Are current ecological restoration practices capturing natural levels of genetic diversity? A New Zealand case study using AFLP and ISSR data from mahoe (*Melicytus ramiflorus*)

Mark I. Stevens[1,2†], Andrew C. Clarke[3,5†], Fiona M. Clarkson[4], Mary Goshorn[4] and Chrissen E.C. Gemmill[4*†]

[1]School of Pharmacy and Medical Sciences, University of South Australia, SA 5000, Adelaide, Australia
[2]South Australian Museum, North Terrace, GPO Box 234, SA 5000, Adelaide, Australia
[3]Allan Wilson Centre for Molecular Ecology and Evolution, Institute of Molecular BioSciences, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand
[4]School of Science, University of Waikato, Private Bag 3105, Hamilton 3240, New Zealand
[5]Present address: McDonald Institute for Archaeological Research, University of Cambridge, Downing Street, Cambridge, CB2 3ER, United Kingdom

*Author for correspondence (Email: Gemmill@waikato.ac.nz)

[†]These authors contributed equally

Published online: 26 March 2015

Abstract: Sourcing plant species of local provenance (eco-sourcing) has become standard practice in plant-community restoration projects. Along with established ecological restoration practices, knowledge of genetic variation in existing and restored forest fragments is important for ensuring the maintenance of natural levels of genetic variation and connectivity (gene flow) among populations. The application of restoration genetics often employs anonymous 'fingerprinting' markers in combination with limited sample sizes due to financial constraints. Here, we used two such marker systems, AFLPs (amplified fragment length polymorphism) and ISSRs (inter-simple sequence repeats), to estimate population-level genetic variation of a frequently used species in restoration projects in New Zealand, mahoe (*Melicytus ramiflorus,* Violaceae). We examined two rural and two urban forest fragments, as potential local source populations, to determine whether the māhoe population at the recently (re)constructed ecosystem at Waiwhakareke Natural Heritage Park, Hamilton, New Zealand, reflects the genetic variation observed in these four potential source populations. Both marker systems produced similar results and indicated that, even with small population sizes, levels of genetic variation at the heritage park were comparable with in situ populations. However, the AFLPs did provide finer resolution of the population genetic structure than ISSRs. ISSRs, which are less expensive and technically less demanding to generate than AFLPs, may be sufficient for restoration projects where only a broad level of genotypic resolution is required. We recommend the use of AFLPs when species with a high conservation status are being used, due to the greater resolution of this technique.

Keywords: eco-sourcing; genetic structure; native plants; provenance; restoration.

Introduction

Restoration of ecologically functioning plant communities is becoming increasingly sophisticated, with practices to facilitate the maintenance of biodiversity at multiple levels, from genes and species to entire ecosystems (Lesica & Allendorf 1999; Kraus & Koch 2004; McKay et al. 2005). Knowledge of the local landscape, both historical and ecological, is often used to determine the optimal composition of plant species to be used in the restoration, along with specific planting practices, such as using a sequence of planting that mimics ecological successional stages (e.g. Janzen 1985; Hobbs & Norton 1996; Higgs 1997; Malaval et al. 2010; see also Choi 2007).

In 1989 Roger MacGibbon and colleagues of the Taupo Native Plant Nursery coined the term 'eco-sourcing' as a means 'to describe the concept of genetic integrity and the importance of local genetic provenances' (R. MacGibbon pers. comm.); eco-sourcing is now popularised within New Zealand. The use of locally collected, eco-sourced seed and plants is becoming standard practice in a range of restoration projects (e.g. McKay et al. 2005; Ramp Neale et al. 2008; Sinclair & Hobbs 2009; Malaval et al. 2010; Overdyck et al. 2013). Although most eco-sourcing practitioners argue that collecting seed locally from similar ecological microhabitats is an important criterion (Janzen 1985; Higgs 1997; Moore 2000; Jones et al. 2001; Wilkinson 2001; but see Sackville Hamilton 2001), questions remain regarding the spatial extent of what is meant by 'local', from both ecological and genetic perspectives. This general problem exists due to numerous factors, including what spatial scales are important for the species involved, lack of standard eco-sourcing guidelines, logistical constraints of procuring eco-sourced materials, nursery practices, and the extent of natural linkages (gene flow) between restored and existing fragmented populations.

Use of locally adapted ecotypes from local populations potentially conflicts with the aim to maximise genetic diversity. This is increasingly important, given the fragmentation of forests combined with the decline of native pollinators and seed dispersers (Williams et al. 2014). For example, some eco-sourcing practices may limit genetic variation in restored fragments especially when a low number of highly fecund individuals or populations are targeted for seed collections. If seeds are collected from a single productive 'mother' plant, seedlings will be closely related, from full- to half-siblings,

creating a population with limited genetic variation (due to inbreeding), which does not bode well for the long-term persistence of the restored population (Jamieson et al. 2008).

Evolutionary theory predicts that higher levels of genetic diversity help ensure species' abilities to respond to changing selective pressures (evolutionary 'potential') (e.g. Harrisson et al. 2014), and therefore increase the likelihood of long-term persistence of the restored populations. Thus, for the long-term viability of restored populations, genetic variation should reflect levels found in local natural populations. Therefore, even a basic understanding of the myriad factors that shape population-level variation is likely to enhance the scope and success of any restoration project. However, little has been done to test these theories. By providing quantitative measures of genetic diversity within and among local source populations these data can be used to guide the source of restored populations. Only then can one objectively assess how population genetic structure is correlated with provenance, including geographic location and ecological circumstance (Moore 2000; Sackville Hamilton 2001; McKay et al. 2005; Malaval et al. 2010, Mijangos et al. 2015).

Here, we assessed the genetic variation of *Melicytus ramiflorus* J.R.Forst. & G.Forst. (Violaceae; Fig. 1), commonly known as māhoe, which is found in Waikato forest fragments and regularly used in North Island New Zealand restoration projects. Population genetic analyses were performed using two DNA fingerprinting techniques commonly applied to ecosystem restoration (see Mijangos et al. (2015) for review), amplified fragment length polymorphism (AFLP; Vos et al. 1995) and inter-simple sequence repeats (ISSR; Zietkiewicz et al. 1994). Eco-sourced māhoe from the restored ecosystem at Waiwhakareke Natural Heritage Park (WNHP), Hamilton, New Zealand (Fig. 1), was compared with māhoe from two urban and two rural forest fragments often used for seed collection by eco-sourcers. Our aim was to determine whether the māhoe population at WNHP reflects the genetic variation observed in these four potential source populations.

Materials and methods

Study species and site selection

Māhoe was first described by Forster and Forster in 1776, hence is one of the first species of New Zealand's flora to be described. Common names also include hinahina, inaina, inihina, moeahu and whiteywood (Breitwieser et al. 2010), the latter referring to its pale bark. Māhoe is a dioecious tree species, reaching to 10 m, with small, nectariferous, sexually dimorphic flowers (Fig. 1d, e). Flowers are produced over the late austral spring and summer in a number of pulses, with fruits produced mainly in late summer and autumn (Powlesland et al. 1985). Pollination

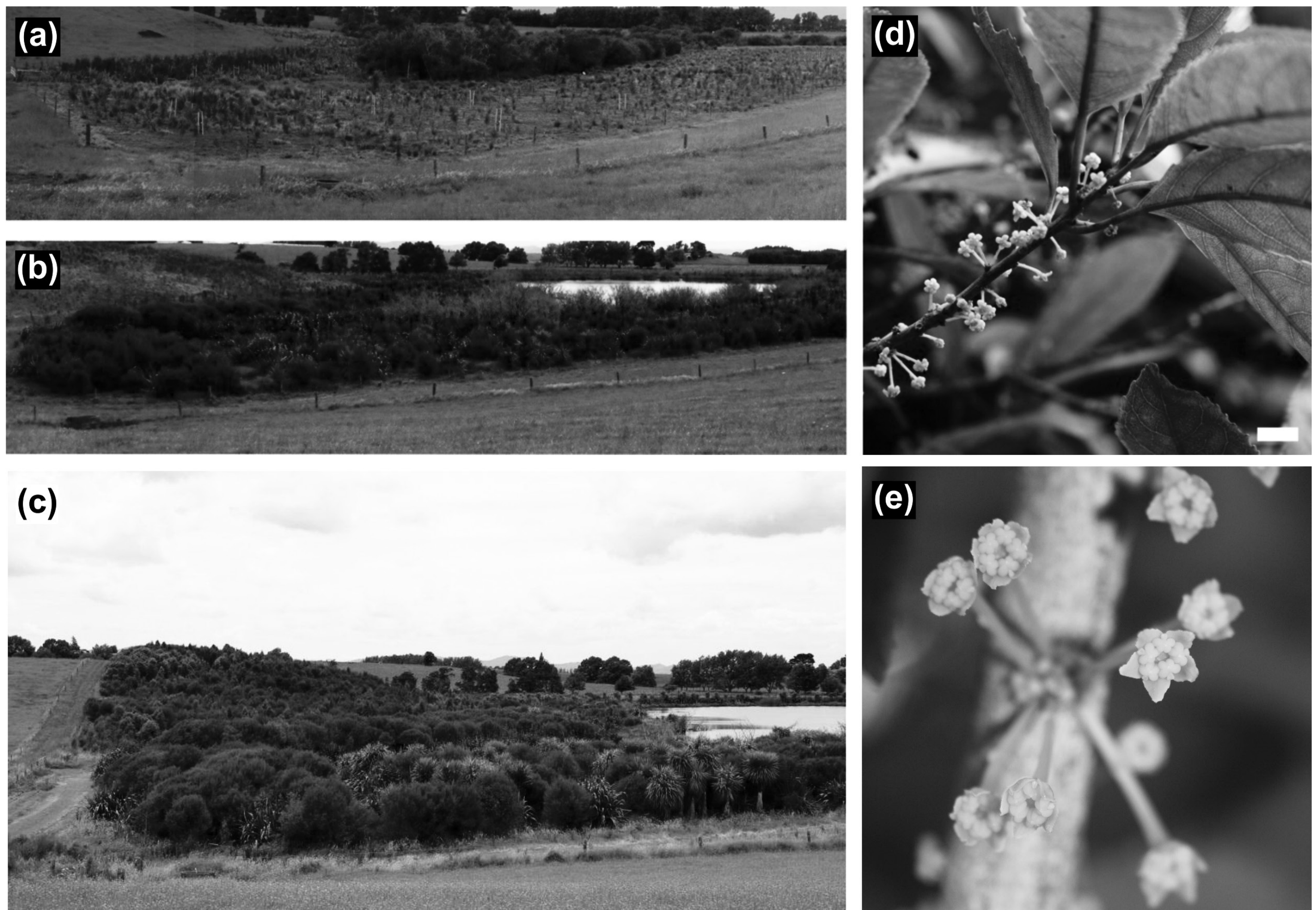

Figure 1. Waiwhakareke Natural Heritage Park showing revegetation progress from 2005 (a) to 2009 (b) and 2012 (c). Photos by Elizabeth Overdyck, Toni Cornes, and Catherine Kirby respectively. (d,e) Māhoe (*Melicytus ramiflorus*; Violaceae) is a small, dioecious tree growing to 10 m, with the small insect-pollinated flowers borne directly on the branches. Scale bar = approx. 1 cm.

of the unspecialised māhoe flowers occurs by a diversity of insects (Powlesland 1984). The numerous small purple berries contain 3–10 seeds (Kirk 1899) and are dispersed by birds, including native kererū (*Hemiphaga novaeseelandiae*), bellbirds (*Anthornis melanura*), silvereye (*Zosterops lateralis*) and tūī (*Prosthemadera novaeseelandiae*) (Powlesland et al. 1985; Burrows 1995; Kelly et al. 2010). The seeds exhibit autoinhibition and germinate once a brown exudate is rinsed away sufficiently by rain (Partridge & Wilson 1990; but see Herron & Clemens 2001). Hence, māhoe is outcrossing and gene flow occurs at two spatial scales with pollen locally, and fruits and seeds over greater distances. Chromosome studies report 2n = 32 (Beuzenberg & Hair 1959; Beuzenberg 1961). On the basis of their phylogenetic analyses, Mitchell et al. (2009) suggested that dioecious *Melicytus* species are the result of ancient polyploidisation (palaeodiploid with 2n = 16) and should actually be considered as tetraploids. Lyttle et al. (2011) report that apomictic races may exist; however, more rigorous study is required to confirm this.

We selected four intact forest fragments within the Waikato Ecological District from which to collect material for DNA analyses in addition to the restoration site, WNHP. Two collection sites were urban native forest fragments within Hamilton City (Claudelands Bush and Hammond Bush) and two were rural sites situated in countryside to the north of the city (Pukemokemoke Bush Reserve and Waingaro Forest) (Fig. 2). Site choice was based on knowledge of eco-sourcing practices used to collect local seeds for the WNHP restoration project (see Clarkson & McQueen 2004) and completeness of site historical information.

Waiwhakareke Natural Heritage Park is a 60-ha site situated within the urban landscape of Hamilton City that has been under ongoing native ecosystem restoration from agricultural pasture since 2004, and is the largest inland restoration project in New Zealand. Five different native ecosystems are being assembled to represent most of the original local species diversity (http://www.waiwhakareke.co.nz). The five ecosystems are based on the different topographical units within the park and the restoration plan is guided by fundamental ecological research on natural succession and assembly rules (Clarkson & McQueen 2004; Grove et al. 2006; Clarkson et al. 2007b). The restoration plantings at WNHP were all provided by the Hamilton City Council nursery and were grown from seed sourced from within and around Hamilton City using typical eco-sourcing practices. The plants used at WNHP were produced via eco-sourcing; however, as is often the case, no records had been maintained about the number or location of source populations, the number of individuals from which seed was collected, or when the seed collections were made.

Claudelands Bush (CB) is the largest urban forest fragment in Hamilton (5.2 ha) and consists of semi-swamp forest located on a floodplain that was once part of an extensive (324 ha) tract of native forest (Gudex 1955). Logging in 1864 removed most of the podocarps except for some kahikatea (*Dacrycarpus dacrydioides*) (Whaley et al. 1997). Hammond Bush (HB) is a 1-ha forest remnant, and is one of only two surviving examples of mixed tawa/tītoki (*Beilschmiedia tawa*/*Alectryon excelsa*)–pukatea/swamp maire (*Laurelia novae-zelandiae*/*Syzygium maire*) forest types within the Hamilton basin (de Lange 1996). Both of the urban populations have varying proportions of native and introduced species, and restoration efforts to date include ongoing native planting and weed control (D Stephens et al. 2000; JM Stephens et al. 2005; Clarkson et al. 2007a).

Waingaro Forest (WF) is an 8-ha block of native mixed podocarp forest located in Waingaro, north-west of Hamilton. The forest was logged in the 1900s, leaving a remnant of smaller trees, and in 2002 was fenced to prevent grazing and encourage regeneration. Pukemokemoke Bush Reserve (PBR) is a 40-ha mixed broadleaved tawa–podocarp forest located in Whitikahu, north-east of Hamilton, which was logged for large podocarps in the late 1940s (Clarkson et al. 2007a).

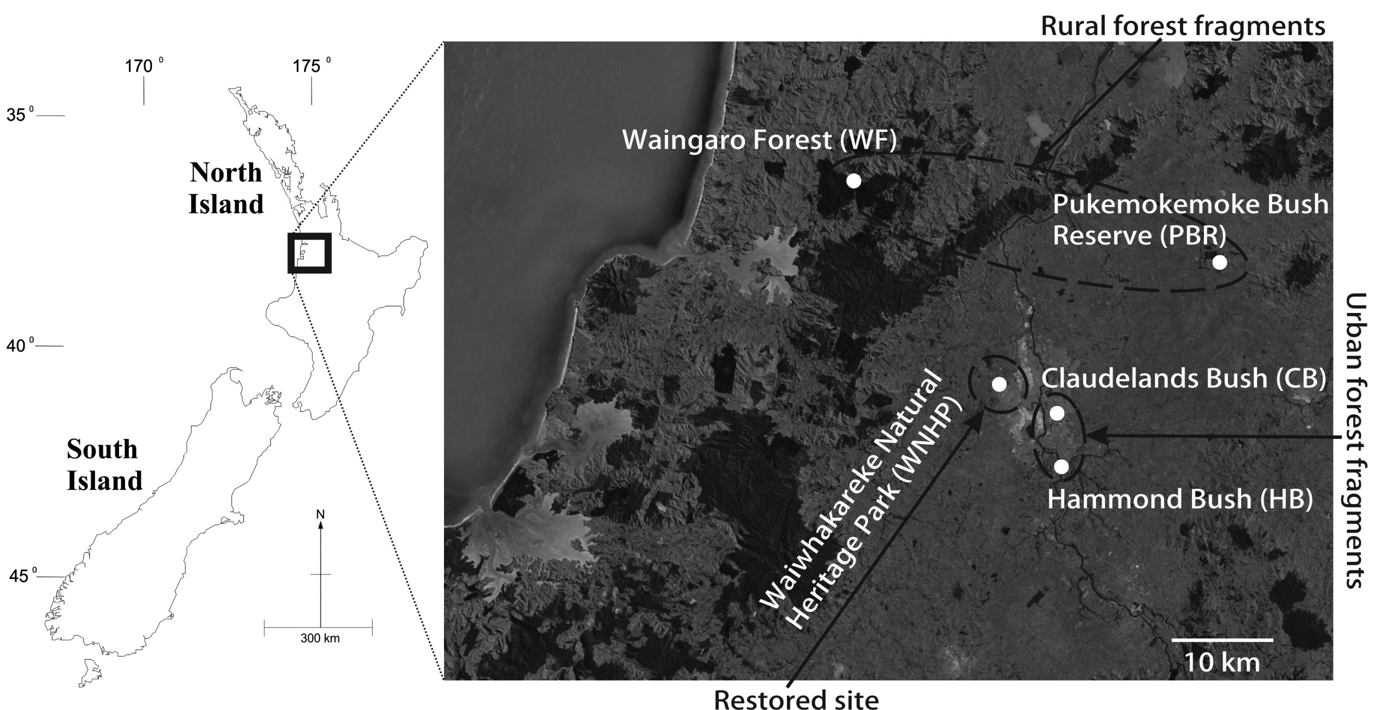

Figure 2. The five forest fragments within Waikato Ecological District sampled for māhoe (*Melicytus ramiflorus*): one 'restored' site (WNHP), two 'urban forest' fragments within Hamilton City (CB and HB), and two 'rural forest' fragments (PBR and WF).

Molecular methods

Up to 5 g of fresh leaf material was randomly collected from each of clearly distinct individuals distributed at least 10 m apart at each of the five sites (Table 1). Leaf tissue was stored at −80°C. Total genomic DNA was extracted from frozen leaf tissue using a standard CTAB-based method (Rogers & Bendich 1985). All samples were treated with RNase, and the quality and quantity of DNA was assessed by agarose gel electrophoresis. The same DNA extract was used in both the ISSR and AFLP analyses. In total 100 individuals were sampled with the aim to generate AFLP and ISSR profiles from the same individual plants within each population. However, not all DNA was of sufficient quality, resulting in 59 samples for the AFLP analyses and 87 for the ISSR analyses; 49 individuals had both AFLP and ISSR profiles.

Amplified fragment length polymorphisms were generated according to the original protocol of Vos et al. (1995) modified for detection of fluorescently-labelled markers on a capillary sequencing instrument (see Meudt & Clarke 2007; Michel et al. 2008; protocol at http://clarkeresearch.org/aflp_2012-01-26/AFLP_Protocol.pdf). AFLPs were amplified from māhoe (59 individuals) using selective primer combinations (6FAM-Eco-ACT + Mse-CCC, VIC-Eco-AGC + Mse-CAC, NED-Eco-ATA + Mse-CAG and PET-Eco-AAG + Mse-CCC). The choice of selective primers was based on a primer screen of a small number of primers that have been shown to work well in multiple plant species (Clarke 2009). AFLP fingerprints were generated, electrophoresed and scored as described elsewhere (Meudt & Clarke 2007; Michel et al. 2008; Meudt et al. 2009). To ensure reproducibility, a subset of individuals (10%) were duplicated for all selective primers (all replicates were removed after determining less than 0.05% variation and nearest-neighbour pairing between the replicated pair; sensu Holland et al. 2008). The fluorescently-labelled selective amplification products were pooled, along with a GeneScan™ 500 LIZ™ size standard, on a 3730 Genetic Analyzer (Applied Biosystems). Capillary electrophoresis was carried out at the Allan Wilson Centre Genome Service, Massey University. Automated scoring was performed using GeneMapper3.7 (Applied Biosystems) with the following settings: 50–500 bp peak range, 50 rfu peak height threshold and 0.5 bp bin width. All other settings were as in Holland et al. (2008). A bin width of 0.5 bp is expected to be optimal for the experimental set-up used here (Holland et al. 2008). Optimum minimum fragment length and peak height threshold for population genetics would need to be determined in a method similar to Holland et al. (2008) but the resolution metrics required for population genetics are different to those for phylogenetics, and exploring this further was beyond the scope of the current project. Automated scoring resulted in a binary matrix of diallelic data (1 = present, 0 = absent).

Fifteen ISSR primers (Invitrogen) were screened and assessed for clarity, reproducibility, and number of polymorphisms. Eight of these were then amplified in 87 individuals of māhoe. The eight primers were: 1: $(CA)_6GG$, 2: $(CT)_8AC$, 3: $(CT)_8TG$, 4: $(CA)_6AC$, 6: $(CA)_6AG$, 7: $(CA)_6GT$, 9: $(GT)_6GG$, 10: $(GA)_7CC$, 14: $(CTC)_3(GC)_3$. Final concentrations of PCR mixtures were as follows: 1 × PCR Buffer (Invitrogen), 3 mM $MgCl_2$, 0.2 µM primer, 80 µM of each dNTP, 0.08 mg ml^{-1} bovine serum albumin (BSA), 0.5 U Platinum *Taq* DNA polymerase (Invitrogen) and 0.1 µl DNA in a total volume of 25 µl. PCR was carried out in an Eppendorf thermocycler program of: 4 min at 94°C; 35 cycles of 40 s at 94°C, 45 s at 50°C, 1 min 30 s at 72°C followed by a final extension of 5 min at 72°C. Positive and negative controls were included on all runs. The primer sequences that produced reproducible fingerprints that could be reliably scored were: ISSR primers 1: $(CA)_6GG$, 2: $(CT)_8AC$, 3: $(CT)_8TG$, 4: $(CA)_6AC$, 6: $(CA)_6AG$, 7: $(CA)_6GT$, 9: $(GT)_6GG$, 10: $(GA)_7CC$, 14: $(CTC)_3(GC)_3$. The resulting PCR products were mixed with 8 µl of loading dye, and 16 µl was loaded on a 2% agarose/1×TBE gel containing ethidium bromide, with a 100-bp DNA ladder size standard (Invitrogen). Each gel was run for 2 to 4 h at 2.5 V cm^{-1}. Products that were amplified for a second time, to check reproducibility and/or improve upon band quality, were run side-by-side with the original products on additional gels. Each gel was visualised under UV light using an Alpha Imager and an image immediately obtained for scoring. Negative controls were completely contaminant free and the fragment profiles were highly reproducible.

Each gel was scored by eye for presence/absence of alleles. Bands of similar/dissimilar molecular weight/mobility were estimated among individuals with the aid of rerunning gels and a 100-bp DNA ladder size standard. A data matrix was

Table 1. Locations of the māhoe (*Melicytus ramiflorus*) populations sampled and population genetic statistics for the amplified fragment length polymorphism (AFLPs; n = 59) and inter-simple sequence repeat (ISSRs; n = 87) analyses. # P loci (PLP) = number of polymorphic loci (percentage of loci polymorphic); total number of loci for each species shown alongside the marker system (in parentheses); gene diversity shown ± one standard deviation.

		Forest type				
		Restored	Urban forest fragments		Rural forest fragments	
Site		Waiwhakareke Natural Heritage Park	Claudelands Bush	Hammond Bush	Waingaro Forest	Pukemokemoke Bush Reserve
Grid reference		37°46′13.17"S, 175°13′33.33"E	37°46′28.90"S, 175°17′28.27"E	37°48′25.44"S, 175°19′13.74"E	37°40′24.60"S, 174°58′22.18"E	37°35′20.68"S, 175°22'24.68"E
Sample size	AFLP (59)	13	16	15	3	12
	ISSR (87)	14	19	17	18	19
# P loci (PLP)	AFLP (1440)	982 (68.2%)	822 (57.1%)	824 (57.2%)	300 (20.8%)	697 (48.4%)
	ISSR (66)	44 (66.7%)	52 (78.8%)	57 (86.4%)	52 (78.8%)	50 (75.8%)
Gene diversity	AFLP	0.231 ± 0.119	0.183 ± 0.093	0.177 ± 0.090	0.139 ± 0.104	0.167 ± 0.087
	ISSR	0.250 ± 0.133	0.236 ± 0.123	0.327 ± 0.170	0.262 ± 0.137	0.252 ± 0.131

constructed of fragments, which were assigned to loci for each primer and were scored as diallelic (1 = present, 0 = absent). Only individuals that could be scored with <5% missing data per individual were included in the analyses.

For each population (1) the percentage of loci that were polymorphic (PLP) and (2) expected heterozygosity (H_e) under an assumption of Hardy–Weinberg equilibrium (average gene diversity over loci) were calculated. Population structuring (within and among) was performed using analysis of molecular variance (AMOVA), fixation indices (F_{ST}) (Wright 1921) were based on pairwise distance between individuals (number of shared AFLP peaks or shared ISSR bands), and exact tests (Tajima D) based on an infinite-site model were carried out to detect any departures from neutrality. These measures were calculated in ARLEQUIN ver. 3.11 (Excoffier et al. 2005) with significance set to $P < 0.05$.

Results

AFLP population genetic diversity and structure

A total of 1440 AFLP markers were scored for 59 individuals among five populations (Table 1), with an average of 353 alleles per individual. This high number of AFLP markers is consistent with research where high resolution automated scoring parameters are employed (e.g. Holland et al. 2008). The percentage of polymorphic loci among māhoe populations ranged from 68.2% at WHNP to 20.8% at WF (likely to be low due to small sample size), but was relatively similar among the other three populations (48.4–57.2%) (Table 1). No departures from neutrality were detected (Tajima D-test; $P > 0.99$ in all cases).

Exact tests found no significant differentiation among genotypic frequencies ($P > 0.99$; 6000 Markov steps) and no significant differentiation among any population pair ($P > 0.99$ in all cases; 10 000 steps). Pairwise F-statistics were non-significant ($F_{ST} = 0$, $P > 0.05$ in all cases; Table 2). To examine population structure further, the AMOVA was partitioned into three groups (restored (WHNP), urban (CB, HB) and rural (WF, PBR)). AMOVA indicated that there was no structure that could be explained among these groups (0.85%; $P = 0.472$), with 5.73% of variation among populations within groups and 93.4% of the variation within populations (both $P < 0.001$) explained (Table 3). These data indicate that individuals are well mixed within populations, and although some population variation occurs there is no significant difference among the restored, urban and rural groups (Table 3).

There were 66 ISSR loci that could be reliably scored for māhoe (87 individuals among five populations) (Table 1). The percentage of polymorphic loci among populations ranged from 86.4% at HB to 66.7% at WNHP, but was relatively similar among the other three populations (75.8–78.8%). These data were also supported by higher gene diversity indices (0.327) for māhoe at HB compared with all other populations (Table 1). There were no departures from neutrality detected (Tajima D-test; $P > 0.99$ in all cases).

Table 2. Population structure from the five populations of māhoe (*Melicytus ramiflorus*) using (a) amplified fragment length polymorphism (AFLP) analyses for 59 māhoe individuals and (b) inter-simple sequence repeat (ISSR) analyses for 87 māhoe individuals. Pairwise population φ-$_{st}$ values between populations; all $P > 0.05$.

	Population	Restored	Urban		Rural	
		Waiwhakareke	Claudelands	Hammond	Waingaro	Pukemokemoke
AFLP	Waiwhakareke NHP	-				
	Claudelands Bush	0.050	-			
	Hammond Bush	0.082	0.066	-		
	Waingaro Forest	0.093	0.090	0.048	-	
	Pukemokemoke Bush Reserve	0.085	0.052	0.031	0.081	-
ISSR	Waiwhakareke NHP	-				
	Claudelands Bush	0.121	-			
	Hammond Bush	0.139	0.125	-		
	Waingaro Forest	0.080	0.069	0.059	-	
	Pukemokemoke Bush Reserve	0.143	0.115	0.097	0.098	-

Table 3. Population structure from the five populations of māhoe using (a) amplified fragment length polymorphism (AFLP) analyses for 59 māhoe individuals and (b) inter-simple sequence repeat (ISSR) analyses for 87 māhoe individuals: Analysis of molecular variance (AMOVA) (Excoffier et al. 1992), as implemented in Arlequin ver. 3.513 (Excoffier et al. 2005). The groups used were as in Table 1, 'Restored', 'Urban forest', and 'Rural forest' fragments. Statistical significance of variance components in AMOVA tested with 16 000 permutations.

	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P
AFLP	Among groups	2	521.166	1.229	0.85	0.472
	Among populations within groups	2	437.042	8.264	5.73	<0.001
	Within populations	54	7274.165	134.707	93.42	<0.001
	Total	58	8232.373	144.200		
ISSR	Among groups	2	46.875	-0.140	-1.44	0.799
	Among populations within groups	2	58.330	1.121	11.53	<0.001
	Within populations	82	716.772	8.741	89.91	<0.001
	Total	86	821.977	9.722		

Exact testing of sample differentiation based on genotypic frequencies found no significant differentiation ($P > 0.99$; 6000 Markov steps) with no significant differentiation among any population pair ($P > 0.99$ in all cases; 10 000 steps). Population pairwise F-statistics were non-significant ($F_{ST} = 0$, $P > 0.05$, in all cases; Table 2). To examine population structure further the AMOVA was partitioned into the same three groups that were used for the AFLP analyses (restored (WHNP), urban (CB, HB) and rural (WF, PBR)). AMOVA indicated that there was no structure that could be explained among these groups (0%; $P = 0.799$), with 11.5% of variation among populations within groups and 89.9% of the variation within populations (both $P < 0.001$) explained (Table 3). These data indicate that individuals are well mixed; although some population variation occurs, there is no significant difference among the restored, urban and rural groups (Table 3).

Discussion

This study contributes novel genetic data to a significant, long-term restoration project and, to our knowledge, is the first published study comparing AFLP and ISSR data for a forest restoration project in New Zealand. Overall, these data indicate that the restored population of māhoe at WNHP is genetically similar in terms of diversity and structure to nearby urban and rural forest fragments (Tables 2 and 3). The highest level of polymorphism based on AFLPs for māhoe was found at WNHP, while results based on ISSRs indicated that this occurred at Hammond Bush. It is likely because WNHP is a mix of individuals from several populations that any incongruence between the AFLPs and ISSRs for māhoe was caused by the greatly reduced number of ISSR loci scored and potential lower level of resolution obtained with that technique. The number of ISSR loci scored is not unusual, and is comparable with other studies using ISSRs (e.g. Culley et al. 2007; Fant et al. 2008; Ramp Neale et al. 2008).

Sample size is also an important factor that varied across all populations for the species examined here. The AFLP data are based on fewer individuals (3–16, mean of 11.7) than the ISSR data (14–19, mean of 17.4) (Table 1). Sinclair and Hobbs (2009) examined the effects of sample size for ecological restoration using AFLPs (from 117 loci). They found that small sample sizes would underestimate population genetic diversity while population structuring would be overestimated, and a minimum sample size of 30 individuals would be required to maintain 'natural' levels of genetic diversity and population subdivision (Sinclair & Hobbs 2009). This conclusion is echoed by a number of studies using anonymous (dominant) markers (e.g. Krauss & Koch 2004; McKay et al. 2005) and potentially highlighted in the present study with significant population structure (Tables 2 and 3).

However, AFLPs have become the anonymous marker of choice for some very clear reasons (e.g. Bussell et al. 2005; Meudt & Clarke 2007; Sinclair & Hobbs 2009). Firstly, the number of loci the technique can yield is far greater (when AFLPs are characterised using a capillary electrophoresis system; sensu Meudt & Clarke 2007) than other techniques such as RAPDs and ISSRs. For example, in our study 66 ISSR loci were obtained, compared with 1440 AFLP loci. Secondly, as a benefit of many more loci, the resolving power of analyses is much greater, allowing finer levels of population genetic structure within each population to be revealed. This study shows that, with sufficient markers, genetic structure can be resolved even when sample sizes are low. This is potentially important for future research where relatively few individuals can be sampled (e.g. due to costs, or for species that are rare or difficult to sample). It is important to note, however, that the ability to accurately determine population structure with a low number of individuals will depend on the levels of intra- and inter-population heterogeneity, i.e. the extent to which genetic variability in a few individuals accurately reflects the variability of a population as a whole.

Inter-simple sequence repeat analyses are typically less informative (lower resolution); however, in our analyses the population structure, differentiation and population diversity indices (Tables 1–3) were similar when compared with the AFLP analyses. This is likely due to the greater sample sizes analysed, which required a fraction of the resources needed for the AFLPs and yet still provide a broad level of genotypic resolution that is likely to be adequate to guide restoration efforts in most situations.

Although AFLPs revealed similar structure as ISSRs with smaller sample sizes, this requires a much larger resource commitment in terms of time, technical ability and money (c. 4× the cost of ISSR), and this may limit the application of AFLP for some restoration projects. Managers of restoration projects will have to balance the scale (fine or coarse) of the data required and the financial and technical resources available (see Lesica & Allendorf 1999; Hufford & Mazer 2003) including the number of individuals per population, the number of populations and the number of primers. For example, AFLPs may be preferable for rare or difficult-to-obtain species that result in low sample sizes or when delineation of precise seed provenances is required. Other considerations will include taxonomic range, discriminatory power, reproducibility, technical difficulty, budget, and ease of interpretation and standardisation (see also Savelkoul et al. 1999; Bussell et al. 2005).

Conclusions

In this study, WNHP represents a unique opportunity to investigate retrospectively how current eco-sourcing practices are shaping the genetic variation of a common species in a developing ecosystem, while at the same time providing the opportunity to fine-tune these practices for future restoration plantings. When assessing genetic variation, restoration practitioners will need to weigh the costs and benefits of the various molecular tools at their disposal. If a general estimate of genetic variation is needed then ISSR analyses are likely to be sufficient. Increasing the number of loci and/or samples is likely to increase the resolution of genetic structure. When detailed knowledge for a specific taxon is required, AFLPs should be used to recover fine-scale genetic structuring and may be requisite for delineating precise seed provenance zones.

In evaluating eco-sourcing success at the WNHP restoration site, our use of estimates of AFLP and ISSR genetic variation has shown that the site successfully represents the natural variation in local populations; but care must be taken to understand natural population variation and the scale at which this operates when sourcing seed for the purpose of restoration (see also Malaval et al. 2010; Mijangos et al. 2015). This is an important consideration for ensuring the long-term persistence of these populations. Furthermore, levels of genetic variation observed in urban fragments (which are typically avoided as source populations) were comparable with that seen in rural

populations (for both AFLPs and ISSRs). The conservation value of urban fragments has been suggested to be high (Roberts et al. 2007) and these should be an important additional source for future eco-sourcing collections, especially if they are close to the restoration site (and consequently contain local ecotypes). Therefore, along with the usual primary criterion of locality of collection, knowledge of genetic variation of population fragments is useful to guide eco-sourcing efforts. Genetic data complement and underpin existing ecological knowledge and are important considerations for how seed is collected for the purpose of long-term restoration projects.

Acknowledgements

We thank Arvina Ram (University of Waikato), Lara Shepherd, Trish McLenachan and Pete Lockhart (Massey University) for lab support; Gerard Kelly, Kemble Pudney and Gavin Vile (Hamilton City Council) for information on the sourcing of plants for WNHP; and Nicolas Rawlence and John Weyland ('Exo') for help with data analyses. We thank two anonymous reviewers, Rob Smissen, Priscilla Wehi, Catherine Kirby, Sarah Wright and Steve Pratt for constructive comments on the manuscript. We are also indebted to Bruce Clarkson (University of Waikato) for continual support throughout the project and comments on the manuscript. This work was supported through New Zealand Foundation for Research, Science and Technology funding to the University of Waikato (UOWX0501).

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Editorial Board member: Hannah Buckley
Received 30 May 2014; accepted 1 January 2015

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