these 24 the thirteen most promising loci were optimized. Eight primers were fluorescently labelled at their 5′-end and five were labeled using an M13-tag at its 5′-end following Schulke (2000)

(Table 1). Polymorphism of the thirteen selected primers were evaluated using all adult trees of 89 *M. oppositifolia* samples collected from the four different sites on Mahé.

Leaf genomic DNA was extracted from *M. oppositifolia* ($n = 89$) using the QIAGEN DNeasy Plant Maxi Kit, following the manufacturer's protocol. PCRs for the fluorescence labeled primers were carried out in 10 μL reactions with, 2μl of 1X PCR buffer (Promega colorless Flexi GoTaq PCR buffer), 2 mM $MgCl_2$, 0.2 μM dNTPs, 0.6μM of each primer, 0.025 U *Taq* polymerase (Promega), and 1 μL DNA template (*c.* 10 ng). Cycling conditions were as follows: $1\times$ (94 °C for 3 min), $35\times$ (94 °C for 40 sec, primer-specific temperature (57°C) for 40 sec, 72 °C for 30 sec), $1\times$ (72 °C for 4 min) (Table 1) carried out in a Bio-Rad Dyad Cycler. PCRs for the M13 primers were carried out in 10μl reactions with 2μl of 1X PCR buffer (Promega colorless Flexi GoTaq PCR buffer), 15 mM MgCl2, 0.2μM dNTPs, 0.2 μL of the 0.04μM M13 forward primer, 0.8μl of the 0.16μM reward primer and 0.8μl of the 0.16μM M13 primer, 0.025 U *Taq* polymerase (Promega), and 2μL DNA template (*c.* 10 ng). Cycling conditions were as follows: $1\times$ (95 °C for 15 min), $30\times$ (95 °C for 30 sec, primer-specific temperature (56°C) for 45 sec, 72 °C for 45 sec), $8\times$ (95 °C for 30 sec, primer-specific temperature (53°C) for 45 sec, 72 °C for 45 sec), $1\times$ (72 °C for 30 min) (Table 1) carried out in a Bio-Rad Dyad Cycler. We used an ABI3730 for genotyping and Genemapper 3.5 software (Applied Biosystems) for fragment analysis.

Descriptive statistics (number of alleles, observed and expected heterozygosities), deviations from Hardy–Weinberg equilibrium (HWE) were generated using GenAlEx 6.2 (Peakall and Smouse 2006). The polymorphism information content (PIC) was calculated in Cervus 3.0 (Kalinowski et al. 2007). Linkage disequilibrium was implemented in GENEPOP (Raymond and Rousset 1995). All thirteen loci were polymorphic with 2–15 alleles and a total number of 85 alleles detected over all populations for *M. oppositifolia*. Observed heterozygosity values ranged from 0.14 to 0.77. There was no evidence for scoring error due to stuttering and no evidence for large allele dropout according to microchecker 2.2.3 (Oosterhout et al. 2004) but evidence for null alleles were detected for loci A107 and A114. Significant deviations from Hardy– Weinberg equilibrium (HWE, $P < 0.05$) were detected in the same 2 loci (A114, A107). No significant linkage disequilibrium was detected suggesting that all 13 loci segregate independently of each other. These results indicate that these 13 primers will provide a valuable tool for evaluating genetic diversity and the reproductive ecology of this rare and emblematic tree species.

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Table 1: Characteristics of thirteen polymorphic microsatellite loci in *Medusagyne oppositifolia*. Abbreviations: F: forward primer; R: reverse primer; T_a: annealing temperature; A: mean number of alleles; H_o: observed heterozygostiy; H_e: expected heterozygosity; PIC: Polymorphism information content; 89 individuals were analysed for each locus.

Locus	GenBank Accession no.	Primer sequence (5′-3′)	Repeat motif	Size range (bp)	Ta (°C)	A	H_o	H_e	PIC
A114	GU045577	F AAG CAA GCA CCT AAG ACA GTC R TTG ATT CGT TTA CGG TTG AG	$(TC)_{18}(CA)_{16}$	85-115	57	7	0.28	0.42	0.73
B107	GU045578	F ATG TCC AAT CTC TCT CGT CAA C R ACC CCT GTC GTT TAG AAT CTG	$(CT)_{17}$	146-161	57	6	0.80	0.56	0.53
C107	GU045580	F TTG CTT TTG GCA CTG AAT C R CTT CCC TCC TTT TCT GAC G	$(GTT)_{12}$	173-182	57	2	0.35	0.28	0.29
D12	GU045574	F GTT ATT GCA CTA AAG CCT CATC R CTT CGT ATA GAT TGC CTT AAC G	$(TAGA)_6$	226-242	57	5	0.58	0.49	0.60
D118	GU045581	F TTG GCC CTT GCT TTC ATA G R GGT GAC CTC TTG ATC CAA CAT	$(CTAT)_9$	103-119	57	5	0.48	0.37	0.70
A107	GU045576	F GAT GCC GAT TTC ATG TAC G R TCG ATT CAC AAC GCT CTT C	$(CA)_{19}$	133-139	57	4	0.21	0.39	0.39
D6	GU045573	F GCA CGT ATT GCA CAC ACA C R TCG AAA AGC ATA GAC TAA CAG C	$(TAGA)_9$	289-305	57	4	0.51	0.42	0.42
B112	GU045579	F ACC CAT AAA AGC CAC CAC R GCT CAG GTT GAA CAG GTT AG	$(GA)_{19}$	150-202	57	15	0.69	0.59	0.86
A9 (M13)	GU045571	F CTT CCT CAC CCA CCT GAC R GCA ATC TCT CAT TCC TTT CTT	$(GT)_{19}$	240-276	56	11	0.59	0.59	0.66
B8a (M13)	GU045575	F ACC CCC TCT CTA TCT TCT TTC R CAA AAT CGC CAC AAA TTC	$(CTGT)_3(CT)_{12}$	166-178	56	7	0.70	0.64	0.50
C12 (M13)	GU045572	F ACC GAA TTA GGG CTA GGA TG R ACA GCA ACA GCA ACT CCT GT	$(GTT)_7$	206-218	56	2	0.17	0.12	0.03
A4 (M13)	GU045570	F: GCA ATC CTC TCA TCA ATA CCA R: ACG CAA ATA GTT TAC TCC TTC G	$(CA)_{15}(TA)_4(GA)_8$	235-273	56	10	0.52	0.66	0.74
A7 (M13)	GU045569	F ATC CGT AAA GTG CAT GGT TTC R AAG AAG TGG ATC GGA GTG ATG	$(CACG)_4(CA)_{29}(TA)_8$	254-286	56	7	0.40	0.43	0.63

Appendix B

Development of polymorphic microsatellite markers of the endangered and endemic *Vateriopsis seychellarum* (Dipterocarpaceae), a relict canopy tree of the Seychelles

with S. Ismail, J. Ghazoul and C.J. Kettle

ABSTRACT

The Dipterocarpaceae are a globally significant family of tropical timber trees. They are especially dominant in lowland rainforests of Southeast Asia, but have a pan tropical distribution. *Vateriopsis seychellarum* is the sole representative of this family on the Seychelles. Historically one of the dominant canopy trees on Mahé, extensive overexploitation of this species for its timber has led to its virtual extinction. The last individuals of this species are found in small fragmented populations at lower altitudes (up to 400 m) on the island of Mahé where the total number of known reproductive adults is 112. We developed ten polymorphic microsatellite loci for this species to enable us to quantify the levels of diversity in remnant populations and to study genetic structure and contemporary gene flow. In addition we tested for cross amplification of these alleles in the closely related but geographically disjunct species *Vateria indica*. In *Vateriopsis seychellarum* the number of alleles per locus ranged from 6-20 (mean of 11.4 per locus) with an average polymorphic information content of 0.73 across loci. Expected heterozygosity ranged from 0.40-0.71 with 3 of the 10 loci showing deviation from Hardy-Weinberg expectations. 8 of the 10 primers showed cross amplification in *Vateria indica*. These markers will help to provide a better understanding of the significance of historic distributions, gene flow and recent anthropogenic habitat degradation for the survival of widespread species in recently fragmented landscapes.

PRIMER NOTE

Vateriopsis seychellarum is an endangered and endemic tree species of the Seychelles, occurring in lowlands up to 400m on the main island of Mahé. It is the sole representative of the family of Dipterocarpaceae in the Seychelles. Historically this species dominated the forest canopy across much of the Seychelles island of Mahé (Procter 1984), but extensive overexploitation for timber has led to its virtual extinction. Today there are only 112 known adults left, dispersed in nine populations on Mahé, at least two of which are known to be planted. Understanding the distribution and extent of the remaining genetic diversity in these populations, as well as the contemporary potential for gene dispersal by pollen and seed, will help to inform in-situ and ex-situ conservation. *Vateriopsis seychellarum* also provides a useful study system for exploring the genetic consequences of habitat fragmentation of highly endangered dominant tropical canopy species.

To this end we developed microsatellite markers for *V. seychellarum*. Enriched libraries were established from size selected genomic DNA ligated into SAULA/SAULB-linker (Armour et al. 1994) using magnetic bead selection with biotin-labelled (CT)13 and (GT)13 oligonucleotide repeats (Gautschi et al. 2000a,b). Of 352 recombinant colonies screened, 255 gave a positive signal after hybridization. Plasmids from 72 positive clones were sequenced and primers were designed for 24 microsatellite inserts, of which 18 were tested for polymorphism. Of these, only the ten most variable loci were optimized and labeled with an M13-tag at its 5′-end described by Schulke (2000) (Table 1). Polymorphism of the ten PCR-primers generating the expected PCR products was tested with 98 *V. seychellarum* adult tree samples collected from six different sites on Mahé.

Genomic DNA was extracted from silica dried leaves of *V. seychellarum* (n = 98) using the QIAGEN DNeasy Plant Maxi Kit, following the manufacturer's protocol. PCR was carried out in 10μl reactions with 2μl of 1X PCR buffer (Promega colorless Flexi GoTaq PCR buffer), 15 mM MgCl2, 0.2μM dNTPs, 0.2 μL of the 0.04μM M13 forward primer, 0.8μl of the 0.16μM reward primer and 0.8μl of the 0.16μM M13 primer, 0.025 U *Taq* polymerase (Promega), and 2μL DNA template (*c.* 10 ng). Cycling conditions were as follows: 1× (95 °C for 15 min), 30× (95 °C for 30 sec, primer-specific temperature (56°C) for 45 sec, 72 °C for 45 sec), 8× (95 °C for 30 sec, primer-specific temperature (53°C) for 45 sec, 72 °C for 45 sec), 1× (72 °C for 30 min) (Table 1) carried out in a Bio-Rad Dyad Cycler. We used an ABI3730 for genotyping and genemapper 3.5 software (Applied Biosystems) for fragment analysis.

Descriptive statistics (number of alleles, observed and expected heterozygosities), deviations from Hardy–Weinberg equilibrium (HWE) were generated using GenAlEx 6.2 (Peakall and Smouse 2006). The polymorphism information content (PIC) was calculated in Cervus 3.0 (Kalinowski et al. 2007). Linkage disequilibrium was tested using GENEPOP (Raymond and Rousset 1995). All ten loci were polymorphic with 6–20 alleles and a total number of 114 alleles detected over all analysed populations for *V. seychellarum*. Observed heterozygosity values ranged from 0.25 to 0.71. There was no evidence for scoring error due to stuttering and no evidence for large allele dropout according to microchecker 2.2.3 (Oosterhout et al. 2004) but evidence for the presence of null alleles in 2 loci (12 and 23). Significant deviations from Hardy–Weinberg equilibrium (HWE, $P < 0.05$) were detected in three loci (12, 20 and 23). No significant linkage disequilibrium was detected suggesting that all 10 loci segregate independently of each other. These results indicate that the 10 primers will provide a valuable tool for evaluating genetic diversity and the reproductive ecology of this rare and emblematic tree species.

In our preliminary test for cross species amplification with *Vateria indica* we applied the same PCR conditions and used 1.3 uL of template DNA. Of the 10 microsatellites tested 8 amplified, we checked for polymorphism in these 8 loci and found 51 alleles in 23-35 individuals sampled from a single region in the Western Ghats, India (Table 1). The results indicate that this set of 10 microsatellite primers will be valuable for quantifying the genetic diversity and gene flow in the remaining populations of *Vateriopsis seychellarum.* Our preliminary assessment of cross amplification in *Vateria indica* also suggests that these markers may prove useful for studies in other closely related taxa.

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Table 1: Characteristics of ten polymorphic microsatellite loci in *V. seychellarum*. Abbreviations: F: forward primer; R: reverse primer; T_a: annealing temperature; *A*: number of alleles; H_o: observed heterozygosity; H_e: expected heterozygosity; PIC: Polymorphism information content; 98 individuals were analysed for each locus in *Vateriopsis seychellarum* and 23-35 individuals in *Vateria indica*.

Locus	GenBank Accession no.	Primer sequence (5´-3´)	Repeat motif	*Vateriopsis seychellarum*						*Vateria indica*		
				Size range (bp)	Ta (°C)	A	H_o	H_e	PIC	Cross-species amplification	Size range (bp)	A
01	GU591481	F TCATTTCAAACCCAGCAATG R TCATGCTGCTGATGAAGACC	(CA)₁₇	214-234	56	6	0.48	0.45	0.46	Yes	217-227	2
10	GU591482	F TGCGAGAATCAGCCTATGAG R CATAAAGCATGGACCTCAGC	(CT)₁₇	140-178	56	12	0.46	0.54	0.68	Yes	132-158	6
11	GU591483	F TCAAGCCATAGGACACTTGC R GATCGGCCTGCTAAACATTC	(CT)₁₉	201-249	56	20	0.68	0.70	0.87	Yes	191-247	13
12	GU591484	F GGAATCAAGCGCGAATTAAG R TCATCATCTTTACCCATTATCAG	(CT)₁₆	199-223	56	9	0.25	0.51	0.70	Yes	177-215	9
14	GU591485	F CTTTTGCCATATGCATGCTC R ATCGTCACAGCCTCATTACG	(TC)₃TT(TC)₁₆	100-130	56	15	0.71	0.74	0.89	Yes	99-109	4
15	GU591486	F ATTAGGGCTTTGGGTGAGTG R GCCAGAACCAGTGGATGAG	(GA)₂₂	142-174	56	8	0.42	0.39	0.56	Yes	156-225	6
20	GU591487	F TTTACAGTCTCGAAAATTGTGACTAAG R AACAACCTGGGTTGGAGATGC	(GA)₁₅	114-158	56	11	0.65	0.71	0.82	Yes	102-129	8
21	GU591488	F TATTCCCTCATCGTGGAACC R TTCGGGTATAAGAGGGAGGAG	(CT)₄GGTTG(CT)₂₂	188-220	56	12	0.58	0.68	0.82	No	-	-
22	GU591489	F: TTTTGATAAACGTTCAAGGCTTC R: ATTCAGCCATTGTTGTGCAG	(CT)₂₁	168-198	56	10	0.56	0.65	0.73	No	-	-
23	GU591490	F: TATGGCTTCGCTCAATTGTC R: TTCGTCAGTTTTGGAGTTGG	(AG)₂₂	204-230	56	11	0.58	0.70	0.81	Yes	198-226	3

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Appendix C

Development of polymorphic microsatellite markers of the Seychelles endemic tree *Glionnetia sericea* (Rubiaceae)

with C.N. Kaiser-Bunbury and C.J. Kettle

ABSTRACT

Glionnetia sericea (Rubiaceae) is an endemic and rare tree species of the Seychelles, restricted to altitudes between 500m and 900m with less than 1000 remaining individuals. It survives in mist forests but also in smaller populations on granitic outcrops (inselbergs) and is pollinated by hawk moths which might ensure long-distance pollen flow. Understanding the reproductive ecology of this species will allow a better understanding on how such species survive in naturally fragmented habitats and will provide scientifically informed management recommendations. Here we report on ten species specific polymorphic microsatellite loci developed for a study of historic and contemporary gene flow. Based upon a sample of 81 adults, the number of alleles per locus ranged from 3-12 (mean of 6.1 per locus) with an average polymorphic information content of 0.52 across loci. Expected heterozygosity ranged from 0.27-0.82 with two of ten primers showing some deviation from Hardy-Weinberg expectation.

PRIMER NOTE

The rare tree *Glionnetia sericea* (Rubiaceae) is endemic to the Seychelles archipelago. It is found on two Islands, Mahé and Silhouette, where probably less than 1000 individuals survive at ten known discrete sites. The species is classified as vulnerable in the IUCN red list (2011). *G. sericea* is distributed in mist forests at higher altitudes between 500m and 900m and is representative of a plant community occurring in remnants of virgin forest. The current distribution includes some relatively large and continuous populations, consisting of more than 100 individuals, and some smaller more isolated populations found on granitic outcrops (inselbergs). Increase in habitat degradation and invasive species may threaten the long-term survival of this species (Daehler *et al.* 2004) and the inselbergs provide important refugia for *Glionnetia sericea*. The species' naturally patchy population structure on inselbergs provides a useful study system for investigations of the genetic consequences of habitat fragmentation. Seeds are dispersed by wind and it is pollinated by hawk moths (*Agrius convolvuli* and *Cenophodes tamsi*) (Kaiser-Bunbury et al. 2011), traits that might render the species less vulnerable to habitat fragmentation.

Our work aims to investigate the ability of the species to survive in fragmented populations through a better understanding of the reproductive ecology and specifically, historic genetic differentiation, contemporary gene flow by pollen dispersal, and genetic diversity among remaining large continuous and small isolated populations.

Here we describe the characterization of 10 microsatellite markers for *G. sericea.* Enriched libraries were established from size selected (300-750bp) genomic DNA ligated into the *Hin*d III site of a pUC19 plasmid and enriched using magnetic bead capture (CPG, Inc., Lincoln Park, New Jersey) with biotin-labelled CA(15), Biotin-GA(15), Biotin-AAC(12) and Biotin-TAGA(8). Microsatellite-containing clones were identified from two di-nucleotide and two tri-nucleotide repeat libraries. Plasmids from 96 positive clones were sequenced and primers designed for microsatellite-containing clones using DesignerPCR version 1.03 (Research Genetics, Inc), of which a subset were tested for polymorphism. The ten most promising loci were optimized. All primers were labeled using an M13-tag at the 5′-end following Schulke (2000) (Table 1). Polymorphism of the ten selected primers were evaluated using 81 adult trees of *G. sericea* samples collected from seven different sites on Mahé.

Leaf genomic DNA was extracted from *G. sericea* (n = 211) using the QIAGEN DNeasy Plant Maxi Kit, following the manufacturer's protocol. PCRs for the M13 primers were carried out in 10μl reactions with 2μl of 1X PCR buffer (Promega colorless Flexi GoTaq

PCR buffer), 15 mM MgCl2, 0.2μM dNTPs, 0.2 μL of the 0.04μM M13 forward primer, 0.8μl of the 0.16μM reward primer and 0.8μl of the 0.16μM M13 primer, 0.025 U *Taq* polymerase (Promega), and 2μL DNA template (*c.* 10 ng). Cycling conditions were as follows: $1\times$ (95 °C for 15 min), $30\times$ (95 °C for 30 sec, primer-specific temperature (56°C) for 45 sec, 72 °C for 45 sec), $8\times$ (95 °C for 30 sec, primer-specific temperature (53°C) for 45 sec, 72 °C for 45 sec), $1\times$ (72 °C for 30 min) (Table 1) carried out in a Bio-Rad Dyad Cycler. We used an ABI3730 for genotyping and Genemapper 3.5 software (Applied Biosystems) for fragment analysis.

The number of alleles and deviations from Hardy–Weinberg equilibrium (HWE) were generated using GenAlEx 6.2 (Peakall and Smouse 2006). The polymorphism information content (PIC), observed and expected heterozygosities was calculated in Cervus 3.0 (Kalinowski et al. 2007). Linkage disequilibrium was implemented in GENEPOP (Raymond and Rousset 1995). All ten loci were polymorphic with 3–12 alleles and a total number of 61 alleles detected over all populations for *G. sericea*. Observed heterozygosity values ranged from 0.24 to 0.78 and expected heterozygosity from 0.27 to 0.82. There was no evidence for scoring error due to stuttering, no evidence for large allele dropout and no evidence for null alleles according to microchecker 2.2.3 (Oosterhout et al. 2004). Significant deviations from Hardy-Weinberg equilibrium (HWE, $P < 0.05$) were detected in loci B109 and C105. No significant linkage disequilibrium was detected after Bonferroni correction suggesting that all 10 loci segregate independently of each other. The results indicate that these 10 primers will provide a valuable tool for evaluating genetic diversity and the reproductive ecology of this rare tree species.

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Table 1: Characteristics of ten polymorphic microsatellite loci in *Glionnetia sericea*. Abbreviations: F: forward primer; R: reverse primer; T_a: annealing temperature; A: mean number of alleles; H_o: observed heterozygosity; H_e: expected heterozygosity; PIC: Polymorphism information content; 81 individuals were analysed for each locus.

Locus	GenBank Accession no.	Primer sequence (5´-3´)	Repeat motif	Size range (bp)	Ta (°C)	A	H_o	H_e	PIC
B6	JN377940	F CCACCCTGGAAAGAAAGTG R GGGACTGTTGCTACTGAAGG	$(AG)_{18}$	239-264	56	11	0.76	0.79	0.76
C104	JN377941	F CAGCCATCAGAACCTTACA R ATAGCCGACCCCACATAG	$(CAA)_8$	131-143	56	3	0.44	0.46	0.38
A104	JN377942	F GCGATTTTGTTCAGGGTC R ATTTTAGCAGACACAGGATGAC	$(CA)_{18}$	161-183	56	6	0.49	0.66	0.61
B110	JN377943	F TCCCTCCTATGAAAATTACTG R TATCCCTTTAGCATTGGAACA	$(AG)_{13}$	219-227	56	5	0.70	0.73	0.68
B102	JN377944	F ATTAGCATCTAACGCACGATA R AGCAAAGCCATTACTTGTAGTC	$(TC)_5(CT)_{14}$	295-315	56	4	0.33	0.39	0.35
C10	JN377945	F ATAGCCGACCCTACATAGTGG R TGATTGCTGGAGTACCTTCTG	$(GTT)_7$	211-217	56	3	0.24	0.28	0.26
C12	JN377946	F TGGTGGAACTACTTGAGCA R TTGCGTTAGGTTGACAGC	$(ACA)_7$	138-148	56	3	0.30	0.27	0.24
A106	JN377947	F CACCAACCAAATTAACAAGAT R GTCAGCACAAATCAATCTATCC	$(CA)_{13}$	194-222	56	12	0.78	0.82	0.80
B109	JN377948	F GCAAAATCAAATCAGGTGAC R GCTCCAAAGAGAGGAAGAAAAG	$(CT)_{18}$	108-130	56	11	0.58	0.82	0.79
C105	JN377949	F CTGTCCTTTCACATTGTTCTG R GCGAAACATCATCCATATAGC	$(CAA)_6$	128-134	56	3	0.28	0.48	0.37

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Damien Doudee and Terence Valentin at Bernica

Damien Doudee and Terence Valentin at Mt. Planneau

Terence Valentin and Chris Kaiser-Bunbury at Copolia

Terence Valentin at Mt. Sebert

Terence Valentin and Aline Finger at L'Abondance

Jaboury Ghazoul, James Mougal and Terence Valentin at Mt. Jasmin

Terence Valentin at L'Abondance

Aline Finger and Chris Kaiser-Bunbury at Casse Dent

Chris Kettle at Mt. Cotton

Chris Kaiser-Bunbury and Chris Kettle at Mt. Jasmin

Steven Azemia and Aline Finger at Mt. Sebert

Pollen collection for cross-pollination

Terence Valentin and Aline Finger at Mt. Jasmin

Pollination bags at Copolia

Hand pollination with brush


Labelled fruits from hand pollination flowers

Aline Finger and Damien Doudee at the Biodiversity Centre on Mahé

Glionnetia sericea (left) and *Medusagyne oppositifolia* (right) seedlings at the Biodiversity Centre

SNAPSHOT FROM THE FIELD
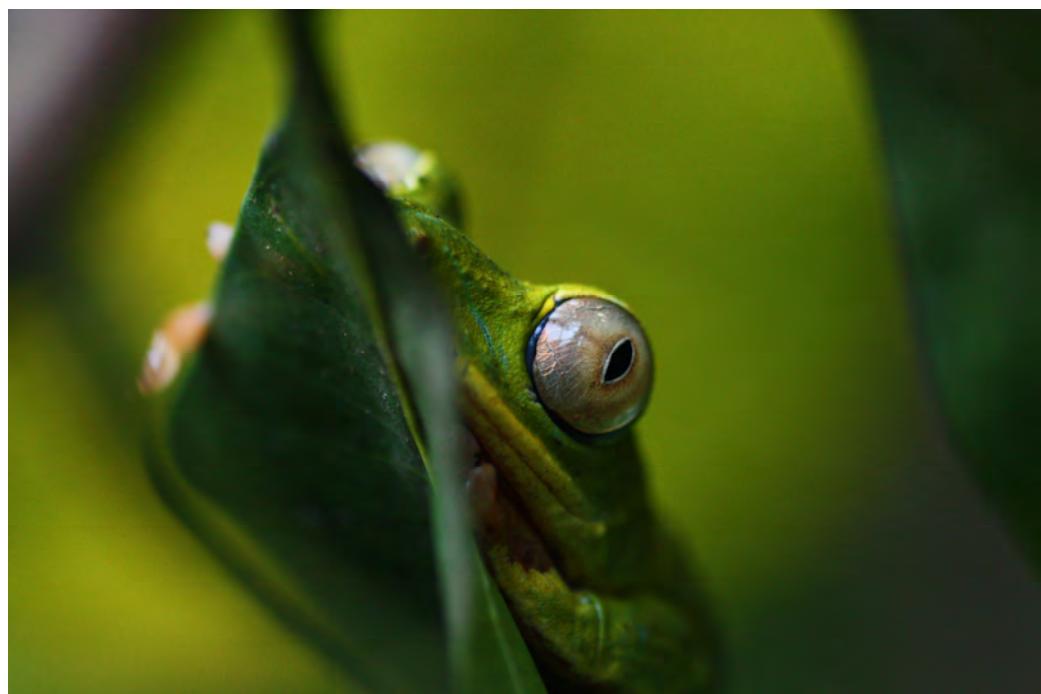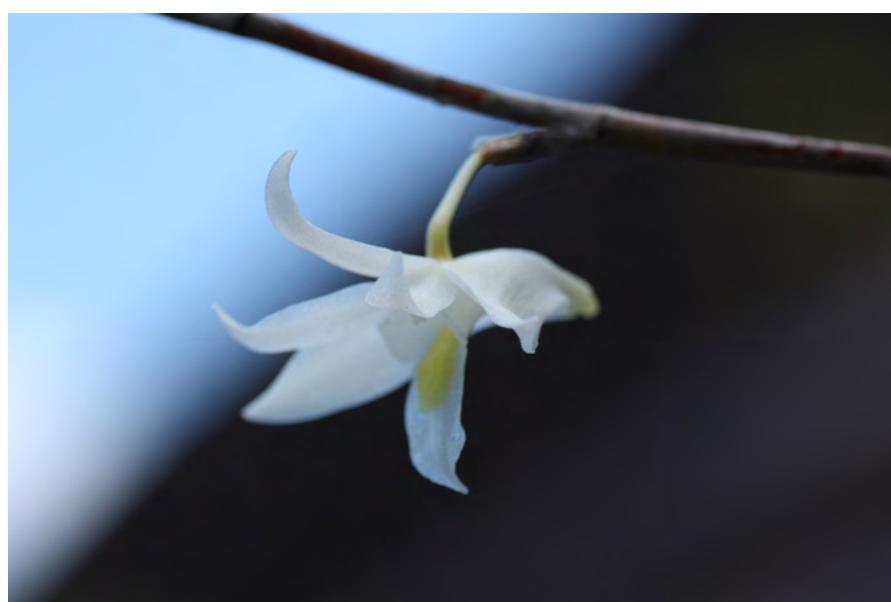
Personal Details

Aline Finger
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Employment Record

Since March 2012

SNF funded post-doctoral fellow at the Genetics and Conservation section of the Royal Botanic Garden Edinburgh, working on *Araucaria* species of New Caledonia

January 2008 – December 2011

PhD student in the group of Ecosystem Management; title of thesis: **Ecological and genetic processes underlying Allee effects among plant populations in the context of divergent population histories**

April 2007 - October 2007

Participation to the project „Natural history of butterflies" at the Musée National d´histoire Naturelle in Luxemburg

Internship

October 2003 - January 2004

School of Biological, Earth & Environmental Sciences, University of New South Wales, **Sydney/Australia: Participation to the project**: Saving wildlife, saving people on our roads

Education

October 2000 - April 2007

University of Trier
Studies of Applied Biogeographie

Major subject: **Biogeographie, Geobotany**
Minor subject: **Chemistry, Geologie**

Graduation: **25th of April 2007**

Diploma thesis: **Conservation genetics of *Lycaena helle*, High genetic diversity and differentiation of an endangered boreal relict species in Central Europe**

Skills

Programs:
- MS Office
- Most population genetic programs
- Basic knowledge of R
- Basic knowledge of ArcGIS

Languages:
- German (native speaker)
- Good command of english
- Fluent in french

External Fundraising

2011: SNF grant for a post-doctoral fellowship for prospective researchers over **42.800CHF** for a period of 18 months

2005: Stipend from the Ministerium für Umwelt, Forsten und Verbraucherschutz Rheinland-Pfalz of **1500€** as financial support for field work during the diploma thesis

PhD Courses

- Methods for statistical analysis of siring success and relatedness in plants and animals
- An introduction to data analysis for ecologists using R
- Writing a post-doctoral grant
- Field course at Beinn Eighe National Reserve, Scotland
- Ecology of Alpine Plants

Reviewer of the journals: Biotropica, Biological Conservation, International Journal of Molecular Sciences, Conservation Genetics

LIST OF PUBLICATIONS

In preparation

Finger, A., Kettle, C.J., Kaiser-Bunbury, C.N., Valentin, T. & Ghazoul, J. (in prep.) Genetic connectivity in the moth pollinated tree *Glionnetia sericea* (Rubiaceae) in a highly fragmented habitat.

Finger, A., Kettle, C.J., Kaiser-Bunbury, C.N. & Ghazoul, J. (in prep.) Vulnerability of tree species to the genetic consequences of habitat fragmentation: the importance of divergent population histories.

2012

Finger, A., Kettle, C.J., Kaiser-Bunbury, C.N., Valentin, T., Mougal, J. & Ghazoul, J. (2012) Forest fragmentation genetics in a formerly widespread island endemic tree: *Vateriopsis seychellarum* (Dipterocarpaceae). *Molecular Ecology* **21**: 2369-2382. doi: 10.1111/j.1365-294X.2012.05543.

2011

Finger, A., Kaiser-Bunbury, C.N., Kettle, C.J. (2011) Development of polymorphic microsatellite markers of the Seychelles endemic tree *Glionnetia sericea* (Rubiaceae). *Conservation Genetics Recourses*. doi: 10.1007/s12686-011-9515-3

Finger, A., Kettle, C.J., Kaiser-Bunbury, C.N., Valentin, T., Doudee, D., Matatiken, D. & Ghazoul, J. (2011) Back from the brink: barriers to reproduction and the potential for genetic rescue in the jellyfish tree. *Molecular Ecology* **20**: 3773–3784. doi: 10.1111/j.1365-294X.2011.05228.x

2010

Habel, J. C., Schmitt, T., Meyer, M., **Finger, A.**, Rödder, D., Assmann, T. & Zachos, F. E. (2010) Biogeography meets conservation: the genetic structure of the endangered lycaenid butterfly *Lycaena helle* (Denis & Schiffermüller, 1775). *Biological Journal of the Linnean Society* **101**: 155–168

Habel, J.C., **Finger, A.**, Schmitt, Th. & Nève, G. (2010) Survival of the endangered butterfly *Lycaena helle* in a fragmented environment: Genetic analyses over 15 years. *Journal of Zoological Systematics and Evolutionary Research* **49** (1): 25-31. doi: 10.1111/j.1439-0469.2010.00575.x.

Finger A., Ismail S., Ghazoul J. & Kettle C.J. (2010) Development of polymorphic microsatellite markers of the endangered and endemic *Vateriopsis seychellarum* (Dipterocarpaceae), a relict canopy tree of the Seychelles. *Conservation Genetic Resources* **2**, Supplement 1: 309-311 Doi: 10.1007/s12686-010-9213-6

Finger, A. & Klank, C. (2010) Molecular methods – Blessing or curse? In: J.C. Habel & T. Assmann (Eds) *Relict Species - Phylogeography and Conservation Biology*. Springer Heidelberg, p.309-320.

Finger, A., Ghazoul, J., Todd, M., Kaiser-Bunbury C. & Kettle, C.J. (2010) Development of thirteen polymorphic microsatellite markers for the Seychelles endangered and endemic jellyfish tree *Medusagyne oppositifolia* (Medusagynaceae). *Conservation genetic resources* **2**: 173–175. doi 10.1007/s12686-009-9120-x.

2009

Finger, A., Schmitt, T., Meyer, M., Assmann, T., Zachos, F.E. & J.C. Habel (2009) The genetic status of the Violet Copper *Lycaena helle*, a relict of the cold past in times of global warming. *Ecography* **32**: 382-390

Habel, J.C., **Finger, A.**, Meyer, M., Louy, D., Zachos, F., Assmann, T. & T. Schmitt (2009) Unprecedented long-term genetic monomorphism in an endangered relict butterfly species. *Conservation Genetics* **10**: 1659-1665

2008

Habel, J., **Finger, A.**, Meyer M. & Assmann, T. (2008) Polymorphic microsatellite loci in the endangered butterfly *Lycaena helle* (Lepidoptera: Lycaenidae). *European Journal of Entomology* **105**(2): 361-362

Schmitt, T., Augenstein, B. & **Finger, A.** (2008) The influence of changes in viticulture management on the butterfly (Lepidoptera) diversity in a wine growing region of southwestern Germany. *European Journal of Entomology* **105**: 249-255

2007

Wipking, W., **Finger, A.** & Meyer, M. (2007) Habitatbindung und Bestandssituation des Blauschillernden Feuerfalters *Lycaena helle* (Denis & Schiffermüller) in Luxemburg (Lepidoptera, Lycaenidae). *Bull. Soc. Nat. Luxemb.* **108**: 81-87.

Oral presentations

"Island endemics close to extinction: Conservation on the cutting edge" at the GPPC conference in St. Louis, Missouri, USA (July 05 - 07, 2011).

„Conservation genetics of the Jellyfish tree, a Seychelles flagship species", at the GTÖ - Society of Tropical Ecology in Frankfurt, Germany (February 21 - 24, 2011).

„Conservation genetics of the Jellyfish tree, a Seychelles flagship species", at 54th Annual Meeting of the Ecological Genetics Group at the University of Stirling (April, 06 – 08, 2010). Student prize winner for the best oral presentation.