Aquatic Botany 90 (2009) 296–302

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

Concordance between life history traits, invasion history, and allozyme diversity of the Everglades invader *Melaleuca quinquenervia*☆

F. Allen Dray Jr.[a,*], Rebecca E. Hale[b], Paul T. Madeira[a], Bradley C. Bennett[c], Ted D. Center[a]

[a] U.S. Department of Agriculture, Agricultural Research Service, Invasive Plant Research Lab, 3225 College Avenue, Fort Lauderdale, FL 33314, USA
[b] Florida State University, Department of Biological Sciences, Tallahassee, FL 32306, USA
[c] Florida International University, Department of Biological Sciences and Center for Ethnobiology and Natural Products, University Park, Miami, FL 33199, USA

ARTICLE INFO

Article history:
Received 6 June 2008
Received in revised form 19 November 2008
Accepted 20 November 2008
Available online 27 November 2008

Keywords:
Allozyme
Biological control
Cellulose acetate gel electrophoresis
Genetic diversity
Invasive species
Life history trait
Melaleuca quinquenervia
Myrtaceae

ABSTRACT

During the century following its initial introduction in 1886, the Australian tree *Melaleuca quinquenervia* (Myrtaceae) dispersed from a few introduction points to occupy over 200,000 ha, primarily in historic Everglades wetlands of southern Florida. Cellulose acetate gel electrophoresis (CAGE) was used to investigate the allozyme diversity and population genetic structure of 208 individuals in a dozen populations resulting from this invasion. The analyses showed that these populations have a high (82%) rate of polymorphic loci and an average of 2 alleles/locus. There was substantial heterozygosity (mean H_e = 0.356), which concords well with recent studies reporting a greater number of introduction events and sources than generally recognized. The introduction history and distributional patterns within Florida have led to geographic structuring (G_{ST} = 0.419) in which the Gulf Coast metapopulation has a greater effective number of alleles and greater heterozygosity than the Atlantic Coast metapopulation. The gene diversity in *M. quinquenervia* was comparable to other tropical woody species. Its strong population divergence was reminiscent of pioneer species and consistent with its status as a plant invader in Florida.

Published by Elsevier B.V.

1. Introduction

Conventional wisdom long held that founder effects and genetic bottlenecks result in pioneer populations of invading species which harbor reduced levels of genetic variation relative to source populations (Sakai et al., 2001; Novak and Mack, 2005). The loss of genetic diversity restricts the suite of potential responses to environmental variation and stochastic events (Barrett, 2000), thus rendering small founder populations more susceptible to extinction (Hanski and Gilpin, 1997). This may help explain why many invaders fail to establish persistent populations.

Researchers have more recently recognized that successful invasions commonly are associated with multiple introduction events (Novak and Mack, 2005). Multiple introductions from a broader gene pool would be expected to reduce founder effects and result in substantial genetic differentiation among invasive populations (Dlugosch and Parker, 2008). A number of studies have described populations of naturalized, introduced species that exhibit substantial amounts of genetic variation in their adventive ranges (e.g., Ward, 2006; Facon et al., 2008). Genetic variation and population differentiation can strongly influence how species will respond to control attempts and to environmental changes. Thus, it is increasingly being recognized that understanding the extent of this variation is an important component in the arsenal of information required to properly manage invaders (Sobhian et al., 2003).

Melaleuca quinquenervia (Cav.) Blake is a large paperbark tree predominantly occurring in seasonally and permanently inundated wetlands in its native Australia. The species was introduced into the United States over a century ago, but naturalized quickly (Dray et al., 2006). It ultimately invaded over 200,000 ha of Florida's prized Everglades ecosystem, replacing diverse wetland communities with dense monocultures that have little value to native species (Bodle et al., 1994; O'Hare and Dalrymple, 1997). It has long been assumed that *M. quinquenervia* in Florida derived primarily from two sources (Meskimen, 1962; Kaufman and Smouse, 2001). Populations along Florida's Atlantic Coast purportedly arose from seeds imported by John Gifford in 1906 from the Royal Botanical Gardens in Sydney, Australia. Populations along the Gulf Coast presumably came from seeds imported by A.H. Andrews from a Melbourne, Australia, seed house during 1912. With a small number of founder events, and the likelihood of high

☆ Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

* Corresponding author. Tel.: +1 954 475 6556; fax: +1 954 476 9169.
E-mail address: allen.dray@ars.usda.gov (F.A. Dray Jr.).

0304-3770/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.aquabot.2008.11.006

levels of inbreeding resulting from a paucity of suitable pollinators, Florida populations have generally been assumed to harbor limited genetic diversity.

Recent scrutiny of early horticultural catalogs and USDA plant introduction records has expanded the number of sources known to have contributed to populations along both coasts (Dray et al., 2006). These same data indicate that progeny from several pioneer populations have been widely distributed throughout the state. Further, an influx of indiscriminant pollinators occurred during the second quarter of the 20th century when apiculturists began overwintering their honey bee colonies in southern Florida (Wilder, 1940). It thus seems reasonable to propose that Florida populations of *M. quinquenervia* harbor greater genetic diversity than previously anticipated.

This study describes the allozyme diversity in 12 populations of *M. quinquenervia* distributed throughout its naturalized range in Florida, and is part of a larger effort to understand factors that may influence biological control agent establishment and efficacy. Our results confirmed hypotheses (based on invasion history, life history characteristics, and observed trait variation) that populations of *M. quinquenervia* in Florida would harbor substantial genetic diversity and show strong population differentiation. Population genetic patterns also corroborated hypotheses regarding possible invasion routes for a previously unprovenanced population.

2. Material and methods

2.1. Plant material

Infructescences were collected from up to 20 open-pollinated individuals at each of 12 *M. quinquenervia* populations. Some early introduction sites had fewer than 20 reproductive individuals in which case all available trees were sampled. Samples were restricted to the third/fourth youngest infructescences because these cohorts have the highest seed viabilities (Rayamajhi et al., 2002). The infructescences from each individual were bagged separately and transported to the laboratory where they were allowed to dehisce. Seeds were planted in germination flats containing ProGro Potting Soil for Starting Seeds (Scotts-Sierra Horticultural Products, Marysville, OH). The flats were kept in shallow (8 cm) water-filled trays to supply constant moisture. The flats were replaced by 0.5 L pots into which the seedlings were transplanted after 6 weeks. Each tray received 1 L of fertilizer solution (Excel 15-5-15 plus Mg, Ca, and minors; Scotts-Sierra Horticultural Products, Marysville, OH; mix: 0.26 tbsp/L) on the day of planting and again after 90 days. Leaves for this study were harvested from the seedlings on week 20.

2.2. Allozyme analysis

We collected approximately 40 mg of leaf material from each seedling and sampled one seedling per maternal lineage. The leaf material from each seedling was stored at −80 °C. Enzymes were extracted using the Butcher et al. (1992) modification of the Cheliak and Pitel (1984) extraction buffer which was prepared in advance, adjusted to pH 7.0, and stored at 10 °C. Bovine serum albumin and sucrose (at 10 and 100 mg mL^{-1}, respectively) were added immediately prior to extraction. To extract the enzymes, the frozen leaf tissue was homogenized in 1 mL of extraction buffer using a chilled pestle and mortar. The resulting slurry was centrifuged for 5 min at 13,500 × *g*, after which the supernatant was drawn off and stored at −80 °C.

Allozymes were separated using cellulose acetate gel electrophoresis (CAGE) and a Tris-Glycine (TG) continuous buffer system. Gel plates (76 mm × 76 mm) were soaked overnight in 0.5× TG buffer prior to use. Electrophoresis was carried out at room temperature, and lasted 30–45 min at 200 V with a current of 6 mA. Enzyme activity was detected using stains described in Hebert and Beaton (1993), occasionally modified following Acquaah (1992). Where possible, stain components were prepared in advance and stored frozen until needed.

Preliminary evaluations of 11 enzyme systems yielded seven that resolved in clear and consistent banding patterns: aspartate aminotransferase [AAT; EC (Enzyme Commission) 2.6.1.1], arginine kinase (ARK; EC 2.7.3.3), glucose-6-phosphate dehydrogenase (GPI; EC 5.3.1.9), malate dehydrogenase (MDH; EC 1.1.1.37), mannose-6-phosphate dehydrogenase (MPI; EC 5.3.1.8), phosphoglucomutase (PGM; EC 2.7.5.1), and trehalase (TRE; EC 3.2.1.28). A total of ten loci were scored: *Aat-1, Ark-1, Ark-2, Gpi-1, Gpi-2, Mdh-1, Mpi-1, Pgm-1, Pgm-2,* and *Tre-2*.

2.3. Data analysis

Allele frequencies were calculated from genotype arrays, and PopGene (version 1.32; Yeh et al., 1999), was used to calculate standard measures of genetic diversity including the average number of alleles per locus (A) and per polymorphic locus (A_P), the effective number of alleles (A_e), observed heterozygosity (H_o) and an unbiased estimate of expected heterozygosity (H_e; Nei, 1978). The latter two parameters were used to calculate local inbreeding coefficients (Hartl, 2000). Allele frequency estimates for the Royal Palm Nursery site were excluded from these analyses because only two reproductive trees were available at the site. Sample sizes were small (20 or fewer individuals per population), so loci were considered polymorphic when the most frequent allele occurred in less than 95% of the individuals in a population (Nei, 1987). This was expressed as the average proportion of polymorphic loci (P_p). *t*-Tests were used to compare mean allelic variation of the populations occurring along Florida's Atlantic Coast with those along the Gulf Coast—the principle distributional pattern of *M. quinquenervia* in the state.

Genetic structure was examined using Wright's *F*-statistics as modified by Goudet (2002) in the software program FSTAT. Overall genetic variation (H_t) was partitioned into gene diversity within subpopulations (H_s) and among subpopulations (D_{st}) using Nei's genetic diversity indices. Among-population diversity was expressed relative to overall gene diversity as the coefficient of gene differentiation ($G_{st} = D_{st}/H_t$; Nei, 1987). The metrics D_{st} and G_{st} are both dependent on the number of subpopulations (Goudet, 2002), which in the present study was only twelve. To compensate for this small sample size, Goudet's adjusted measures for these metrics were employed. Among-population differences in allele frequency at each locus were examined using chi-square analysis: $\chi^2 = 2NG_{st}(a-1)$, $df = (a-1)(n-1)$, where N is total number of individuals examined, a is the number of alleles at a locus, and n is the number of populations (Workman and Niswander, 1970).

Geographic trends in genetic structure were investigated through hierarchical analysis of population structure. For this analysis, G_{st} was calculated for the two regions (Atlantic and Gulf coasts) using FSTAT. Total G_{st} was then parsed into two components: diversity among regions and diversity among-populations within regions, following Butcher et al. (1992). We also looked for correlations between genetic distance and Euclidian distance by regressing pairwise $F_{st}/(1-F_{st})$ against the natural log of geographic distance (in km) between sites using the program SigmaPlot (version 11.0; Systat Software Inc., San Jose, CA). An unweighted pair-group clustering based on arithmetic averages (UPGMA) of Nei's genetic distances was generated with PopGene. Clustering programs force objects into clusters whether or not these clusters actually exist, so we tested the goodness of fit

Table 1
Origins of the seedlots used to assess allozyme variation in *Melaleuca quinquenervia* in Florida. Sites 2, 3, 6, 11, and 12 represent early importations. Sites 1, 2, 4, 5, 7, and 8 comprise the Atlantic Coast metapopulation. Sites 3, 9, 10, 11, and 12 comprise the Gulf Coast metapopulation.

Site no.	Site name	County	No. of trees	Latitude	Longitude
1	Conservation Area 2A	Broward	20	26° 09.46′ N	80° 21.95′ W
2	Lange Park	Broward	20	26° 03.80′ N	80° 14.00′ W
3	Royal Palm Nursery	Manatee	2	27° 26.80′ N	82° 32.77′ W
4	Loxahatchee National Wildlife Refuge	Palm Beach	20	26° 29.80′ N	80° 16.36′ W
5	Everglades National Park	Miami-Dade	20	25° 41.33′ N	80° 29.83′ W
6	Lake Okeechobee	Glades	12	26° 47.04′ N	80° 57.14′ W
7	Krome Avenue	Miami-Dade	20	25° 55.83′ N	80° 27.03′ W
8	Gramercy Park	Palm Beach	20	26° 45.61′ N	80° 07.24′ W
9	Corkscrew Wellfields	Collier	20	26° 27.49′ N	81° 42.10′ W
10	Estero Bay State Buffer Preserve	Lee	20	26° 28.15′ N	81° 53.84′ W
11	Koreshan State Park	Lee	18	26° 26.13′ N	81° 48.71′ W
12	Caribbean Gardens	Collier	16	26° 10.20′ N	81° 47.28′ W

of the clustering by comparing the cophenetic value matrix to the original distance matrix using NTSYSpc (Rohlf, 2000).

3. Results

3.1. Sampled populations

Five of the populations (Lange Park, Royal Palm Nursery, Lake Okeechobee, Koreshan State Park, and Caribbean Gardens; see Table 1) selected for sampling were chosen to represent early *M. quinquenervia* importations in Florida (Dray et al., 2006). A further six sites were selected to ensure that sampled populations were distributed throughout the adventive range of *M. quinquenervia* in southern Florida. One additional site (Estero Bay Preserve) was selected to represent populations that grow in brackish waters (most melaleuca populations in Florida, as in Australia, occur in permanently or seasonally inundated freshwater communities). Voucher specimens from the study populations are deposited in the Australian National Herbarium (FRI), where Dr. Lyn Craven confirmed our determinations, and at Fairchild Tropical Botanical Gardens (FTG).

3.2. Allelic variation

Three of the 36 alleles identified in Florida populations of *M. quinquenervia* were rare, occurring at frequencies of 0.05 or less for the species as a whole. Interestingly, each of these alleles (*Pgm-1c*, *Mpi-1c*, and *Gpi-2a*) was rare in populations along the Atlantic Coast but entirely absent from populations along the Gulf Coast. One additional allele (*Tre-2a*) that was absent from Gulf Coast populations was relatively common in Atlantic Coast populations. Each of the ten loci included in this study was polymorphic in at least one of the populations surveyed. Overall, these loci were polymorphic (frequency of most common allele < 0.95) 81.6% of the time (Table 2). There was no difference between Atlantic and Gulf Coast populations in number of monomorphic loci ($\chi^2 = 0.80$, $P = 0.492$).

Allelic richness as measured by mean number of alleles per locus (A) varied from 1.8 to 2.5 (mean = 2.00) in Florida, whereas the effective number of alleles (A_e) varied from 1.48 to 1.89 (mean = 1.73). Absence of the rare alleles in Gulf Coast populations contributed to regional differences in A_e (1.82 vs. 1.66 on Atlantic Coast; $t = 2.85$, $P = 0.019$). Polymorphic loci (A_P) averaged 2.49 alleles (Table 2). Observed heterozygosity (H_o) within each population was similar to expected heterozygosity (H_e; $t = 0.16$, $P = 0.872$), although mean H_o was substantially greater on the Gulf Coast than the Atlantic Coast (Table 2; $t = 3.11$, $P = 0.012$). The inbreeding coefficients show some populations had heterozygote deficits and others excesses (Table 2). This differed geographically, as the Atlantic Coast metapopulation exhibited a heterozygote deficit but the Gulf Coast metapopulation showed an excess. The species as a whole exhibited a slight heterozygote deficit (Table 2).

Fifty-one of 96 individual fixation indices were significantly different from zero ($P < 0.05$), which is ten-fold greater than would be expected by chance alone. So, individual loci and populations did not meet Hardy–Weinberg expectations. Fixation indices for *Mdh-1*

Table 2
Estimates of genetic diversity for 11 populations (Royal Palm Nursery is excluded—see text for explanation) of *Melaleuca quinquenervia* in Florida based on 10 allozyme loci.

	P_p (%)	A	A_p	A_e	H_o (±S.D.)	H_e (±S.D.)	F
Population variation							
1	90.0	2.50	2.67	1.78	0.280 (0.331)	0.405 (0.196)	0.309
2	87.5	2.25	2.43	1.71	0.309 (0.283)	0.359 (0.228)	0.139
4	70.0	2.20	2.71	1.68	0.237 (0.351)	0.306 (0.278)	0.225
5	70.0	2.40	3.00	1.67	0.310 (0.378)	0.319 (0.275)	0.028
6	80.0	2.30	2.62	1.89	0.567 (0.368)	0.418 (0.253)	−0.356
7	100.0	2.50	2.50	1.67	0.325 (0.310)	0.334 (0.215)	0.027
8	60.0	1.80	2.33	1.48	0.290 (0.395)	0.252 (0.251)	−0.151
9	70.0	2.10	2.43	1.84	0.400 (0.375)	0.365 (0.272)	−0.096
10	100.0	2.30	2.30	1.77	0.453 (0.296)	0.390 (0.196)	−0.162
11	90.0	2.30	2.44	1.86	0.371 (0.344)	0.389 (0.229)	0.046
12	80.0	2.00	2.00	1.72	0.312 (0.337)	0.374 (0.230)	0.166
Metapopulation variation							
Atlantic	79.6	2.28	2.61	1.67	0.292 (0.031)	0.329 (0.051)	0.112
Gulf	84.0	2.20	2.36	1.82	0.421 (0.096)	0.387 (0.020)	−0.088
Overall species variation							
	81.6	2.06	2.49	1.73	0.350 (0.093)	0.356 (0.049)	0.017

P_p: Percent polymorphic loci; A: total alleles per locus; A_e: effective alleles per locus; A_p: alleles per polymorphic locus; H_o: observed heterozygosity; H_e: expected heterozygosity; F: inbreeding coefficient; S.D.: standard deviation.

Table 3
Distribution of genetic diversity among *Melaleuca quinquenervia* populations in Florida. Diversity is expressed as total genetic diversity (H_t), within-population diversity (H_s), differentiation among-populations (D_{st}), and the proportion of total diversity attributable to among-population variation ($G_{st} = D_{st}/H_t$) at each of ten loci representing seven enzyme systems. χ^2 tests the interpopulational homogeneity of gene frequencies.

Locus (alleles)	H_t	H_s	D_{st}	G_{st}	χ^2 (df)[a]
Pgm-1	0.46	0.232	0.249	0.517	417.7 (22)***
Pgm-2	0.699	0.604	0.104	0.147	178.2 (33)***
Mpi-1	0.349	0.241	0.118	0.329	254.0 (22)***
Tre-2	0.707	0.370	0.368	0.498	573.7 (33)***
Ark-1	0.485	0.214	0.296	0.580	468.6 (22)***
Ark-2	0.774	0.549	0.245	0.309	499.3 (44)***
Gpi-1	0.569	0.442	0.139	0.239	193.1 (22)***
Gpi-2	0.652	0.192	0.501	0.723	876.3 (33)***
Aat-1	0.598	0.224	0.407	0.645	497.9 (22)***
Mdh-1	0.625	0.493	0.144	0.226	260.4 (33)***
Metapopulation variation					
Atlantic Coast	0.539	0.328	0.254	0.436	3631.9 (385)***
Gulf Coast	0.565	0.385	0.225	0.368	1848.1 (341)***
Overall species variation					
	0.592	0.356	0.257	0.419	5631.4 (385)***

*** Significant at $P < 0.001$.

were significant, and for *Mpi-1* non-significant, in all populations. Otherwise, deviations from Hardy–Weinberg expectations presented no discernable pattern across populations and loci.

3.3. Population structure

χ^2 analyses (Table 3) showed that allele frequencies were highly heterogeneous ($P < 0.001$) among-populations at all ten loci. G_{st} values for individual loci ranged from 0.147 to 0.723, suggesting a substantial *M. quinquenervia* population differentiation in Florida (Table 3). However, when the 42% of genetic diversity attributable to population differentiation (G_{st}) was parsed into inter- and intraregional components, only about 16% (i.e., 7% of total diversity) resulted from differences between Gulf and Atlantic Coast populations.

Genetic identity (I) among-populations was strongest (0.843) between Loxahatchee Wildlife Refuge and Caribbean Gardens (see Fig. 1). Trees at Koreshan Unity and Estero Bay Preserve exhibited similarly high genetic identity (0.830). Royal Palm Nursery and Koreshan Unity exhibited the lowest genetic identity (0.324). Genetic identity averaged 0.585 (S.D. = 0.118) overall. Genetic identity between populations in different regions averaged 0.581 (S.D. = 0.111), which was similar to the mean genetic identity within each metapopulation ($I = 0.606$, S.D. = 0.109, Atlantic Coast; $I = 0.574$, S.D. = 0.145, Gulf Coast). Geographic distance explained 35% of the variation in genetic distance within the Gulf Coast metapopulation ($F = 6.874$, $P = 0.021$). It explained none of the genetic distance within the Atlantic Coast metapopulation ($R^2 = 0.074$, $F = 1.044$, $P = 0.326$), however, and only marginally ($F = 2.898$, $P = 0.094$) explained 4% of the genetic distance among-populations throughout the state. A dendogram was constructed based on genetic distances (Nei, 1972) to aid visualization of these relationships (see Fig. 1 inset). Goodness of fit between the original distance matrix and a cophenetic matrix representing the phenogram was marginal ($r_{cs} = 0.71$; Unmack, 1999), but tree architecture is supported by historical and chemometric data (Dray, 2003). So the low cophenetic values likely reflect the limited number of loci being examined.

4. Discussion

The invasion history of an exotic plant species has a strong influence on its success as an invader, with invasion success often (but not exclusively) related to the number of introductions (Lockwood et al., 2005; Novak and Mack, 2005). Multiple introductions increase the likelihood that pioneers represent a diversity of genotypes, which enhances the ability of a species to respond both to local environmental conditions and to stochastic events (Dlugosch and Parker, 2008). The successful encroachment of *M. quinquenervia* into southern Florida was, in part, promoted by more than a dozen introduction events involving at least six different seed sources (Dray et al., 2006). One consequence of the significant propagule pressure generated by the plethora of sources has been that this notorious Everglades invader harbors substantial genetic diversity as measured by analysis of ten allozyme loci using cellulose acetate gel electrophoresis. This finding compares favorably with reports of substantial variation in phenotypic traits within both Florida and Australia populations (Wang and Littell, 1983; Kaufman and Smouse, 2001; Dray, 2003).

M. quinquenervia in Florida exhibits levels of intraspecific genetic variability representative of other species with similar life history traits. For example, a substantial proportion (61%) of allozyme loci tends to be polymorphic in tropical woody plants (Hamrick et al., 1979; Hamrick and Loveless, 1989). *M. quinquenervia* exhibits a similarly high level of allozyme polymorphism (mean $P_p = 82\%$), one that is identical to that reported by Hamrick and Godt (1996) for *Eucalyptus*, another tropical genus in the family Myrtaceae. Likewise, mean number of alleles ($A = 2.06$) in this study falls well within the range ($A = 1.00$–3.12) reported by Hamrick et al. (1979) for tropical trees. Further, tropical woody species often harbor substantial heterozygosity ($H_e = 0.211$; Hamrick and Loveless, 1989). This was also true of *M. quinquenervia* ($H_e = 0.356$) which exhibited greater heterozygosity than *Eucalyptus* ($H_e = 0.222$; Hamrick and Godt, 1996). Thus, the gene diversity within populations of *M. quinquenervia* in Florida is typical specifically of the family Myrtaceae and more generally of many large, woody perennial species growing in tropical and subtropical regions of the world.

M. quinquenervia populations in Florida are strongly divergent from one another ($G_{st} = 0.419$), which contrasts sharply with what has been reported for other tropical woody species. Hamrick and Godt (1996) reported a mean $G_{st} = 0.134$ for *Eucalyptus* species. Hamrick and Loveless (1989) examined 14 tropical trees and shrubs (not including *Eucalyptus*) and reported levels of interpopulation differentiation ($G_{st} = 0.022$–0.090) an order of magnitude lower than found among *M. quinquenervia* populations in Florida. High G_{st} values are, however, common among early successional species (mean $G_{st} = 0.411$; Loveless and Hamrick, 1984). Successful invaders such as *M. quinquenervia* perform like traditional pioneer species when encroaching into new territory, even though they may not fill that niche in their native ranges. Thus, the data from this study suggest that other invasive plant species are likely to exhibit high levels of interpopulation differentiation in their adventive ranges for a considerable time (it has been over 120 years since *M. quinquenervia* was first imported into Florida) after initial invasion. This prediction is corroborated by North American leafy spurge (*Euphorbia esula* L.) populations, which show a similarly high differentiation among populations ($G_{st} = 0.460$; Rowe et al., 1997) and Chinese populations of bitterbush [*Chromolaena odorata* (L.) King & H.E. Robins] which demonstrate even greater differentiation ($G_{st} = 0.736$; Ye et al., 2004).

The high among-population diversity is also reflective of the distribution of *M. quinquenervia* in its adventive range. Although the tree has a reasonably restricted geographic range in Florida, the heart of the Everglades ecosystem apparently serves as a natural geographic barrier that has forced a disjunction in the populations here. Butcher et al. (1992) demonstrated that a similarly small (<100 km) disjunction in the distribution of *M. alternifolia*

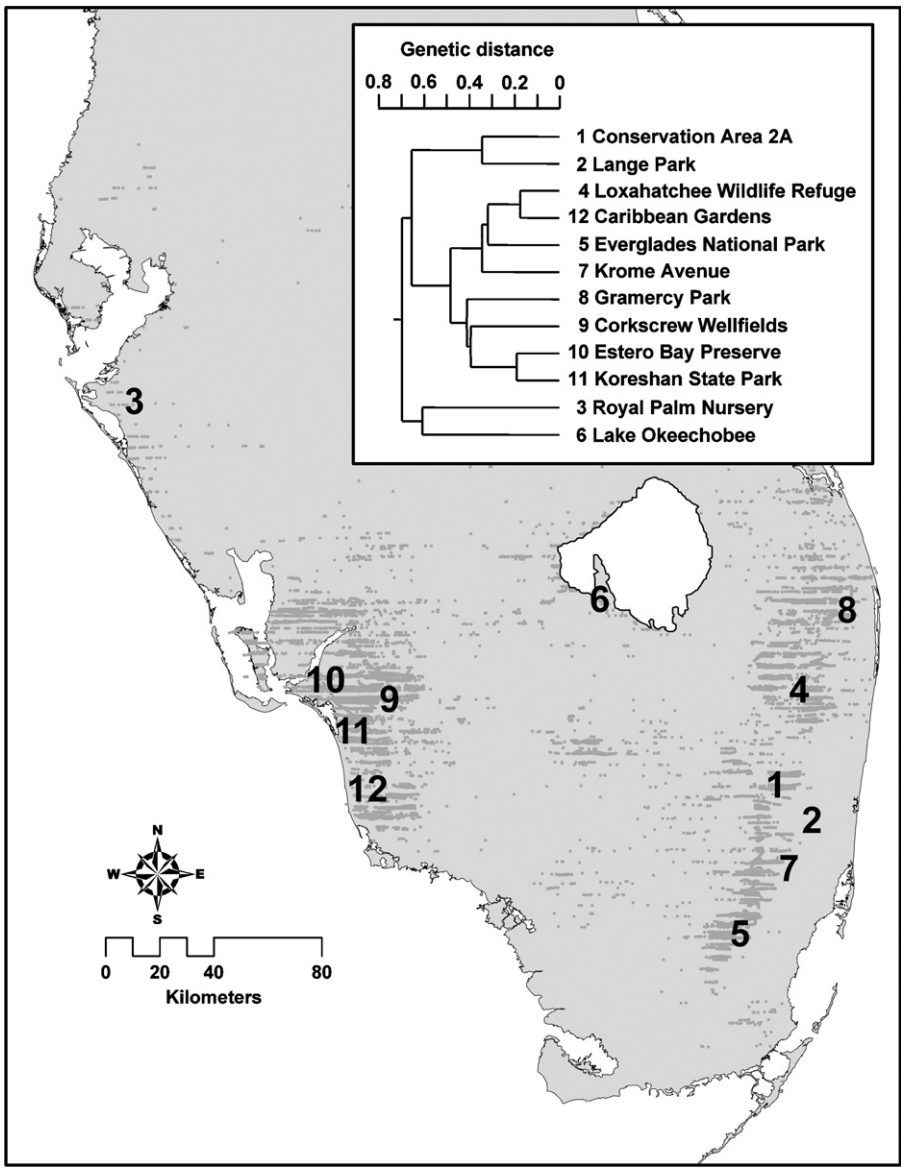

Fig. 1. Geographic distribution (grey dots) of *Melaleuca quinquenervia* in Florida (derived from systematic reconnaissance flight data found at http://tame.ifas.ufl.edu/old/index.htm) showing locations of populations included in this study, and cluster analysis (inset) based on Nei's genetic distance and the UPGMA algorithm. Numbers correspond to the sites in Table 1.

populations in Australia has resulted in their separation into two genetically distinct metapopulations. Likewise, the two principle *M. quinquenervia* metapopulations in Florida (occurring along the Atlantic and Gulf coasts) are genetically distinct (see also Kaufman and Smouse, 2001). The high G_{st} concords well with the fact that two principal chemical phenotypes found in Florida seem to be disproportionately represented among these metapopulations (Dray, 2003; but see Wheeler et al., 2007). It is also consistent with observations by Wang and Littell (1983) of marginally significant ($P = 0.069$) differences in caloric content of *M. quinquenervia* tissues from plants in Lee Co. versus Miami-Dade Co.

It is somewhat surprising that the Everglades presents such a substantial barrier to gene flow between Atlantic and Gulf Coast populations because the infested areas around Lake Okeechobee would seem to offer an ideal corridor for gene exchange between the two metapopulations. Natural range expansion is restricted because *M. quinquenervia* seeds are small (Rayamajhi et al., 2002) and gravity-dispersed, and so are deposited within a short distance (<170 m) of the parent tree (Meskimen, 1962). However, Vardaman (1994) reported that honey bees (*Apis mellifera*) are the principle pollinators of *M. quinquenervia* in Florida, and Ward et al. (2005) presented evidence that insect pollinators can distribute pollen many kilometers from a source tree. Further, the historic data (Dray et al., 2006) show that human-vectored dispersal was, at times, substantial. Thus, the apparently low gene flow represents a conundrum that warrants additional study.

The origin of the Lake Okeechobee populations has never been clear. The U.S. Army Corps of Engineers (Corps) established a melaleuca nursery on the southern shore of the lake during 1938–1941, as a source for trees to be planted along the levee to protect it from wave action (Dray, 2003). The seeds from which this nursery was established came from an unknown source. However, the close genetic relationship between the Royal Palm plants and those along Lake Okeechobee (Fig. 1 inset) suggests the Reasoner brothers (see Dray et al., 2006) provided the seeds to start the Corps nursery. This hypothesis is supported by historical evidence that Norman Reasoner (son of a Royal Palm Nurseries founder) worked as an Agronomic Consultant for the Corps at about this same time (Dray, 2003).

Despite the evidence for regional differences, only a small proportion (7%) of the total genetic variation present among *M. quinquenervia* populations could be ascribed to the disjunction in the adventive range of this species. In contrast, the high level of differentiation within metapopulations (35%) means that gene flow, even assisted by human agents, has thus far failed to homogenize independent gene pools established a century ago by discrete introduction events. Each introduction consisted of a small envelope of seeds, each of which likely derived from a single maternal seed source (Dray et al., 2006). Limited numbers of colonization events, each consisting of only a few propagules, is a recipe for founder effects (Hartl, 2000). Nei (1987) says such genetic bottlenecks can temporarily increase estimates of genetic distance, which may help explain the high G_{st} values within *M. quinquenervia* metapopulations in Florida.

Historical evidence (Dray et al., 2006) suggests that much of the range expansion of *M. quinquenervia* in Florida occurred through human agency. This transport of plant material between regions was largely unidirectional, with "truckloads" of saplings being transported from nurseries on the Gulf Coast to developing towns on both coasts. The strong relationship between genetic distance and geographical distance within the Gulf Coast metapopulation, but absence of a similar relationship within the Atlantic Coast metapopulation or within the species as a whole in Florida seems to concord well with these historical data. Further, absence in the Gulf Coast metapopulation of four alleles that are present in the Atlantic Coast metapopulation, and especially lack of the *Tre-2a* allele (which is common along the Atlantic Coast), supports this interpretation of the historical data. This unidirectional propagule flow helps explain the somewhat greater among-population differentiation within the Atlantic Coast metapopulation.

The *M. quinquenervia* populations in this study were seldom in Hardy–Weinburg equilibrium. One likely contributor to this result is the mating system of *M. quinquenervia*. Vardaman (1994) demonstrated that this species is highly self-compatible with 70% of artificially selfed flowers, and 25% of autogamously pollinated flowers, successfully producing fruit. She also noted, however, that the highest rates of fruit set were obtained from cross-pollinated flowers, and that *M. quinquenervia* does not appear to be pollinator limited in Florida. Thus, it is likely that *M. quinquenervia* employs a mixed mating system in Florida. Also, some of the seeds imported into Florida derive from locations (e.g., Ventimiglia, Italy; Nice, France; Ivoloina, Madagascar; see Dray et al., 2006) outside the native range of *M. quinquenervia* and undoubtedly endured genetic bottlenecks of their own. This strengthens the possibility that seeds comprising different introduction events in Florida harbored vastly disparate genomes. These two factors likely account for the observed heterozygote deficiency.

Our understanding of the population genetics of *M. quinquenervia* in Florida contributes to the growing body of evidence (e.g., Novak and Mack, 2005; Dlugosch and Parker, 2008; Facon et al., 2008) that alien plants often present strong genetic diversity in their adventive ranges. The propagule pressure resulting from many introductions at multiple sites over time (Dray et al., 2006) was clearly sufficient to establish multiple genetic lineages that gene flow and human-vectored dispersal have thus far failed to homogenize. The two principle metapopulations demarcated by Florida's two coasts and largely (but not exclusively) segregated by the Everglades ecosystems that bisect them can now be characterized by genetic as well as phenotypic traits (Wang and Littell, 1983; Kaufman and Smouse, 2001; Dray, 2003). Recent management efforts aimed at dramatically reducing the geographic distribution and reproductive potential of *M. quinquenervia* in Florida (Pratt et al., 2005) may help maintain the isolation of these metapopulations by further restricting gene flow. Finally, although the allelic diversity among *M. quinquenervia* populations in Florida concords well with other tropical, long-lived, woody plant species, its recent invasion history has contributed to a high genetic diversity that is more reminiscent of pioneer species.

Acknowledgements

The authors thank Drs. Min Rayamajhi and Thai Van (USDA, ARS Invasive Plant Research Lab) for their kind assistance with seedlot collections. Luke Kasarjian provided excellent care of our plant cultures. Marie Wells and Michael Vacek aided with sample processing. Drs. Blanca Cortez (Florida International University) and Dan Brazeau (University of Florida) helped sort out allozyme techniques. Dr. Lyn Craven (Australian National Herbarium) graciously verified the identities of *Melaleuca* specimens. Two anonymous reviewers provided encouraging and helpful suggestions for the improvement of this manuscript.

References

Acquaah, G., 1992. Practical Protein Electrophoresis for Genetic Research. Dioscorides Press, Portland, OR.

Barrett, S.C.H., 2000. Microevolutionary influences of global changes on plant invasions. In: Mooney, H.A., Hobbs, R.J. (Eds.), Invasive Species in a Changing World. Island Press, Washington, DC, pp. 115–139.

Bodle, M.J., Ferriter, A.P., Thayer, D.D., 1994. The biology, distribution, and ecological consequences of *Melaleuca quinquenervia* in the Everglades. In: Davis, S.M., Ogden, J.C. (Eds.), Everglades: The Ecosystem and Its Restoration. St. Lucie Press, Delray Beach, FL, pp. 341–355.

Butcher, P.A., Bell, J.C., Moran, G.F., 1992. Patterns of genetic diversity and nature of the breeding system in *Melaleuca alternifolia* (Myrtaceae). Aust. J. Bot. 40, 365–375.

Cheliak, W.M., Pitel, J.A., 1984. Genetic control of allozyme variants in mature tissues of white spruce trees. J. Hered. 75, 34–40.

Dlugosch, K.M., Parker, I.M., 2008. Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple invasions. Mol. Ecol. 17, 421–449.

Dray Jr., F.A., 2003. Ecological genetics of *Melaleuca quinquenervia* (Myrtaceae): population variation in Florida and its influence on performance of the biological control agent *Oxyops vitiosa* (Coleoptera: Curculionidae). Ph.D. dissertation. Florida International University, Miami, Florida.

Dray Jr., F.A., Bennett, B.C., Center, T.D., 2006. Invasion history of *Melaleuca quinquenervia* (Cav.) S.T. Blake in Florida. Castenea 71, 210–225.

Facon, B., Pointier, J.P., Jarne, P., Sarda, V., David, P., 2008. High genetic variance in life-history strategies within invasive populations by way of multiple introductions. Curr. Biol. 18, 363–367.

Goudet, J., 2002. Fstat, version 2.9.3.2. Institute of Ecology, Lausanne, Switzerland. http://www2.unil.ch/popgen/softwares/fstat.html.

Hamrick, J.L., Godt, M.J.W., 1996. Effects of life history traits on genetic diversity in plant species. Philos. Trans. R. Soc. Lond., Ser. B: Biol. Sci. 351, 1291–1298.

Hamrick, J.L., Loveless, M.D., 1989. The genetic structure of tropical tree populations: associations with reproductive biology. In: Bock, J.H., Linhart, Y.B. (Eds.), Plant Evolutionary Ecology. Westview Press, Boulder, CO, pp. 131–146.

Hamrick, J.L., Linhart, Y.B., Mitton, J.B., 1979. Relationships between life history characteristics and electrophorectically detectable genetic variation in plants. Ann. Rev. Ecol. Syst. 10, 173–200.

Hanski, I.A., Gilpin, M.E. (Eds.), 1997. Metapopulation Biology: Ecology, Genetics, and Evolution. Academic Press, New York, NY.

Hartl, D.L., 2000. A Primer of Population Genetics, third ed. Sinauer Associates, Sunderland, MA.

Hebert, P.D.N., Beaton, M.J., 1993. Methodologies for Allozyme Analysis Using Cellulose Acetate Electrophoresis. Helena Laboratories, Beaumont, TX.

Kaufman, S.R., Smouse, P.E., 2001. Comparing indigenous and introduced populations of *Melaleuca quinquenervia* (Cav.) Blake: response of seedlings to water and pH levels. Oecologia 127, 487–494.

Lockwood, J.L., Cassey, P., Blackburn, T., 2005. The role of propagule pressure in explaining species invasions. Trends Ecol. Evol. 20, 223–228.

Loveless, M.D., Hamrick, J.L., 1984. Ecological determinants of genetic structure in plant populations. Ann. Rev. Ecol. Syst. 15, 65–95.

Meskimen, G.F., 1962. A silvical study of the melaleuca tree in South Florida. Masters thesis. University of Florida, Gainesville, FL.

Nei, M., 1972. Genetic distance between populations. Am. Nat. 106, 283–292.

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

Nei, M., 1987. Molecular Evolutionary Genetics. Columbia University Press, New York.

Novak, S.J., Mack, R.N., 2005. Genetic bottlenecks in alien plant species. In: Sax, D.F., Stachowicz, J.J., Gaines, S.D. (Eds.), Species Invasions: Insights into Ecology, Evolution, and Biogeography. Sinauer Associates, Sunderland, MA, pp. 201–228.

O'Hare, N.K., Dalrymple, G.H., 1997. Wildlife in southern Everglades wetlands invaded by melaleuca (*Melaleuca quinquenervia*). Bull. Fla. Mus. Nat. Hist. 41, 1–68.

Pratt, P.D., Rayamajhi, M.B., Van, T.K., Center, T.D., 2005. Herbivory alters resource allocation and compensation in the invasive tree *Melaleuca quinquenervia*. Ecol. Entomol. 30, 316–326.

Rayamajhi, M.B., Van, T.K., Center, T.D., Goolsby, J.A., Pratt, P.D., Racelis, A., 2002. Biological attributes of the canopy-held melaleuca seeds in Australia and Florida. J. Aquat. Plant Manage. 40, 87–91.

Rohlf, F.J., 2000. Ntsys: Numerical Taxonomy and Multivariate Analysis System, version 2.1. Applied Biostatistics Inc., Port Jefferson, NY.

Rowe, M.L., Lee, D.J., Bowditch, B.M., Masters, R.A., 1997. Genetic variation in North American leafy spurge (*Euphorbia esula*) determined by DNA markers. Weed Sci. 45, 446–454.

Sobhian, R., Ryan, F.J., Khamraev, A., Pitcairn, M.J., Bell, D.E., 2003. DNA phenotyping to find a natural enemy in Uzbekistan for California biotpes of *Salsola tragus* L. Biol. Control 28, 222–228.

Sakai, A.K., Allendorf, F.W., Holt, J.S., Lodge, D.M., Molofsky, J., With, K.A., Baughman, S., Caabin, 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. Ann. Rev. Ecol. Syst. 32, 305–332.

Unmack, P.J., 1999. Biogeography of Australian freshwater fishes. Master's thesis. Arizona State University, Tempe, AZ. http://www.peter.unmack.net/biogeog/pdf/thesis.pdf.

Vardaman, S.M., 1994. The reproductive ecology of *Melaleuca quinquenervia* (Cav.) Blake. M.S. thesis. Florida International University, Miami, FL, USA.

Wang, S.-C., Littell, R.C., 1983. Phenotypic variation in calorific value of melaleuca materials from south Florida. Econ. Bot. 37, 292–298.

Ward, M., Dick, C.W., Gribel, R., Lowe, A.J., 2005. To self, or not to self—A review of outcrossing and pollen-mediated gene flow in neotropical trees. Heredity 95, 246–254.

Ward, S., 2006. Genetic analysis of invasive plant populations at different spatial scales. Biol. Invasions 8, 541–552.

Wheeler, G.S., Pratt, P.D., Giblin-Davis, R.M., Ordung, K.M., 2007. Intraspecific variation of *Melaleuca quinquenervia* leaf oils in its naturalized range in Florida, the Caribbean, and Hawaii. Biochem. Syst. Ecol. 35, 489–500.

Wilder, J.J., 1940. Beekeeping in Florida, Bulletin No. 6. Florida Dept. Agriculture, Tallahassee.

Workman, P.L., Niswander, J.D., 1970. Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. Am. J. Hum. Genet. 22, 24–49.

Ye, W.H., Mu, H.P., Cao, H.L., Ge, X.J., 2004. Genetic structure of the invasive *Chromolaena odorata* in China. Weed Res. 44, 129–135.

Yeh, F.C., Yang, R., Boyle, T., 1999. Popgene, Version 1.32. University of Alberta, Alberta, Canada.