Supplementary Material

Methods

Library construction

Genomic DNA was extracted from 40 mg of homogenized, freeze dried material of a single individual following the method of Doyle and Doyle (1987), with addition of PVP-40T polyvinyl pyrrolidine to the extraction buffer and two chloroform extraction steps. Genomic DNA was 454 shotgun sequenced using 1\8 of a picotiter plate on a GS-FLX Titanium machine (454 Life Sciences, Connecticut, USA) by the Australian Genome Research Facility (Adelaide, Australia). The program QDD v. 2 (Meglecz et al. 2010) was used to screen the raw sequences with ≥ eight di-, tetra- or penta-base repeats, remove redundant sequences, and design primers (automated in QDD using Primer3, [Rozen and Skaletsky 2000]) for loci with PCR product lengths of 80-480 base pairs.

PCR optimization

Initial screening of 24 selected microsatellite primers was performed using a CFX96 Touch Real-Time PCR Detection System (Bio Rad Laboratories, Hercules, USA), 5 µl SsOAdvanced Universal SYBR Green supermix (Bio Rad Laboratories, Hercules, USA), 0.2 µM each forward and reverse primers, 5–10 ng genomic DNA in 10 µl reaction volume. Initially, screening included reliable amplification of a single sample across a range of temperatures (55 - 65°C) using a Veriti thermocycler (Thermo Fisher Scientific, Waltham, USA) to determine the most appropriate annealing temperature, followed by evidence of polymorphism among eight randomly selected individuals, as visualized using an ABI 3500 (Thermo Fisher Scientific Waltham, USA) sequencer. Twelve of the 24 tested primer pairs

produced clear and variable loci (Ma01, Ma02, Ma06, Ma07, Ma08, Ma09, Ma12, Ma13, Ma15, MA20, Ma21, Ma24, Table 1).

DNA extraction and genotyping

Seed were germinated on agar at 20°C, with a 12 hr day/night cycle and DNA extracted when cotyledons had emerged. Genomic DNA was extracted from all dried leaf material and seedling tissue using a Carlson method (Carlson et al. 1991) with modifications outlined by Anthony et al., (2016).

Forward primers were fluorescently labelled (G5 label set, FAM, VIC, NED or PET) and microsatellite regions were amplified for all individuals by PCR within three multiplex mixes containing 5.1µl of 2x Multimix and 1.9µl of 5x Q sol (Type-It Microsatellite PCR kit; Qiagen, Hinden, Germany), 1.0l of primer mix and 2.0µl of 5-10ng DNA in a 10µl reaction. Primer Mix (PM) 1 contained the primers *Ma08*, *Ma09*, *Ma15*; PM2 contained *Ma06*, *Ma07*, *Ma13*, *Ma20*, *Ma21*; PM3 contained *Ma01*, *Ma12*, using the following PCR conditions, an initial one minute denaturation at 95^{o}C, 35 cycles of 94^{o}C for 10 sec, 64^{o}C (PM1 and PM2) or 60^{o}C (PM3) for 30 seconds and 72^{o}C for 45 sec followed by a final extension of 15 min at 72^{o}C. Loci *Ma01* and *Ma12* were pooled with PM3 for electrophoresis and amplified using the following conditions; 2 µl 5X polymerase buffer containing dNTPs' (Fisher Biotec, Perth, Australia), 2 mM $MgCl_2$, 0.16 µM each primer and 0.5 U *Taq* polymerase (Fisher Biotec, Perth, Australia) in a 10 µl reaction volume and PCR conditions were an initial two minutes of denaturation at 94^{o}C; 35 cycles 94^{o}C for 30 sec, 56^{o}C for 30 s and72^{o}C for 30 sec, with a final extension of 30 min at 72^{o}C. One µl of PCR product was added to 9 µl of HiDi formamide with GeneScan™ LIZ®500(-250) size Standard (Applied Biosystems, Waltham, USA), and electrophoresis was performed using the ABI 3500 sequencer, and allele sizes were determined using Geneious V 7.1 (Biomatters, Auckland, New Zealand). Multiple

replicate samples were included to ensure the accuracy of the final dataset. Samples that did not amplify well were re-amplified at least once. All maternal trees and seedlings were genotyped in this way. Maternal trees were genotyped at all 12 loci, while seedling progeny were genotyped with a subset of eight of the most reliable loci consisting of Ma06, Ma07, Ma08, Ma09, Ma13, Ma15, Ma20 and Ma21. These eight loci were considered more than adequate for the estimation of various mating system parameters, based on the sample size of at least 200 seedling progeny per site (Ritland and Leblanc 2004).

References

Anthony, J., R. Allcock and S. Krauss. 2016. Isolation and characterisation of 13 microsatellites for the rare endemic shrub *Tetratheca erubescens* (Elaeocarpacaeae). *Applications in Plant Science* 42: apps.1500102. doi:10.3732/apps.1500102.

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Rozen, S. and H.J. Skaletsky. 2000. Primer3 on the WWW for general users and for biologist programmers. Pages 365-386 in S. Krawetz and S. Misene (eds), *Bioinformatics Methods and Protocols: Methods in Molecular Biology*. Totowa, NJ: Humana Press.

Table S1. Potential null allele frequencies for alleles at eight loci as identified in adult individuals of eight populations of *Melaleuca acuminata*. Brookfield Estimator 1 frequencies are reported.

Locus	Population							
	Chingarrup remnant	**Chingarrup restoration**	**Chereninup remnant**	**Chereninup restoration**	**Peniup remnant**	**Peniup restoration**	**Monjebup North remnant**	**Monjebup North restoration**
Ma02					0.1171			
Ma06					0.0975			0.1606
Ma12	0.2650	0.4079	0.4653	0.1090	0.3153	0.3798	0.3189	0.3277
Ma13	0.1014	0.1467	0.2103		0.1507			
Ma15								0.1385
Ma20	0.1160		0.2773	0.2928	0.1593	0.1942		0.3171
Ma21	0.2115			0.1199	0.2079	0.1692	0.2542	0.0975
Ma24	0.2475	0.1867	0.2129	0.1598	0.1615	0.1970	0.1540	0.2585

Table S2. Statistical parameters for comparisons of genetic diversity estimates among sites and among treatments (restored versus remnant) for adult cohorts of eight populations and progeny cohorts of six populations of *Melaleuca acuminata*. Estimates include the mean number of alleles per locus (Na), the number of effective alleles (Ne), the proportion of polymorphic loci (P), expected (He) and observed (Ho) heterozygosity and the Fixation index (F_{IS}).

	Na	**Ne**	**P**	**He**	**Ho**	**F_{IS}**
Adults						
Among sites						
Df	3.00000	3.00000	3.00000	3.00000	3.00000	3.00000
F	0.414891	1.195522	0.999201	5.67625	5.918272	1.231319
p-value	0.755557	0.443381	0.500255	0.093864	0.089137	0.434121
F critical	9.276628	9.276628	9.276628	9.276628	9.276628	9.276628
Among treatments						
Df	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000
F	0.022784	1.515165	1.00000	0.802593	9.25924	0.206732
p-value	0.889598	0.306057	0.391002	0.436334	0.055739	0.680225
F critical	10.12796	10.12796	10.12796	10.12796	10.12796	10.12796
Progeny						
Among sites						
df	2.00000	2.00000	2.00000	2.00000	2.00000	2.00000
F	2.102924	5.102847	1.00000	0.048499	2.634096	0.356905
p-value	0.322277	0.163858	0.50000	0.953744	0.275172	0.736971
F critical	19.00000	19.00000	19.00000	19.00000	19.00000	19.00000
Among treatments						
df	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000
F	0.043459	0.1613112	1.00000	0.035632	1.405405	1.815445
p-value	0.854167	0.725398	0.42265	0.867697	0.357584	0.310207
F critical	18.51282	18.51282	18.51282	18.51282	18.51282	18.51282

Table S3. Statistical parameters for comparisons of mating system estimates among sites and among treatments (restored versus remnant) for six populations of *Melaleuca acuminata*. Estimates include the multi locus outcrossing rate (tm), the single locus outcrossing rate (ts), the apparent level of selfing due to biparental inbreeding (tm - ts), the correlation of selfing among maternal plants (rs) and the multi locus correlated paternity (rp).

	tm	**ts**	**tm-ts**	**rs**	**rp**
Among sites					
df	2.00000	2.00000	2.00000	2.00000	2.00000
F	4.041986	0.456413	0.941867	1.10484	8.959799
p-value	0.198335	0.686618	0.514968	0.497393	0.100404
F critical	19.00000	19.00000	19.00000	19.00000	19.00000
Among treatments					
df	1.00000	1.00000	1.00000	1.00000	1.00000
F	0.714022	1.821801	2.2826222	1.985739	2.432161
p-value	0.487081	0.309575	0.234757	0.294159	0.259221
F critical	18.51282	18.51282	18.51282	18.51282	18.51282

Table S4. Statistical parameters for comparisons of capsule and seed analysis among sites and among treatments for six populations of *Melaleuca acuminata*.

	Capsule width	**Capsule height**	**Capsule volume**	**Number of seed**	**Seed viability**
Among sites					
df	2.0000	2.0000	2.0000	2.0000	2.0000
F	1.4400	0.3300	0.7600	1.0100	10.6300
p-value	0.4098	0.7545	0.5690	0.4965	0.0860
Among treatments					
df	1.0000	1.0000	1.0000	1.0000	1.0000
F	2.7500	1.4400	2.0100	0.0900	17.3200
p-value	0.2392	0.3526	0.2918	0.7956	0.0532

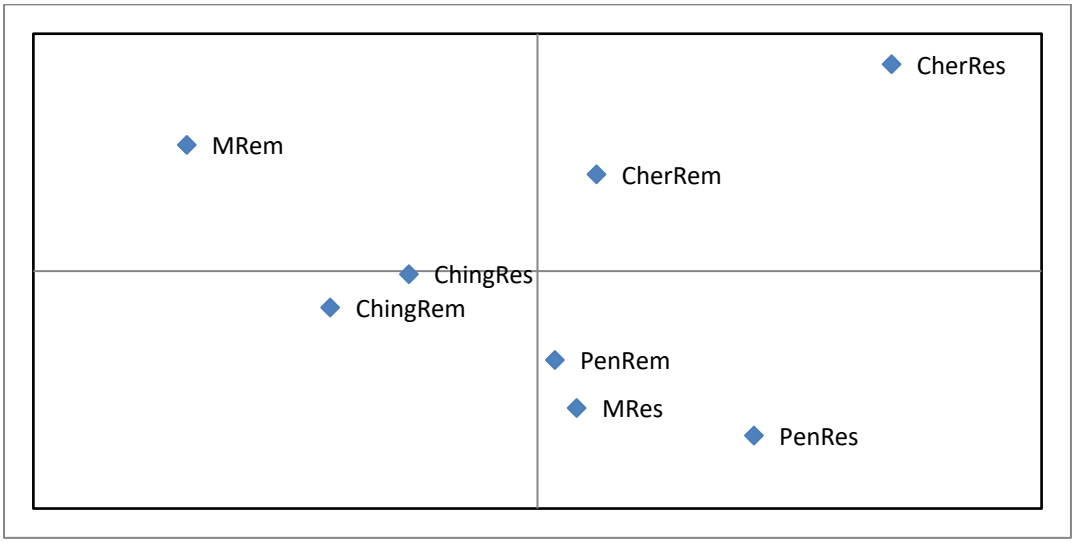

Figure S1. Principal Coordinates Analysis of pairwise F_{ST} values for eight populations of *Melaleuca acuminata*. Populations are ChingRem; Chingarrup remnant, ChingRes; Chingarrup Restoration, CherRem; Chereninup remnant, CherRes; Chereninup restoration, PenRem; Peniup remnant, Pen Res; Peniup restoration, MRem; Monjebup North remnant, MRes; Monjebup North restoration.