BIODIVERSITY RESEARCH

Genetic diversity and structure of the invasive tree *Miconia calvescens* in Pacific islands

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ABSTRACT

Aim This study investigates the amount and distribution of genetic variation within and among populations of the highly invasive tree, *Miconia calvescens* (Melastomataceae; hereafter miconia), in tropical island habitats that are differently impacted (distribution and spread) by this weed.

Location Invasive populations were included from northern and southern Pacific islands including the Hawaiian Islands (Hawaii, Kauai and Maui), Marquesas Islands (Nuku Hiva), Society Islands (Tahiti, Tahaa, Moorea, Raiatea) and New Caledonia.

Methods We used 9 codominant microsatellite and 77 highly variable dominant intersimple sequence repeat markers (ISSRs) to characterize and compare genetic diversity among and within invasive miconia populations. For the codominant microsatellite data we calculated standard population genetic estimates (heterozygosity, number of alleles, inbreeding coefficients, etc.) and described population genetic structure using AMOVA, Mantel tests (to test for isolation by distance), unweighted pair-group method with arithmetic averages (UPGMA) cluster analysis and principal components analysis (PCA). We also tested for the presence of a population bottleneck and used a Bayesian analysis of population structure in combination with individual assignment tests. For the dominant ISSR data we used AMOVA, PCA, UPGMA and a Bayesian approach to investigate population genetic structure.

Results Both markers types showed little to no genetic differentiation among miconia populations from northern and southern Pacific hemispheres (AMOVA: microsatellite, 3%; ISSR, 0%). Bayesian and frequency-based analysis also failed to support geographical genetic structure, confirming considerable low genetic differentiation throughout the Pacific. Molecular data furthermore showed that highly successful miconia populations throughout the Pacific are currently undergoing severe bottlenecks and high levels of inbreeding ($f = 0.91$, ISSR; $F_{IS} = 0.27$, microsatellite).

Main conclusions The lack of population genetic structure is indicative of similar geographical sources for both hemispheres and small founding populations. Differences in invasive spread and distribution among Pacific islands are most likely the result of differences in introduction dates to different islands and their accompanying lag phases. Miconia has been introduced to relatively few tropical islands in the Pacific, and the accidental introduction of a few or even a single seed into favourable habitats could lead to high invasive success.

Keywords
Biological invasions, invasive species, ISSRs, genetic diversity, *Miconia calvescens*, microsatellites.

INTRODUCTION

Introduced alien species that persist and spread in their new environment offer ideal situations to investigate such basic evolutionary processes as drift, gene flow, migration and local adaptation (Sakai *et al.*, 2001). A new environment presents unique conditions to which introduced species normally may have not been exposed historically, in many cases representing

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subadapted environments (lowered fitness) that will exert strong selection on founders. Founding populations typically introduce only a small fraction of the available genetic variation from native gene pools, undergoing, in most cases, severe bottlenecks, likely resulting in reduced fitness. Genetically depauperate populations without pre-adapted histories can only persist if ecological flexibility (phenotypic plasticity) allows genetic assimilation or if postdrift allele frequencies allow local adaptation to occur. Although many introductions fail to persist, others experience a so-called lag phase when species are persisting but not spreading rapidly in the new environment (e.g. Ewel, 1986). This lag phase is thought to partially represent the time lapse that results from the accumulation of sufficient genetic variation and/or from recombination of existing genetic variation to allow local adaptation to occur (Sakai *et al.*, 2001).

Population genetics theory predicts that high genetic diversity predisposes invasive populations to success at establishing and persisting in novel habitats, reducing such lag phases and increasing potential for rapid and successful invasion. A correlation between higher genetic diversities and invasion success has been documented for many species, e.g. in the United States, *Ambrosia artemisiifolia* showed higher within-population genetic diversity compared to native range populations (Genton *et al.*, 2005) and *Tamarix ramosissima* harboured high levels of variation in ecologically important traits and showed evidence for evolution of regional invasive ecotypes (Sexton *et al.*, 2002). Other studies have shown how postintroduction adaptation for phenotypic traits among invasive populations contributed towards their invasiveness (e.g. Huey *et al.*, 2000; Maron *et al.*, 2004). On the contrary, many successful invaders represent bottlenecked populations that typically have low genetic diversity, expected low evolutionary potential and perhaps low reproductive fitness (Frankham, 2005). Such species' success in new environments has been attributed to wide environmental tolerance (e.g. Le Roux *et al.*, 2007), escape from natural enemies (e.g. Wolfe, 2002), reproductive assurance (Rambuda & Johnson, 2004) and/or the presence of pre-adapted genotype(s) (e.g. Hurka *et al.*, 2003). Both genetically diverse and depauperate species have thus been associated with successful invasions, making the supposed role and importance of genetic diversity during biological invasions unclear or at best ambiguous.

The purpose of this study was to characterize the amount of neutral genetic variation harboured within and among populations of a highly invasive plant in Pacific island habitats. *Miconia calvescens* DC (Melastomataceae, herein miconia) has been introduced outside of its native range to various tropical Pacific islands as a popular ornamental for its attractive foliage, and is now considered one of the most destructive invaders in insular tropical rain forest habitats (Gagné *et al.*, 1992; Meyer, 1996). For example, after the introduction of a single tree in 1937 to Tahiti, miconia infestations are now characterized by extensive monotypic stands found over a large portion of the island (Meyer, 1996). Currently, miconia has spread into 70% (*c.* 780 km^2) of Tahiti's forested land surface, forming dense nearly monotypic stands over 25% (*c.* 260 km^2) of the island (Fig. 1b), significantly impacting native biodiversity (Meyer & Florence, 1996). The

Figure 1 Photographs illustrating (a) less dense stands of *Miconia calvescens* as is typically observed in Hawaii and (b) more dense monotypic stands found widely distributed over Tahiti.

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remaining land surface (*c.* 30%) represents urbanized areas or those unsuitable (with low rainfall) to support miconia invasion. Miconia was also secondarily introduced from Tahiti to other Society Islands such as Moorea, Raiatea and Tahaa and to the Marquesas Islands in Nuku Hiva and Fatu Hiva. Furthermore, miconia also naturalized and is spreading in the Province Sud in New Caledonia. In the North Pacific, miconia invasions resulted in dense nearly monotypic stands in low and mid-elevation rain forests in the Hawaiian Islands (Fig. 1a). Miconia was introduced to Hawaii and Oahu in 1961 (24 years later than in Tahiti) and secondarily introduced to Maui in the early 1970s (Medeiros *et al.*, 1997). On Oahu original populations of miconia were confined to two botanical gardens located in areas receiving only marginal rainfall to support miconia spread. These populations were subsequently removed by 1995 (Medeiros *et al.*, 1997) but viable seed still persist in seed banks (J.J. Le Roux, pers. obs.). On the island of Kauai, two small populations of miconia that resulted from seed dispersal from a single mature tree from a nearby nursery became established (Conant, 1996). Miconia has spread over 2% (*c.* 222 km^2) on the island of Hawaii (Invasive Weed Awareness Coalition, 2007), with monotypic stands found in substantially smaller areas (*c.* 12.14 km^2 [Kaiser, 2006]) than in Tahiti (*c.* 260 km^2). These dense monotypic stands pose the biggest threat to island forest ecosystems as they deprive the native flora from available light and resources (Meyer & Florence, 1996). In addition to distributional differences in predominance between Hawaii and Tahiti, Tahitian infestations can also be found at wider elevational ranges (10–1400 m) compared to Hawaiian infestations (10–760 m) (Meyer, 1998b).

Even though miconia is considered one of the biggest threats to Hawaiian forest ecosystems, the extent and spread of invasions is evidently less than in Tahiti. This might be a reflection of the rigorous containment and control efforts against miconia in Hawaii. Nevertheless, miconia infestations in steep mountainous areas that are inaccessible for control efforts remain relatively sparse compared to some of the Society Island infestations (Julie Leialoha, Big Island Invasive Species Committee, pers. comm.). Ecological and climate similarities between these northern and southern Pacific islands raised the question whether Hawaii could potentially experience and expect invasions to the spatial extent that Tahiti has (Medeiros *et al.*, 1997). Other than control and containment efforts, the attributes potentially contributing to differential spread and distribution of miconia among these islands remain undetermined. Whether differences in introduction times, lag phases, differential adaptive potential as a result of differences in genetic diversities or the introduction of differently pre-adapted genotypes play a role, remain unknown.

Using microsatellite and hyper-variable intersimple sequence repeat (ISSR) markers, the following questions were addressed: First, do Hawaiian miconia populations differ from southern Pacific island populations (Moorea, Raiatea, Tahiti, Nuku Hiva and New Caledonia) in the amount of genetic diversity they harbour? Such genetic comparisons would allow us to infer whether these areas are invaded by miconia from similar or different geographical source(s). Second, are invasions throughout the Pacific characterized by inbred and genetically depauperate or genetically diverse populations? We discuss the implications of our findings with regard to the management of miconia, especially biological control.

METHODS

Study species

Miconia calvescens DC, herein miconia, is a diploid (2n = 32) member of the Melastomataceae with a neotropic native range extending 40° of latitude from southern Mexico to northern Argentina and southern Brazil (Meyer, 1996). Miconia is a fast-growing small tree (up to 16 m tall in its native range) that readily outcompetes other species for available light owing to its large (up to 1 m in length) leaves. Pollen to ovule ratios indicate that miconia is facultative xenogamous, having a mixed breeding system (Meyer, 1998a). Dispersal of fruits and seeds is accomplished passively through wind and water, or actively over long distances by frugivorous birds.

Population sampling and DNA extraction

Eight miconia populations were sampled from northern Pacific Hawaiian Islands and eleven populations from southern Pacific Society Islands, Marquesas Islands and New Caledonia (Fig. 2). For each population, leaf material from 10–30 individual plants was collected. Leaf material was collected in 2006 throughout all southern Pacific Islands by J.-Y. Meyer. Plant material from the Province Sud of New Caledonia was collected and donated by Jérôme Munzinger (Laboratoire de Botanique, IRD, Nouméa, New Caledonia). Plant material was placed and dehydrated in sealed plastic pouches containing Drierite™. Hawaiian populations were collected throughout 2005 by J.J. Le Roux and leaf material kept on ice in the field for no longer than 24 h before being transferred to a –80 °C freezer. Locality name, region and where possible, latitude and longitude were recorded for populations sampled (Table 1). In total 500 individuals representing 19 putative populations were collected.

Total genomic DNA was extracted according to the manufacturer's protocol with the DNeasy Plant mini kit (Qiagen) from 40 to 50 mg leaf material that was frozen in liquid nitrogen and ground by hand. All extractions were stored at –80 °C.

Microsatellite analysis

Details concerning the isolation, characterization, and internal repeat structure of the *M. calvescens* microsatellite loci used in this study can be found in Le Roux & Wieczorek (2008). Polymerase chain reaction (PCR) amplification of loci was performed as previously described by Le Roux & Wieczorek (2008). Polymorphisms were screened using an ABI PRISM 377XL DNA sequencer (PE Applied Biosystems) and PCR products sized relative to a molecular size marker (LIZ500, PE Applied Biosystems). DNA fragments were analysed using the GENEMARKER version 1.4 program (SoftGenetics, LLC).

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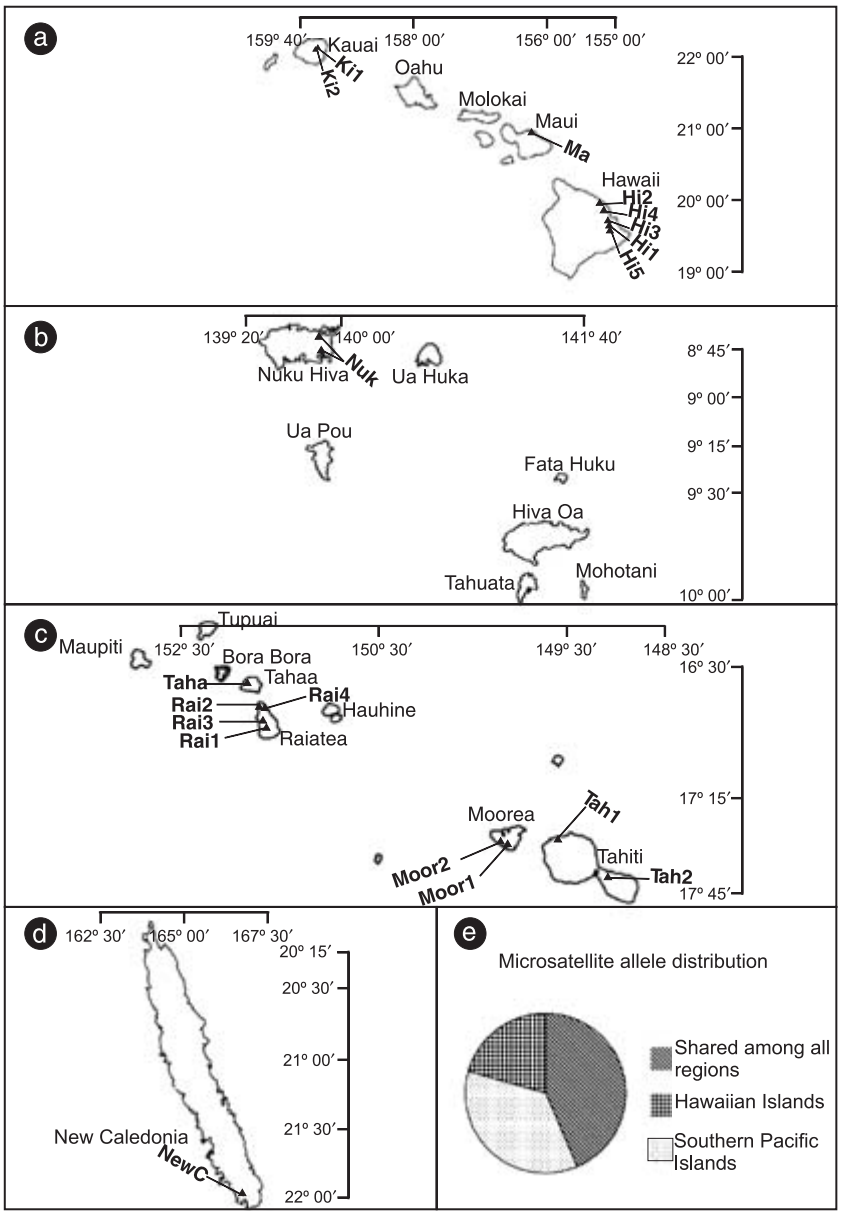

Figure 2 Maps indicating the collection sites in the (a) Hawaiian Islands (b) Marquesas Islands (c) Society Islands and (d) New Caledonia. Inset e illustrates the distribution of 69 microsatellite alleles within and among northern and southern Pacific populations of *Miconia calvescens.*

ISSR amplification and scoring

A subset of the ISSR PCR primers described in Poulin *et al.* (2005) was selected for this study. Three primers were chosen based on high number of bands and the robustness and reproducibility of banding profiles. A subset of populations used in the microsatellite data analysis and the Maui population Ma was used for ISSR analysis (Table 1). For each population ISSR diversity was analysed in 24 individuals. Each 10 μL ISSR PCR reaction contained 5 μL 2X Qiagen Multiplex PCR Mastermix [HotStarTaq DNA Polymerase; Qiagen Multiplex PCR Buffer (6 mM $MgCl_2$, pH 8.7); dNTP mix], 1 μL Q-solution [PCR additive (Qiagen)], 12.5 pmol of individual ISSR primer and approximately 5 ng total genomic DNA. PCR was conducted on an MJ Research PTC 100 cycler with a thermocycle of: initial denaturation of 95 °C for 15 min; 35 cycles at 94 °C for 60 s, primer-specific annealing temperature: Primer1 ($[AC]_7RG$), 52 °C; Primer3 ($[AG]_8TG$), 48 °C; Primer5 ($[CA]_6RY$), 50 °C for 60 s, elongation at 72 °C for 90 s and final extension at 72 °C for 12 min.

The Agilent 2100 Bioanalyser analysis LabChip (Agilent Technologies, Inc.) was used for high-resolution separation of fragments (Banerjea *et al.*, 2003). This analysis allows for detecting smaller differences (sizes) between fragments than allowed by conventional methods and also detects minute quantities of DNA (as low as 0.01 ng/μL). Data were analysed with the 2100 expert program (Agilent Technologies, Inc.) and to ensure that poorly amplified bands were not missed during the scoring of profiles, the 'global height threshold' setting was set to 0.02 in all analysis. Adjusting the contrast of gel images to the appropriate levels furthermore helped to detect potential 'null' (poorly amplified) loci. For each individual, each locus (size fragment) was scored as present or absent ('1' = locus present, '0' = locus absent).

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Table 1 Geographic position of sampling sites with measurements of genetic diversity at nine microsatellite and 77 ISSR loci: *A*, mean number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; F_{IS}, inbreeding coefficient; *hs*, panmictic heterozygosity; *f*, inbreeding coefficient.

				Co-dominant marker genetic diversity				*Dominant marker genetic diversity*	
ID	Island	Locality	Lat/Long‡	*A*	H_O	H_E	F_{IS}	*hs*	*f*†
Northern Pacific									
Hi1	Hawaii	South Hilo	19.830°/–155.108°	2.00	0.443	0.166	0.649	–	–
Hi2	Hawaii	North Hilo	19.869°/–155.271°	2.556	0.081	0.176	0.407	–	–
Hi3	Hawaii	South Hilo	19.811°/–155.115°	1.556	0.016	0.031	0.185	–	–
Hi4	Hawaii*	Honokua Bay	19.980°/–155.253°	2.111	0.039	0.090	0.260	0.261	0.871
Hi5	Hawaii	Leilani Estates	19.676°/–154.989°	2.222	0.068	0.124	0.253	–	–
Ki1	Kauai	UH Research	22.079°/–159.408°	2.222	0.086	0.161	0.190	–	–
Ki2	Kauai	UH Research	22.068°/–159.396°	1.222	0.034	0.104	0.410	–	–
Ma	Maui	Huelo Honokala	20.855°/–156.135°	–	–	–	–	0.2989	0.911
Southern Pacific									
Moor1	Moorea*	Maatea valley	–17.579°/–149.813°	1.778	0.038	0.077	0.403	0.259	0.895
Moor2	Moorea*	Vaianae valley	–17.573°/–149.834°	1.556	0.020	0.020	–0.062	0.237	0.878
Tah1	Tahiti*	Papenoo valley	–17.521°/–149.441°	2.111	0.077	0.084	0.032	0.254	0.912
Tah2	Tahiti*	Taravao plateau	–17.743°/–149.298°	1.333	0.029	0.042	0.286	0.253	0.907
Rai1	Raiatea*	Mount Toomaru	–16.864°/–151.448°	3.333	0.102	0.240	0.532	0.211	0.916
Rai2	Raiatea	Uturaerae valley	–16.747°/151.474°	1.444	0.050	0.047	–0.060	–	–
Rai3	Raiatea	Tetooroa valley	–16.822°/–151.466°	1.333	0.016	0.016	–0.031	–	–
Rai4	Raiatea*	Anatorea	–16.750°/–151.442°	1.444	0.017	0.017	–0.024	0.233	0.850
Taha	Tahaa*	Pueheru valley	–16.598°/–151.501°	1.889	0.193	0.180	0.252	0.260	0.927
Nuk	Nuku Hiva*	Taipivai-Hatiheu	–140.053°/–8.814°	3.000	0.031	0.249	0.873	0.231	0.922
NewC	New Caledonia*	Mount Dore	–22.299°/166.792°	2.556	0.075	0.128	0.298	0.247	0.916

*Populations that were included in both dominant and co-dominant marker data sets.

†*f* is an estimate of F_{IS} for dominant marker data after Holsinger *et al.* (2002).

‡Latitude and longitude coordinates were not determined in the field for southern Pacific populations and values given in the table are approximate locality waypoints estimated from geographical information systems (GIS) maps.

Statistical analysis of microsatellite data

Within-population genetic diversity

The number of alleles and private alleles were calculated for each population using GenAlEx6 (Peakall & Smouse, 2006), pairwise F_{ST} and observed (H_O) and expected (H_E) heterozygosities using Arlequin version 3.01 (Schneider *et al.*, 2000). Statistically significant deficits of heterozygotes from that expected under Hardy–Weinberg equilibrium (HWE), linkage disequilibrium among all pairwise sets of loci and inbreeding coefficient (F_{IS}) values were estimated using Arlequin version 3.01 (Schneider *et al.*, 2000). For HWE a Monte Carlo approximation of the Fisher's exact test (Guo & Thompson, 1992) and a standard Bonferroni correction for multiple comparisons where the Markov chain algorithm was run for 100,000 steps following 10,000 dememorization steps were used.

Transient excess of heterozygosity relative to that expected under mutation-drift equilibrium is a signature of a recent population bottleneck (Cornuet & Luikart, 1997). Populations that have experienced a recent reduction of their effective population size (N_e) exhibit a correlative reduction in numbers of alleles and gene diversity (H_E, or Hardy–Weinberg heterozygosity) at polymorphic loci. However, allele numbers decline more rapidly than gene diversity, so that in recently bottlenecked populations, the observed gene diversity is higher than the expected equilibrium gene diversity (Luikart *et al.*, 1998). An excess of heterozygosity was tested for microsatellite data with the Bottleneck program (Cornuet & Luikart, 1997) under a 100% stepwise mutation model (SMM) and a two-phase mutation model (TPM with 70% SMM). Significance was tested by the sign and Wilcoxon tests (Luikart & Cornuet, 1998). These tests were separately applied to Hawaii ($n = 192$) and southern Pacific islands ($n = 281$) samples.

Among-population genetic diversity

To test for isolation by distance, Mantel tests with 1000 permutations as implemented in Arlequin version 3.01 (Schneider *et al.*, 2000) were used. A matrix of pairwise F_{ST} values was regressed against a matrix of geographical distances between populations calculated from geographical coordinates using GIS-Arcview.

All populations were used in an analysis of molecular variance (AMOVA, Excoffier *et al.*, 1992) to examine the distribution of

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genetic variation at three hierarchical levels: within populations, among populations within southern and northern Pacific regions, and among southern and northern Pacific regions. This test, implemented in GenAlEx6 (Peakall & Smouse, 2006), partitions total genetic variance and calculates Φ_{PT}, an analogue of F_{ST} (Wright, 1965). The significance is determined by comparison with a null distribution derived from permuting haplotypes, individuals or populations at the appropriate hierarchical level (Excoffier *et al.*, 1992). The use of Φ_{PT} enabled us to compare patterns of molecular variance at the same levels between codominant microsatellite and dominant ISSR data (e.g. see Maguire *et al.*, 2002). Finally, pairwise Φ_{PT} values were used in a cluster analysis to generate a dendrogram using the unweighted pair-group method with arithmetic mean (UPGMA). In addition, a principal components analysis (PCA) was used to examine the extent of genetic clustering of populations from throughout the Pacific Ocean using the GenAlEx6 program (Peakall & Smouse, 2006).

Bayesian estimates of population structure

Bayesian assignment techniques were used to test for population structure among populations throughout the Pacific and to assess the geographical scale of population differentiation, using STRUCTURE version 2.2 (Falush *et al.*, 2007). This method identifies clusters of genetically similar individuals from multilocus genotypes without prior knowledge of their population affinities. The model assumes K genetic clusters, with each having a characteristic set of allele frequencies at each locus; the admixture model then probabilistically estimates the proportion of individuals with ancestry in each cluster. A series of pilot runs were used to estimate $\Pr(X \mid K)$, where X represents the data, for K between 1 (the expected value if all populations represent a single panmixic unit) and 18 (the maximum number of populations). Using the options to ignore population affiliation when defining genetic clusters, assuming independence among loci, and allowing admixture, four independent runs of 300,000 iterations were run, following a burn-in period of 100,000, for each value of K (Pritchard *et al.*, 2000). From these initial runs, it was determined that the true value of K (the highest posterior probability) fell between 2 and 10. Pritchard & Wen (2003) warned that $\Pr(X \mid K)$ is in reality only an indication of the number of clusters and an ad hoc guide and potentially difficult to interpret biologically. This is especially true in cases were LnProb values increase with stepwise values of K and can lead to the overestimation of K. To minimize such overestimation, ΔK was calculated (Evanno *et al.*, 2005) by taking into account the shape of the log-likelihood curve with increasing K and variance among estimates among multiple runs. Once the number of genetic clusters was established, each individual was assigned to a cluster, and the overall membership of each sampled individual in the clusters estimated.

ISSR genetic diversity

Within-population genetic diversity was estimated as heterozygosity using a Bayesian approach as implemented in the software Hickory 1.0.4. (Holsinger *et al.*, 2002). Similar to microsatellite data, all populations were used in an analysis of molecular variance (AMOVA, Excoffier *et al.*, 1992) to examine the distribution of genetic variation (Φ_{PT}) within populations, among populations within southern and northern Pacific regions and among southern and northern Pacific regions. Overall, population differentiation was calculated as G_{ST}-B, a Bayesian analogue of the coefficient of gene differentiation among populations, G_{ST} (Nei, 1973). This was performed separately for northern and southern Pacific regions using the Hickory 1.0.4. program (Holsinger & Lewis, 2003). Pairwise genetic distances between populations based on ISSR profiles were calculated according to Huff *et al.* (1993) using the program GenAlEx6 (Peakall & Smouse, 2006). The subsequent genetic distance matrix was used in cluster analysis and a dendrogram generated for the 11 populations using the UPGMA. A phenetic rather than parsimony-based method was used as this study did not verify that all comigrating loci were homologous or that they sorted independently. Similar to microsatellite data, population genetic distances were also used in a PCA to examine genetic clustering of populations from throughout the Pacific using the GenAlEx6 program (Peakall & Smouse, 2006).

RESULTS

Microsatellite genetic diversity

Within-population genetic diversity

In the nine microsatellite loci analysed, a total of 69 alleles were identified, an average of 7.67 alleles per locus. The number of alleles ranged from 5 (locus D101) to 11 (locus D118); within populations the mean number of alleles ranged from 1.2 to 3.0 (Fig. 3). The effective number of alleles (corrected for expected heterozygosity) allows for more meaningful comparisons of allelic diversity across populations with different allele distributions. The effective number of alleles did not differ among sampled populations (Fig. 3) with a large number of alleles, 28 alleles (40.6% of the total), shared among southern Pacific islands and the Hawaiian Islands (Fig. 2), whereas only 26 (37.7% of total) were restricted to southern Pacific islands and 15 (21.7% of total) restricted to the Hawaiian Islands. Expected heterozygosity ranged from 2% to 25%, with a mean of 11% across all loci. Observed heterozygosity ranged from 2% to 44%, with a mean of 8% across all loci. On average, the majority of loci displayed a significant deficit of heterozygotes from that expected under HWE, with some loci conforming to HWE in some populations. No linkage disequilibrium was detected between any of the nine loci across all populations.

A significant excess of heterozygosity was detected under all models tested (full SMM model, TPM model of mutation with 70% SMM) for both northern and southern Pacific regions for the sign and Wilcoxon tests ($P < 0.001$), indicating that *M. calvescens* populations in both Pacific hemispheres are currently going through severe bottlenecks. The average population inbreeding coefficient was high (0.27), further support to severely bottlenecked populations in both regions.

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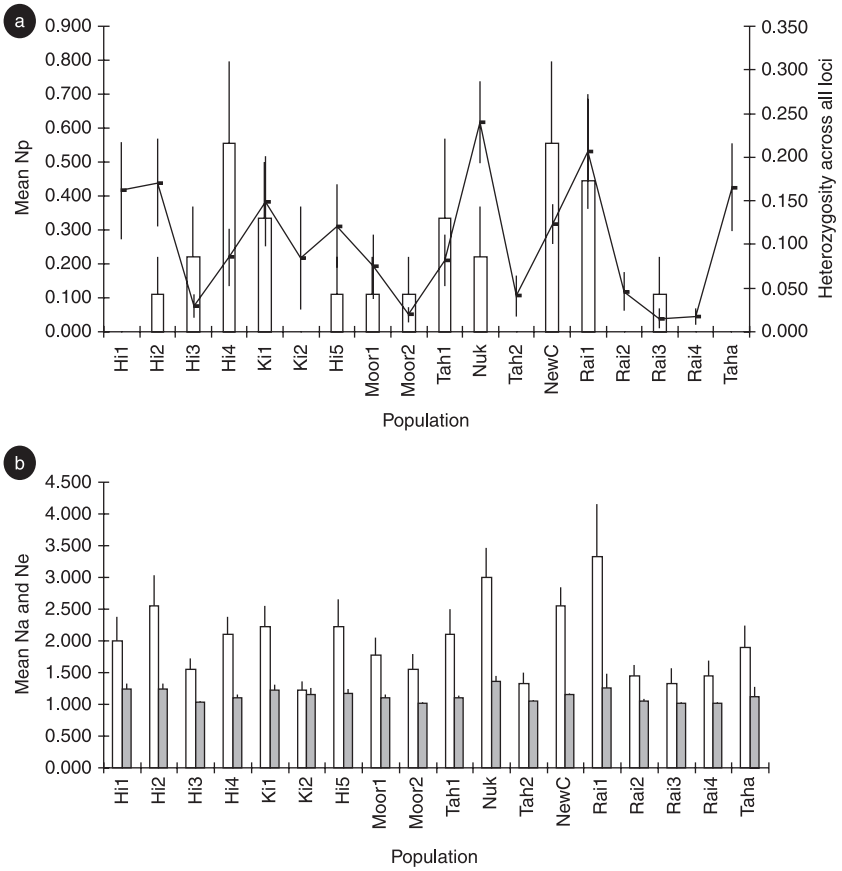

Figure 3 Patterns of microsatellite allelic richness and heterozygosity in 18 populations of *Miconia calvescens* genotyped at nine microsatellite loci. (a) mean ± SD number of private alleles (Np, open bars) and mean ± SD heterozygosities for each population (across all loci) are represented by the black line. (b) mean ± SD number of alleles (Na, open bars), mean ± SD number of effective alleles (Ne, grey bars).

Table 2 Results of hierarchical AMOVA comparing genetic variation within populations, among populations within southern and northern Pacific regions, and among southern and northern Pacific regions invaded by *Miconia calvescens* for both dominant and co-dominant data. Significance was tested against a null distribution of 10,000 random permutations.

Source of variation	d.f.	Sum of squares	Fixation index	Percent variation	*P*-value
Co-dominant microsatellite data					
Among regions	1	77.494	$\Phi_{RT} = 0.028$	3.00	< 0.01
Among populations within regions	16	537.153	$\Phi_{PR} = 0.170$	17.0	< 0.01
Within populations	455	2405.129	$\Phi_{PT} = 0.193$	80.0	< 0.01
Dominant ISSR data					
Among regions	1	32.422	$\Phi_{RT} = 0.000$	0.00	< 0.01
Among populations within regions	5	253.654	$\Phi_{PR} = 0.122$	12.0	< 0.01
Within populations	240	4185.770	$\Phi_{PT} = 0.114$	88.0	< 0.01

Among-population genetic diversity

Pairwise F_{ST} values ranged from 0.009 between population Rai4 (Raiatea) and populations Moor2 (Moorea) and Rai3 (Raiatea) to 0.197 between the Society Island population Rai1 (Raiatea) and Taha (Tahaa). Overall, except for one Raiatea population (Rai1) and the Tahaa population, Taha, these values represented very low population differentiation. Genetic distances among pairs of populations were not significantly correlated to geographical distance between localities (Mantel test, $P > 0.05$).

UPGMA cluster analysis based on genetic distances failed to support geographical clustering (Fig. 4). For example, in cluster 1 some Hawaiian populations (Hi3) showed a closer relationship to many south Pacific populations than to other Hawaiian populations. Cluster 1 contained all populations except for Hawaiian populations Ki2 and Hi5 (cluster 2) and the southern Pacific populations Rai1 and Taha (cluster 3). Genetic distances (Φ_{PT}) were also used in a PCA to investigate the relative position of populations in multidimensional space. The first two principle coordinates (PC) axes explain 73.04 and 14.52% of the genetic

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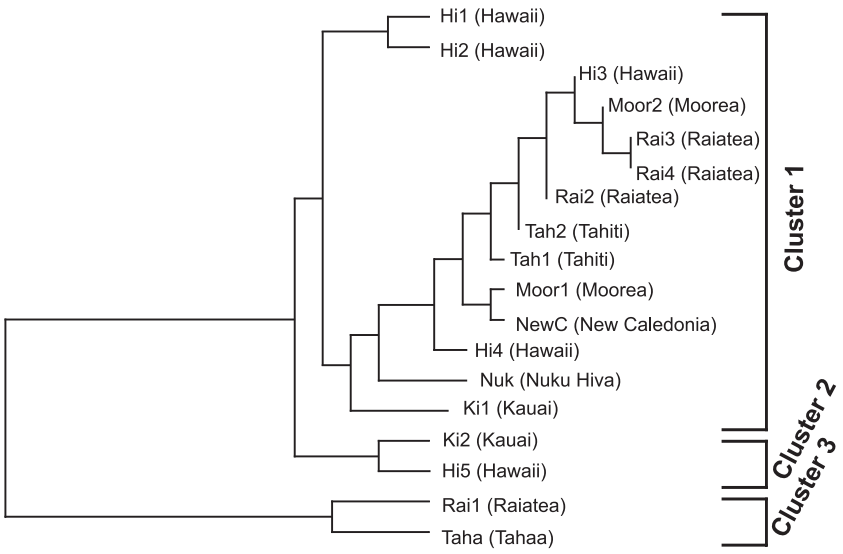

Figure 4 A dendrogram based on pairwise Φ_{PT} values (microsatellite data) constructed by the using the unweighted pair-group method with arithmetic mean (UPGMA) cluster analysis.

variation among populations, respectively, for a total of 87.52%. A scattergram of these two axes showed little geographical correlation (Fig. 5). Although the southern Society Islands of Tahiti and Raiatea were separated from all other populations, primarily the result of displacement along PC Axis 1, the rest of the southern and northern Pacific islands grouped at random with no apparent geographical correlation.

The hierarchical AMOVA based on sampled populations revealed that the majority of genetic variation (81%) resided within populations, 17% was distributed among populations within islands, and 3% of the variation resided among northern and southern Pacific regions (Table 2). Although the proportion of genetic variation accountable at higher levels is small (3%), all fixation indices were statistically significant. Consistent with pairwise F_{ST} values and the PCA, very little genetic variation was attributed to differences among populations between hemispheres, suggesting very little genetic differentiation on this large geographical scale. In addition, most genetic variation at this geographical scale was found at the population level.

Bayesian estimates of population structure

The model-based clustering method implemented in STRUCTURE suggested that the model with $K = 8$ (where K is the number of population genetic clusters) was substantially better than alternative models. The highest posterior probabilities for K varied among multiple runs with the associated K-values ranging from 4 to 10, demonstrating that posterior probability alone is not a good measure of the true K. Values of LnProb (data) showed a pattern of incremental increase with increasing K; leading to potential overestimates of the number of genetic clusters. Evanno *et al.* (2005) suggested the use of ΔK, which takes into account the shape of the log likelihood curve, to overcome this problem. For this study's data, $\Delta K = 8$ was 234.09, the highest value, whereas estimates for all other possible runs were less than 71.08. The genetic clusters identified by STRUCTURE, in congruence with all other estimates, supported low genetic differentiation and high similarity among populations by probabilistically assigning individuals from different hemispheres to the same genetic clusters (Fig. 6).

ISSR genetic diversity

Within- and among-population genetic diversity

The three ISSR primers generated a total of 77 scorable bands (average *c.* 26 bands/primer and range of 13–36) of which 73 (95%) were polymorphic in the 251 genotypes examined. The sizes of bands ranged from 260 base pairs (bp) to 3400 bp.

A Bayesian approach (Holsinger *et al.*, 2002) was used to calculate heterozygosity estimates. The average heterozygosity across populations was slightly higher for Hawaiian Islands populations (0.280) than for southern Pacific islands populations (0.243). The HICKORY 1.0.4. program was used to calculate, *f*, an estimate of F_{IS} (Holsinger *et al.*, 2002). On average values were high (0.91) ranging from 0.850 to 0.927 (Table 1). Holsinger & Lewis (2003) emphasized the problems associated with estimating F_{IS} from the dominant data and warned that programs such as HICKORY can lead to an overestimate of inbreeding, especially when sample sizes are small. However, given the large sample sizes, the bottleneck and high levels of inbreeding detected for microsatellite markers, the high inbreeding coefficients estimated for ISSR data are a further indication of severe inbreeding in these populations.

The Bayesian analogue of Nei's G_{ST}, G_{ST}-B, showed very low differentiation between Maui and Hawaii populations (0.063) compared to all southern Pacific islands (0.1071). Nei's unbiased nuclear genetic distances, based on the absence/presence matrix of ISSR fragments, between northern and southern populations of miconia, ranged from 0.030 (between the New Caledonia population, NewC, and Tahiti population Tah1) to 0.106 (between Moorea population Moor2 and the Nuku Hiva population, Nuk). Consistent with the codominant data, UPGMA cluster analysis based on Nei's unbiased nuclear genetic distances based on ISSR data failed to support geographical clustering. For example,

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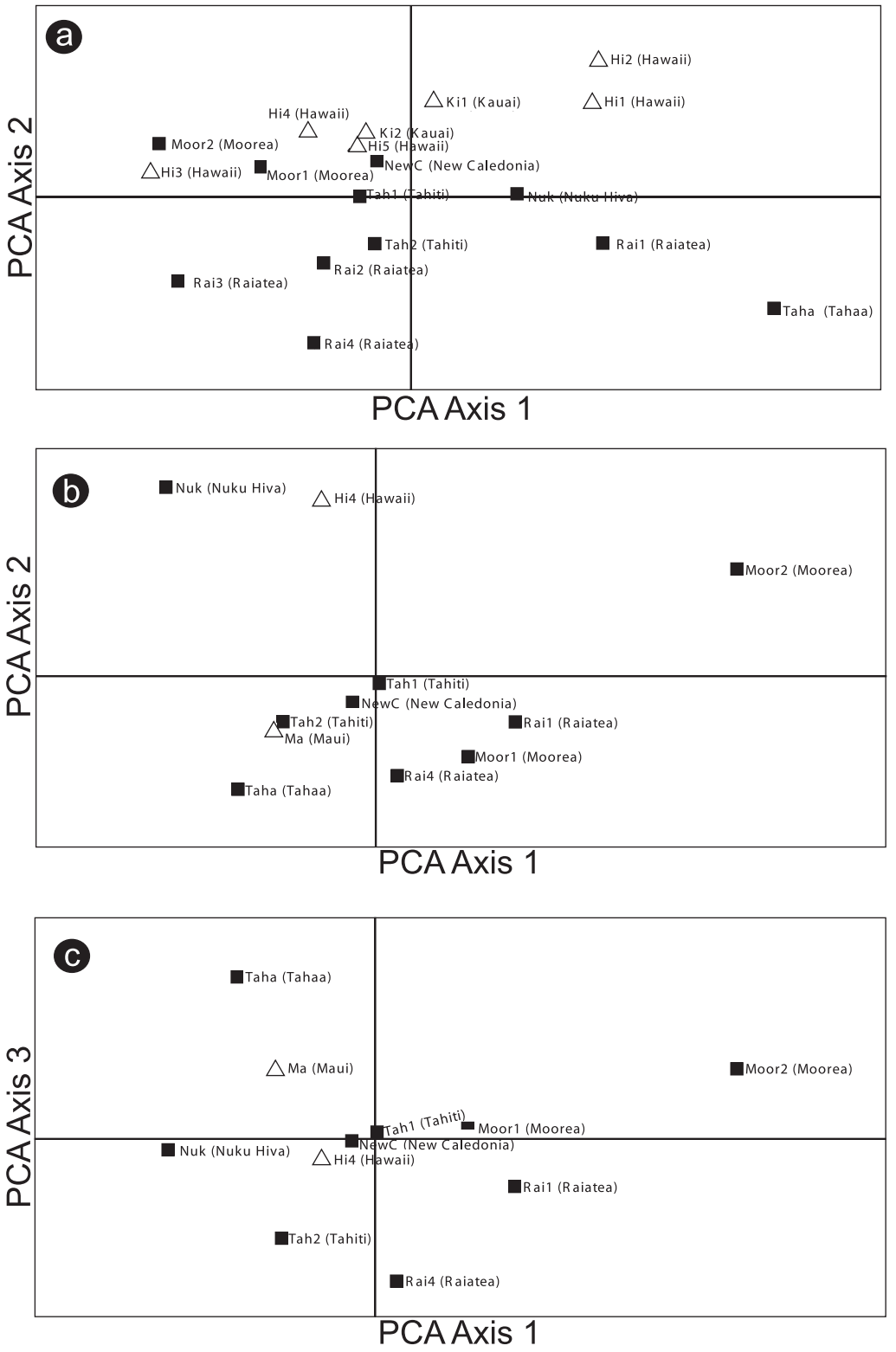

Figure 5 Scattergrams of the first two axes of a PCA of genetic variation for nine microsatellite loci (a) and the first three axes for ISSR data (b and c) based on pairwise Φ_{PT} values in *Miconia calvescens*. Symbols represent northern (open triangles) and southern (black squares) Pacific populations. Clustering of populations is concordant with Bayesian assignment based on microsatellite genotypes (see Fig. 6) and UPGMA analysis of ISSR data (see Fig. 7) in showing no apparent geographical structuring.

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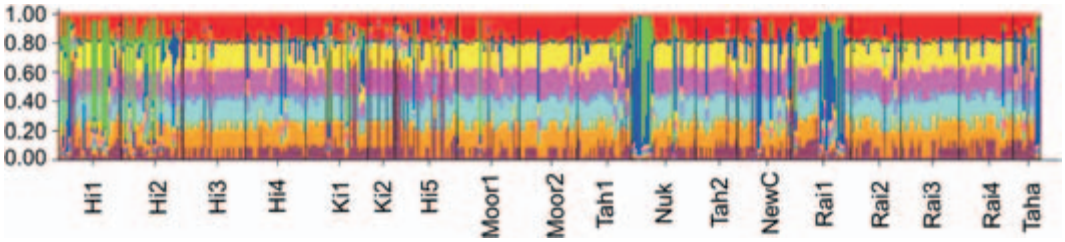

Figure 6 Population structure inferred by Bayesian assignment of northern and southern Pacific individuals of *Miconia calvescens* shown as individual membership coefficients in the STRUCTURE-identified genetic demes. *Miconia* populations can be assigned to eight geographical genetic demes that did not corresponded to geographical location.

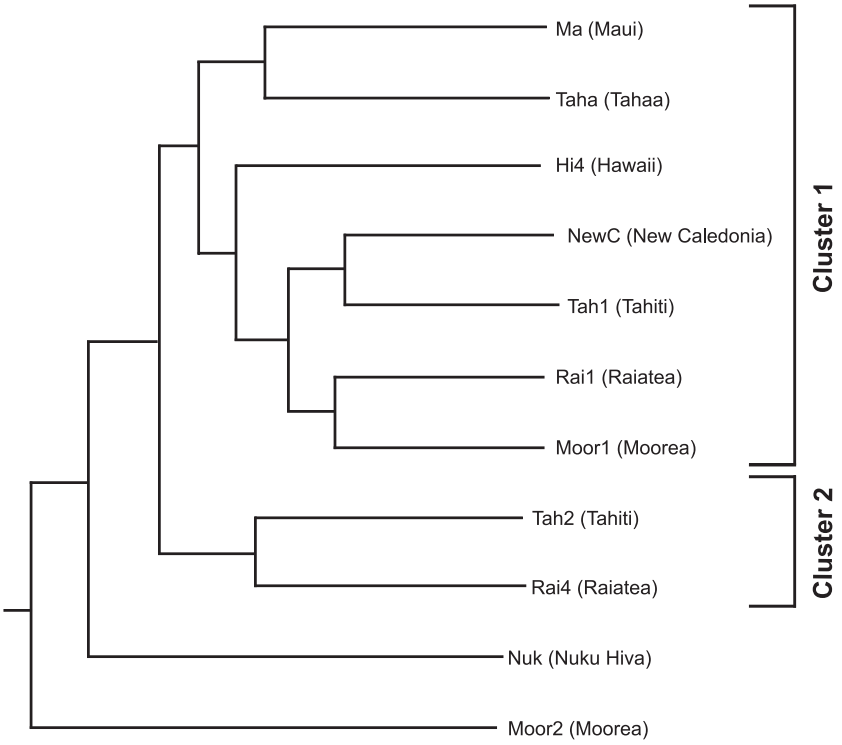

Figure 7 A dendrogram based on Nei's (1978) unbiased genetic distances (ISSR data) constructed by the using the unweighted pair-group method with arithmetic mean (UPGMA) cluster analysis.

Hawaiian populations Hi4 and Ma fell within a southern Pacific cluster (cluster 1, Fig. 7) joined by a more distantly related sister cluster of Society Island populations (cluster 2). These two clusters were basally joined by the Marquesas population, Nuk, followed by Moorea population Moor2.

Similar to microsatellite data, genetic distances (Φ_{PT}) inferred from the ISSR presence/absence matrix were used in a PCA to investigate the relative positions of populations in multidimensional space. The first three PC axes explained 27.83%, 18.83% and 18.48% of the genetic variation among populations, respectively, for a total of 65.14%. Scattergrams of these three axes, similar to that obtained for microsatellite data, showed little geographical correlation (Fig. 5). These results provide further support to the pattern of very little genetic differentiation among southern and northern Pacific populations and regions.

Furthermore, hierarchical AMOVA revealed that the majority of genetic variation (88%) resided within populations, 12% resided among populations within islands, whereas 0% of the variation can be explained by differentiation between northern and southern Pacific regions (Table 2). Once again, these results were similar to those obtained for the codominant marker data set with the exception that none of the genetic variation was partitioned among hemispheres. Although the proportion of genetic variation accountable at higher levels was zero, all fixation indices were statistically significant, supporting very little genetic differentiation on this large geographical scale.

DISCUSSION

Large geographical scales, inbreeding and low genetic differentiation

Throughout the Pacific, invasive miconia populations appear to be highly inbred and genetically similar and depauperate. Areas characterized by wide spatial distributions of large monotypic stands such as Tahiti harbours the same amount of genotypic

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variation as less invaded areas characterized by smaller nearly monotypic stands such as in the Hawaiian Islands or even areas where only a few reproductive trees have naturalized (Nuku Hiva). The obvious explanation for this observation would be a single or small founding source for all Pacific Island populations. Convincing evidence exists in support of this hypothesis for at least the southern Pacific regions. Introduced as a single tree to Tahiti in 1937 from Sri Lanka where it is also considered as naturalized, dense stands were first observed by the early 1970s in Tahiti (Meyer, 1996). During this time frame, miconia was both deliberately and accidentally introduced to the neighbouring island of Raiatea (Meyer, 1998b). Similarly, infestations on neighbouring Tahaa and more distant Nuku Hiva and New Caledonia are thought to be of Tahitian origin (Meyer, 1998b). The origin of Hawaiian miconia infestations is less clear. In Central and South America, native miconia has two distinct leaf morphological types, bi- and monochromatic, with the former restricted to the northern areas of the native range (Mexico, Guatemala and Costa Rica) and the latter to the southern parts of the native range in Argentina and southern Brazil (Csurhes, 1998). Similar to southern Pacific Islands, Hawaii was invaded by miconia with bicoloured leaves and it has been speculated that both regions' invasions are shared/have a common origin (Medeiros *et al*., 1997). The lack of genetic differentiation (i.e. high similarity) based on numerous molecular markers found in this study gives the first experimental support for a similar geographical source for infestations in both Pacific hemispheres. Alternatively, Hawaiian populations of miconia might be the result of a secondary introduction directly from Tahiti (Tracy Johnson, USDA Forest Service, personal communication). Sri Lankan populations of *M. calvescens* originated from Mexico, suggesting that Hawaiian and southern Pacific populations are of Mexican origin. To date, the identification of potential natural enemies to be used in biological control programs aimed at Pacific miconia infestations focused on the native regions in Costa Rica and Brazil and led to the identification of numerous fungal pathogens, witches' broom-inducing phytoplasmas and foliar nematodes (Killgore *et al*., 1999; Seixas *et al*., 2002, 2004). One fungus, *Colletotrichum gloeosporioides* f. sp. *miconiae*, was introduced into Hawaii and the Society Islands and became established. Post-establishment impacts are evident but not nearly sufficient to control or contain the current infestations (Meyer *et al*., 2008). Given the magnitude of miconia's native range (spanning 40° of latitude) it is reasonable to speculate that regional genotypic variants or biotypes exist and these might have different specialized and coevolved natural enemies (e.g. Goolsby *et al*., 2006). More productive and damaging control agents targeting Pacific island infestations are thus more likely to be found in miconia's native ranges in Mexico.

Founders for both northern and southern Pacific regions consisted of only a few individuals or potentially a single plant and, maybe not surprisingly, resulted in invasive populations that are severely bottlenecked. Self-pollination maintains low genetic diversity, especially within miconia populations. Coupled with active seed dispersal by frugivorous birds, it is likely that outlying foci may frequently result from a single propagule. Furthermore, miconia reproduces trimodally each year with mature trees (between 4–5 years of age) capable of producing up to 8 million seeds per cycle (Meyer, 1998a). Seed banks are dense (> 50,000 seeds/m^2), dormant under shaded conditions and remain viable for up to 14 years (J-Y. Meyer, pers. obs.), stimulated by light only when canopy gaps open (Medeiros *et al*., 1997). The resulting generation overlap will increase the effective generation time, preventing genetic decay, the formation of spatial genetic structure between geographically distinct populations and the ability of drift to drive unique alleles to fixation (Loveless & Hamrick, 1984).

Genetically depauperate populations have particularly low adaptive potential and are vulnerable to the effects of subadapted conditions. This suggests that miconia is unlikely to rapidly evolve resistance against effective control mechanisms, including biological control. The presence of high genetic diversity and the subsequent rapid evolution of resistance against control mechanisms have been demonstrated for numerous invasive species. For example, cordgrass, *Spartina alterniflora*, harbours genetic variation in both tolerance and resistance to its introduced biological control agent (Garcia-Rossi *et al*., 2003) whereas the pea aphid, *Acyrthosiphon pisum*, shows genetic variation in resistance to parasitism by its parasitoid (Hufbauer & Via, 1999).

The role of preadapted genotypes in invasion success

Given the high levels of inbreeding and low genetic diversity observed here, miconia's success throughout the Pacific can not be ascribed to high genetic diversity but rather reflect the consequences of pre-adapted genotypes or wide environmental tolerances (phenotypic plasticity), or a combination of both. A lack of genetic variation has been demonstrated for several highly invasive plant species that have wide ecological distributions such as alligator weed (*Alternanthera philoxeroides* [Xu *et al*., 2003]), Japanese knotweed (*Fallopia japonica* [Hollingsworth & Bailey, 2000]) and fountain grass (*Pennisetum setaceum* [Poulin *et al*., 2005; Le Roux *et al*., 2007]). Here, we postulate that pre-adaptations for various life history and physiological traits ensure high fitness and success of miconia throughout its introduced ranges in the Pacific.

Firstly, the mixed breeding system of miconia conforms to Baker's rule (1967) that would allow for a single propagule to disperse, establish foci and spread into the new environment. Although selfing renders reproductive assurance, occasional outcrossing events may relieve the effects of inbreeding depression as a result of selfing. Furthermore, the enormous number of small seeds produced by single mature trees is dispersed both actively and passively. The correlation between reproductive traits such as small seed size, high dispersion capability and invasion success has previously been demonstrated (Rejmanek & Richardson, 1996).

The large leaves of *M. calvescens* (up to 1 m in length) and the absence of its natural foliar herbivores and pathogens would furthermore give it a photosynthetic advantage over Hawaiian natives. Various Hemiptera, Lepidoptera and Coleoptera natural enemies have been found to cause substantial damage to miconia

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plants in native ranges (Picanço *et al.*, 2005). *Clidemia hirta*, another melastome invader in Hawaii in the same tribe, Miconieae, than miconia, showed evidence for enemy release in Hawaii compared to native Costa Rican populations (Dewalt *et al.*, 2004). Dewalt *et al.* (2004) found substantially more damage caused to *C. hirta* by both fungal and insect enemies in native Costa Rican environments compared to invasive environments in Hawaii. Even in the event of enemy release, the highly inbred Pacific populations of miconia are unlikely to have rapidly evolved increased competitive ability (Lee, 2002) by reallocating resources used for defense against natural enemies to growth and reproduction.

Previous work has illustrated that phenotypic plasticity exists in miconia saplings under different light and water stress conditions (Baruch *et al.*, 2000). This may not be surprising given the differences in growth responses of saplings in response to light availability under natural conditions. Plasticity might further contribute to miconia's success in Pacific islands.

Given a species that evolved strategies to cope with competition under low light environments, low genetic diversity throughout its introduced range and favourable environmental conditions in all the introduced ranges, it appears that pre-adapted physiological, morphological and life history traits are the main factors driving miconia's invasion success. The success of plant biological invasions afforded by the introduction of such competitively superior pre-adapted genotypes is well documented (e.g. see Woitke & Diets, 2002; Hurka *et al.*, 2003).

CONCLUSIONS

Miconia calvescens in the Pacific represents an invader with a suit of pre-adapted traits that virtually guarantee high fitness and competitive success in unsaturated tropical insular forest habitats. High output of small seeds and various means of effective dispersal coupled with a mixed breeding system contribute to this species' success throughout the Pacific. This is best manifested by French Polynesian invasions that resulted from a lone tree introduced to Tahiti in 1937. An evolutionary history under shade and dense forest conditions favoured traits that are superior to those in natives from less saturated forests characteristic of the Hawaiian and Society Islands. Coupled with genetic similarity of miconia populations from the Hawaiian and Society Islands, the highly comparable climate, geology, topography, relative geographical location and biota should caution Hawaiian authorities against the potential magnitude of invasions if current control methods are relaxed or outlying foci left unchecked. Recently Kaiser (2006) illustrated the enormous economic repercussions if the spread of miconia is left unabated in Hawaii.

Whether low genetic diversity is a characteristic of miconia populations in general, even in native ranges, remains unknown. Because of the logistical difficulties and budgetary needs we were unable to include native range populations here but collaborations are currently in place with scientists in Costa Rica, Brazil and Australia to investigate genetic diversity in native miconia populations. Whereas a founding event by a single tree (Meyer, 1996) and the subsequent low levels of genetic diversity within and among Society Island populations of miconia found here are maybe not that surprising it has some serious implications: miconia has been introduced to relatively few tropical islands in the Pacific and the accidental introduction of a few or even a single seed into favourable habitats could lead to high invasive success.

Finally, the current differential distribution and spread in northern and some of the southern Pacific regions seems to be the result of two factors. First, rigorous containment and control efforts have been in place for over a decade against miconia infestations in the Hawaiian Islands compared to the relatively less rigorous attempts in the southern Pacific regions. Second, lag phases (*c.* 30 year) associated with miconia infestations in the Pacific are not the result of the accumulation of adaptive genetic diversity or of natural selection on such diversity but rather appear to reflect the consequences of absolute growth rates that accompany small founding populations. Assuming initial establishment of a single propagule, it would take approximately 30 years for a dense population of between 100–1000 trees to establish. If one assumes that 5–10 years as the average time to form a reproductive tree from one seed, 10–20 years will be necessary to build the second generation of approximately 10–100 reproductive trees (Meyer, 1998b). After a period of time between 15–30 years, the third generation will be formed by a dense cover of about 100–1000 trees. This is consistent with observations on the plateau of Taravao in Tahiti, where, in the early 1970s, nearly monotypic stands were first observed about 30 years after its introduction (Meyer, 1998b). Differences in introduction times (*c.* 24 years) have likely played an important role in the differences observed in miconia infestations in northern and southern Pacific Islands.

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REFERENCES

Baker, H.G. (1967) Support for Baker's law – As a rule. *Evolution*, **21**, 853–856.

Banerjea, A., Phillips, S.M., Dorudi, S. & Bustin, S.A. (2003) Colorectal cancers with mononucleotide microsatellite instability can be identified using microfabricated chip technology. *Analytical Biochemistry*, **322**, 130–133.

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Baruch, Z., Pattison, R.P. & Goldstein, G. (2000) Responses to light and water availability of four invasive Melastomataceae in the Hawaiian islands. *International Journal of Plant Science*, **161**, 107–118.

Conant, P. (1996) New Hawaiian pest plant records for 1995. *Bishop Museum Occasional Papers*, **46**, 1–2.

Cornuet, J.M. & Luikart, G. (1997) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics*, **144**, 2001–2014.

Csurhes, S.M. (1998) *Miconia calvescens*, a potentially invasive plant in Australia's tropical and sub-tropical rain forests. *Proceedings of the first regional conference on miconia control* (ed. by J.-Y. Meyer and C.W. Smith), pp. 72–77. Gouvernement de Polynésie française/University of Hawaii at Manoa/Centre ORSTOM de Tahiti.

Dewalt, S.J., Denslow, J.S. & Ickes, K. (2004) Natural-enemy release facilitates habitat expansion of the invasive tropical shrub *Clidemia hirta*. *Ecology*, **85**, 471–483.

Ewel, J.J. (1986) Invasibility: lessons from South Florida. *Ecology of biological invasions of North America and Hawaii* (ed. by H.A. Mooney and J.A. Drake), pp. 214–230. Springer-Verlag, New York.

Excoffier, L., Smouse, P.E. & Quattro, J.M. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Evanno, G., Regnaut, S. & Goudet, J. (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, **14**, 2611–2620.

Falush, D., Stephens, M. & Pritchard, J.K. (2007) Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Molecular Ecology Notes*, **7**, 574–578.

Frankham, R. (2005) Resolving the genetic paradox in invasive species. *Heredity*, **94**, 385.

Gagné, B.H., Loope, L.L., Medeiros, A.C. & Anderson, S.J. (1992) *Miconia calvescens*: a threat to native forests of the Hawaiian Islands. *Pacific Science*, **46**, 390–391.

Guo, S.W. & Thompson, E.A. (1992) A Monte Carlo method for combined segregation and linkage analysis. *American Journal of Human Genetics*, **51**, 1111–1126.

Garcia-Rossi, D., Rank, N. & Strong, D.R. (2003) Potential for self-defeating biological control. Variation in herbivore vulnerability among invasive *Spartina* genotypes. *Ecological Applications*, **13**, 1640–1649.

Genton, B., Shykoff, J.A. & Giraud, T. (2005) High genetic diversity in French invasive populations of common ragweed *Ambrosia artemisiifolia* as a consequence of multiple sources of introduction. *Molecular Ecology*, **14**, 4275–4285.

Goolsby, J.A., De Barro, P.J., Makinson, J.R., Pemberton, R.W., Hartley, D.M. & Frohlicj, D.R. (2006) Matching the origin of an invasive weed for the selection of a herbivore haplotype for a biological control programme. *Molecular Ecology*, **16**, 287–297.

Hollingsworth, M.L. & Bailey, J.P. (2000) Evidence for massive clonal growth in the invasive weed Fallopia japonica (Japanese knotweed). *Botanical Journal of the Linnean Society*, **133**, 463–472.

Holsinger, K.E., Lewis, P.O. & Dey, D.K. (2002) A Bayesian approach to inferring population structure from dominant markers. *Molecular Ecology*, **11**, 1157–1164.

Holsinger, K.E. & Lewis, P. (2003) *HICKORY: a package for analysis of population genetic data V1.0*. Department of Ecology and Evolutionary Biology. University of Connecticut, Storrs, Connecticut, USA. Available at http://www.eeb.uconn.edu.

Huey, R.B., Gilchrist, G.W., Carlson, M.L., Berrigan, D. & Serra, L. (2000) Rapid evolution of a geographic cline in size in an introduced fly. *Science*, **287**, 308–309.

Hufbauer, R.A. & Via, S. (1999) Evolution of an aphid-parasitoid interaction: variation in resistance to parasitism among aphid populations specialized on different plants. *Evolution*, **53**, 1435–1445.

Huff, D.R., Peakall, R. & Smouse, P.E. (1993) RAPD variation within and among natural populations of outcrossing buffalograss *Buchloe dactyloides* (Nutt) Engelm. *Theoretical and Applied Genetics*, **86**, 927–934.

Hurka, H., Bleeker, W. & Neuffer, B. (2003) Evolutionary processes associated with biological invasions in the Brassicaceae. *Biological Invasions*, **5**, 281–292.

Kaiser, B. (2006) Economic impacts of non-indigenous species: *Miconia* and the Hawaiian economy. *Euphytica*, **148**, 135–150.

Killgore, E.M., Sugyiama, L.S., Barreto, R.W. & Gardner, D.E. (1999) Evaluation of *Colletotrichum gloeosporioides* for biological control of *Miconia calvescens* in Hawaii. *Plant Disease*, **83**, 964.

Le Roux, J.J. & Wieczorek, A.M. (2008) Isolation and characterization of polymorphic microsatellite markers from velvet tree, *Miconia calvescens* DC. (Melastomataceae). *Molecular Ecology Resources*, doi: 10.1111/j.1755-0998.2008.02102.x

Le Roux, J.J., Wieczorek, A.M., Wright, M.G. & Tran, C.T. (2007) Super-genotype: global monoclonality defies the odds of nature. *PloS ONE*, **2**, e590.

Lee, C.E. (2002) Evolutionary genetics of invasive species. *Trends in Ecology and Evolution*, **17**, 386–391.

Loveless, M.D. & Hamrick, J.L. (1984) Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics*, **15**, 65–95.

Luikart, G. & Cornuet, J.M. (1998) Empirical evaluation of a test for identifying recently bottlenecked populations from alleles frequencies data. *Conservation Biology*, **12**, 228–237.

Luikart, G., Sherwin, W., Steele, B.M. & Allendorf, F.W. (1998) Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic change. *Molecular Ecology*, **7**, 963–974.

Maguire, T.L., Peakall, R. & Saenger, P. (2002) Comparative analysis of genetic diversity in the mangrove species *Avicennia marina* (Forsk.) Vierh. (Avicenniaceae) detected by AFLPs and SSRs. *Theoretical and Applied Genetics*, **104**, 388–398.

Maron, J.L., Vilà, M., Bommarco, R., Elmendorf, S. & Beardsley, P. (2004) Rapid evolution of an invasive plant. *Ecological Monograph*, **74**, 261–280.

Medeiros, A.C., Loope, L.L., Conant, P. & McElvaney, S. (1997) Status, ecology, and management of the invasive plant *Miconia calvescens* DC (Melastomataceae) in the Hawaiian Islands. *Bishop Museum Occasional Paper*, **48**, 23–36.

© 2008 The Authors

Meyer, J.-Y. (1996) Status of *Miconia calvescens* (Melastomataceae), a dominant invasive tree in the Society Islands (French Polynesia). *Pacific Science*, **50**, 66–76.

Meyer, J.-Y. (1998a) Observations on the reproductive biology of *Miconia calvescens* DC (Melastomataceae), an alien invasive tree on the island of Tahiti (South Pacific Ocean). *Biotropica*, **30**, 609–624.

Meyer, J.-Y. (1998b) Epidemiology of the invasion by *Miconia calvescens* and reasons of a spectacular success. *Proceedings of the first regional conference on Miconia control* (ed. by J.-Y. Meyer and C.W. Smith), pp. 4–26. Gouvernement de Polynésie française/ University of Hawaii at Manoa/Centre ORSTOM de Tahiti.

Meyer, J.-Y. & Florence, J. (1996) Tahiti's native flora endangered by the invasion of *Miconia calvescens* DC. (Melastomataceae). *Journal of Biogeography*, **23**, 775–783.

Meyer, J.-Y., Taputuarai, R. & Killgore, E.M. (2008) Dissemination and impact of the fungal pathogen *Colletotrichum gloeosporioides* f. sp. *miconiae* on the invasive alien tree *Miconia calvescens* (Melastomataceae) in the rain forests of Tahiti (French Polynesia, South Pacific). *Proceedings of the XII international symposium on biological control of weeds, La Grande Motte, France* (ed. by M.H. Julien, R. Sforza, M.C. Bon, H.C. Evans, P.E. Hatcher, H.L. Hinz and B.G. Rector). CAB International Wallingford.

Nei, M. (1973) Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences* USA, **70**, 3321–3332.

Nei, M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**, 583–590.

Peakall, R. & Smouse, P.E. (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, **6**, 288–295.

Picanço, M.C., Barreto, R.W., Fidelis, E.G., Semeão, A.A., Rosado, J.F., Moreno, S.C., de Barros, E.C., Silva, G.A. & Johnson, T. (2005) Biological control of *Miconia calvescens* by phytophagous arthropods. *Pacific Cooperative Studies Unit University of Hawaii at Manoa, Technical Report 134*.

Poulin, J., Weller, S. & Sakai, A. (2005) Genetic diversity does not affect the invasiveness of fountain grass (*Pennisetum setaceum*) in Arizona, California and Hawaii. *Diversity and Distributions*, **11**, 241–247.

Pritchard, J.K. & Wen, W. (2003) *Documentation for* STRUCTURE *software: version 2*. Available from http://pritch.bsd.uchicago.edu.

Pritchard, J.K., Stephens, M. & Donnelly, P. (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Rambuda, T.D. & Johnson, S.D. (2004) Breeding systems of invasive alien plants in South Africa: does Baker's rule apply? *Diversity and Distributions*, **10**, 409–416.

Rejmanek, M. & Richardson, D.M. (1996) What attributes make some plant species more invasive? *Ecology*, **77**, 1655–1661.

Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J., Baughman, S., Cabin, R.J., Cohen, J.E., Ellstrand, N.C., McCauley, D.E., O'Neil, P., Parker, I.M., Thompson, J.N. & Weller, S.G. (2001) The population biology of invasive species. *Annual Review of Ecology and Systematics*, **32**, 305–332.

Schneider, S., Roessli, D. & Excoffier, L. (2000) ARLEQUIN *version 2.000: a software for population genetic data analysis*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Seixas, C.D.S., Barreto, R.W. & Matsuoka, K. (2002) First report of a phytoplasm-associated witches' broom disease of *Miconia calvescens*. *Plant Pathology*, **5**, 801–801.

Seixas, C.D.S., Barreto, R.W., Freitas, L.G., Monteiro, F.T. & Oliviera, R.D.L. (2004) *Ditylenchus drepanocercus* rediscovered in the neotropics causing angular leaf spots on *Miconia calvescens*. *Journal of Nematology*, **36**, 481–486.

Sexton, J.P., McKay, J.K. & Sala, A. (2002) Plasticity and genetic diversity may allow saltcedar to invade cold climates in North America. *Ecological Applications*, **12**, 1652–1660.

Woitke, M. & Diets, H. (2002) Shifts in dominance of native and invasive plants in experimental patches of vegetation. *Perspectives in Plant Ecology Evolution and Systematics*, **5**, 165–184.

Wolfe, L.M. (2002) Why alien invaders succeed: support for the escape-from-enemy hypothesis. *The American Naturalist*, **160**, 705–711.

Wright, S. (1965) The interpretation of population structure by *F*-statistics with special regard to systems of mating. *Evolution*, **19**, 395–420.

Xu, C.Y., Zhang, W.J., Fu, C.Z. & Lu, B.R. (2003) Genetic diversity of alligator weed in China by RAPD analysis. *Biodiversity and Conservation*, **12**, 637–645.

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