

# 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.

**Keywords:** artificial cross-pollinations, genetic rescue, microsatellites, *Medusagyne oppositifolia*, seychelles, spatial genetic structure

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## 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 (Charlesworth & Charlesworth 1999; Leimu *et al.* 2010; Angeloni *et al.* 2011). 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

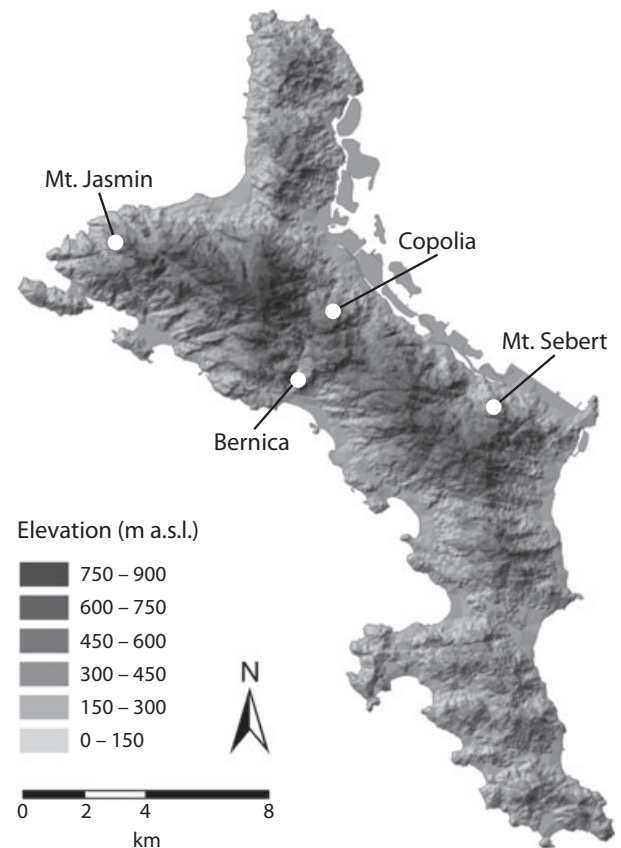
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show a wide range of responses to forest fragmentation (Kramer *et al.* 2008; Bacles & Jump 2011), with species vulnerabilities depending on several reproductive and demographic traits (Ghazoul 2005). For example, tree species that have limited gene 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 (Tallmon *et al.* 2004; Pimm *et al.* 2006). 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 (Richards 2000; Newman & Tallmon 2001; Tufto 2001; Bossuyt 2007; 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



**Fig. 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.

consequences associated with small population size and population isolation. Furthermore, 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 realized 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 realized 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 (Fay *et al.* 1997; Bremer *et al.* 2009). 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, unpublished master thesis). The naturally patchy distribution of *M. oppositifolia* reflects the distribution of inselberg habitats as 'islands' within more extensive forest (Matatiken 2006, unpublished master thesis). 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, personal communication).

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 (480 m a.s.l.), three on Copolia (460 m 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, unpublished master thesis) 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, unpublished 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, unpublished master thesis).

### *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 10 (B107, C107, D12, D118, D6, B112, A9, B8a, A4, A7) nuclear microsatellite loci, details of which are described in Finger (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 10 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 GenAIEx 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. As 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  $\Delta K$  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  $\Delta K = (|mL(K+1) - 2 mL(K) + mL(K-1)|) / \text{SDL}(K)$ , 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*

Spatial genetic structure within the large population (Bernica) was assessed using a Mantel test and 10 000 permutations using SPAGED1 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 (Fine-scale Spatial Genetic Structure),  $F$  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 realized 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 (Marshall *et al.* 1998; Kalinowski *et al.* 2007). Simulations of paternity were run using the allele frequencies of all adult reproductive trees and the following settings: 10 000 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% for proportion of loci geno-

typed. The proportion of candidate parents sampled was set at 99%, 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 (Dow & Ashley 1998; Bacles *et al.* 2006; Nakanishi *et al.* 2009), based on the assumption that seed dispersal is lower 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: (i) Selfing, using pollen sourced from flowers of the same tree; (ii) Within-population crosses using pollen sourced from another tree at Mt. Sebert; (iii) 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 two 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 unfertilized 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). Owing 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 within- and between-population crosses without replacement and repeated the sampling 10 000

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,  $P = 0.316$ ).  $H_E$  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).

### *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).

### *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

**Table 1** Genetic variability of 10 microsatellite loci estimated for all populations of *Medusagyne oppositifolia*

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

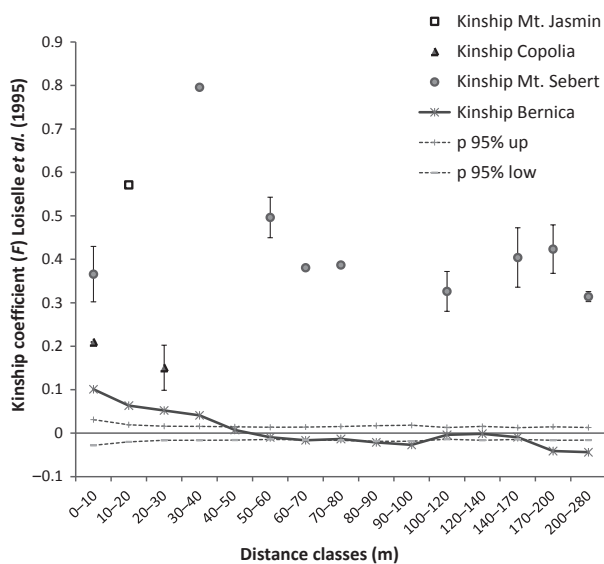
n: number of genotyped individuals;  $N_A$ : mean number of alleles;  $N_E$ : effective number of alleles;  $H_O$ : observed heterozygosity;  $H_E$ : expected heterozygosity;  $R_S$ : allelic richness, based on two diploid individuals;  $P_A$ : total number of private alleles (mean frequency);  $F_{IS}$ : Inbreeding coefficient;  $\pm$  SE. Analysis of variance for  $N_E$ ,  $H_O$ ,  $H_E$  and  $R_S$ :

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

**Table 2** Pairwise  $F_{ST}$  values between populations

	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

\* $P < 0.05$ .



**Fig. 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 80 m. The solid line represents the average  $F$  values. The dotted lines represent the 95% (two-tailed) CI of the average  $F$  distribution calculated from 10 000 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.

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 ( $F = 0.004 \pm 0.002$  SE). In Copolia average individual kinship coefficient was higher ( $F = 0.17 \pm 0.047$  SE) across three pairs, and even higher values were found in the Mt. Sebert ( $F = 0.41 \pm 0.029$  SE; 21 pairs) and Mt. Jasmin ( $F = 0.57$ ; 1 pair) populations (Fig. 2).

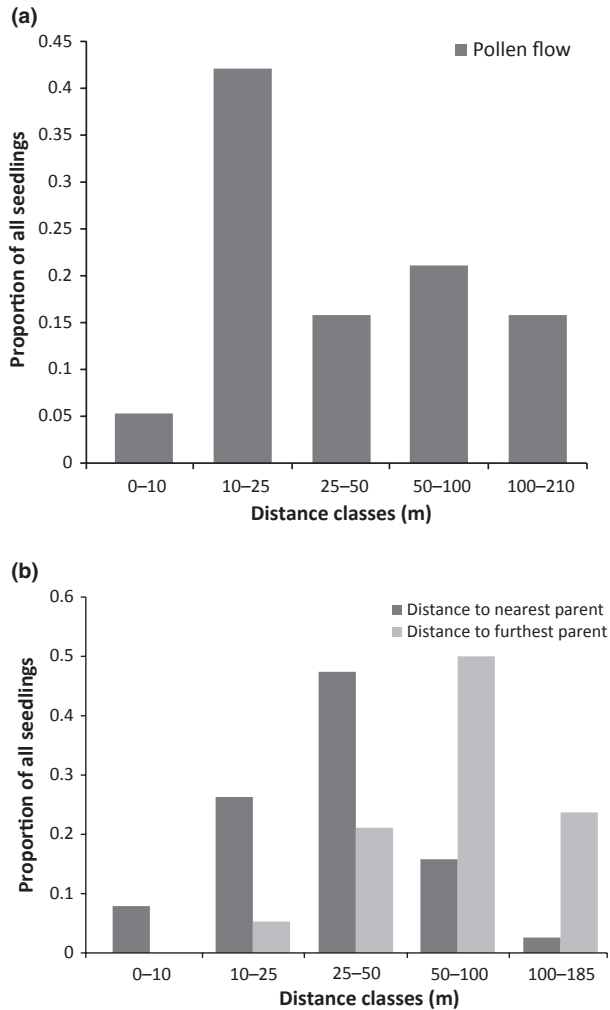
### Contemporary pollen and seed dispersal

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

### Genetic rescue using pollination experiments

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

Seed set values obtained from between-population crosses ( $0.37 \pm 0.02$  SE) and within-population crosses



**Fig. 3** (a) Frequency distributions of realized 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 mother tree, in light grey the scenario that the furthest parent tree is the mother tree.

( $0.32 \pm 0.02$  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 ( $0.003 \pm 0.001$  SE and  $0.01 \pm 0.01$  SE, respectively).

Viable seeds (as a proportion of developed seeds) was highest from between-population crosses ( $0.77 \pm 0.03$  SE) and significantly greater than within-popula-

tion crosses ( $0.52 \pm 0.03$  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 ( $0.03 \pm 0.01$  SE and  $0.07 \pm 0.01$  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 (four 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  $190 \pm 18.5$  SE for between-population crosses,  $155 \pm 14.8$  SE for within-population crosses and  $101 \pm 60.1$  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 (Dutech *et al.* 2004; Ueno *et al.* 2005; Kettle *et al.* 2007; Born *et al.* 2008; Sebbenn *et al.* 2011). 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 km. 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 realized gene flow link remnant populations?*

The parentage analysis of wild seedlings showed that realized 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 (2–200 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  $\pm$  18.5 SE) than within-population crosses (155.5 days  $\pm$  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 realized 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, unpublished 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 emphasizes 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; Eckert *et al.* 2010; Collevatti & Hay 2011) 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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### Data accessibility

DNA sequences: Genbank accessions GU045577-GU045569. Sample locations and microsatellite data: DRYAD entry doi:10.5061/dryad.67529.

### Supporting information

Additional supporting information may be found in the online version of this article.

**Fig. 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).

**Table S1** Summary table of results of pollination experiments. Fruit set, seed set and survival rates are given in mean proportions  $\pm$  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: \* =  $P < 0.05$ ; \*\*\* =  $P < 0.001$ .

**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.