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Analysis of genetic diversity and population structure within Florida coconut (*Cocos nucifera* L.) germplasm using microsatellite DNA, with special emphasis on the Fiji Dwarf cultivar

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Abstract Using 15 simple sequence repeat (SSR) microsatellite DNA loci, we analyzed genetic variation within *Cocos nucifera* germplasm collections at two locations in south Florida, representing eight cultivars. The loci were also used in a parentage analysis of progeny of the 'Fiji Dwarf' variety at both locations. A total of 67 alleles were detected, with eight the highest number at any one locus. These loci identified 83 of the 110 individual palms. Gene diversity of the 15 loci ranged from 0.778 to 0.223, with a mean of 0.574. 'Fiji Dwarf', 'Malayan Dwarf', 'Green Niño' and 'Red Spicata' cultivars resolve as distinct clusters in a neighbor joining tree using modified Rogers distance, while the tall varieties form two aggregates. The highest gene diversity was found in the tall cultivars ($\hat{H} = 0.583$ cumulatively), and the lowest in the 'Malayan Dwarf' ($\hat{H} = 0.202$). After the tall coconuts, the 'Fiji Dwarf' was most genetically diverse ($\hat{H} = 0.436$), and had the largest number of unique alleles. Genetic identity is highest among the 'Malayan Dwarf' phenotypes, and between the tall varieties. The 'Red Malayan Dwarf' is genetically distinct from the 'Green' and 'Yellow Malayan Dwarf' phenotypes, which cannot be distinguished with the SSR loci used. Off-type 'Malayan Dwarf' phenotypes (putative hybrids with talls) can be identified genotypically. Parentage analyses of 30 'Fiji Dwarf' progeny propagated from five adults surrounded by other cultivars estimate that only 20% of the progeny were out-crossed to the other varieties, while 40–46% were possible selfs. This suggests that a seed-production orchard of the variety maintained at reasonable distance from other varieties, will likely yield only 'Fiji Dwarf' genotypes. Our data are discussed in the context of hypotheses of coconut dissemination around the world.

Keywords Arecaceae · Palmae · SSR · Breeding · Parentage analysis · Tropical horticulture · Palm

Introduction

Cocos nucifera L., the coconut, is grown throughout the tropics as a plantation crop yielding several agronomic products that are important to export economies in these regions (Beck and Balick 1990; Harries 1995). In the United States, the coconut is, with minor exception, exclusively a tropical ornamental, much in demand as a signature tropical landscape element (Meerow 1992). In the 1970s, the Lethal Yellowing (LY) phytoplasma devastated the coconut canopy of south Florida. By 1983, the epidemic had destroyed an estimated 100,000 coconut palms (Howard and Barrant 1989). The loss of this palm tree canopy was considered detrimental enough to Florida's tourism economy that the Florida State Legislature funded a research program during the epidemic with two main components. Similar efforts were also established by the Jamaican Coconut Industry Board (Harries 1973). Research centered at the University of Florida Fort Lauderdale Research and Education Center (UF) concentrated on the disease organism and its vector, as well as seeking potential management or curative strategies (McCoy et al. 1983). Subsequently, a coconut breeding program to develop new resistant varieties and seed orchards of known resistant cultivars was established at the USDA Subtropical Horticulture Research Station ("Chapman Field") by the Florida Department of Agriculture and Consumer Services (FLDACS) Division of Forestry. As a result, significant coconut germplasm collections were developed at both locations, though direct funding for both programs was terminated in the 1980s (UF) and 1996 (FLDACS), respectively.

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Management strategies have focused on replacing disease susceptible 'Tall' varieties with resistant 'Dwarf' types or a hybrid ('Maypan') of the 'Panama Tall' and 'Malayan Dwarf' variety (Harries 1970, 1973; Harries and Romney 1974). The 'Malayan Dwarf' is further differentiated by its color phenotypes into 'Red' (known as 'Golden' in Florida), 'Green' or 'Yellow Malayan Dwarf'. Other candidate varieties have been brought into Florida over the last 20 years, including 'Red Spicata', 'Green Niño' and 'Niu Leka', better known in this country as 'Fiji Dwarf'. The latter is of particular interest because of its heavy dense crown of short, dark, broad leaves that is very ornamental. The exact origins of this variety are not known, but the oldest known introductions were from the South Pacific (Whitehead 1966a). This variety has also shown resistance to LY in Florida (Broschat et al. 2002) and is remarkably free of the nutritional deficiencies that plague most other coconut varieties on Florida's relatively infertile substrates. Unlike the dwarf varieties, which are considered to be highly autogamous and thus more homozygous, the 'Fiji Dwarf', despite its name, is primarily out-crossing like the tall varieties (Whitehead 1976; Harries 1978), and thus is more heterozygous (Teulat et al. 2000).

'Fiji Dwarf' germplasm at both the UF and USDA locations is represented by six surviving individuals from the initial import of seed nuts from Jamaica in the 1980s, five at UF and one at USDA, and progeny from the UF population at both locations. These individuals vary to the degree with which they match the phenotype described for the variety by Whitehead (1966a).

Genetic characterization of coconut varieties has been performed with allozymes (Carpio 1982), randomly amplified polymorphic DNA (RAPD) (Ashburner et al. 1997), restriction fragment length polymorphisms (RFLP) (LeBrun et al. 1998), amplified fragment length polymorphism (AFLP) (Perera et al. 1998) and inverse sequence-tagged repeats (ISTRs) (Rohde et al. 1995). Two groups have developed simple sequence repeat (SSR) primers from *C. nucifera* (Perera et al. 1999, 2000; Rivera et al. 1999; Teulat et al. 2000). Rivera et al. (1999) tested primers for 41 SSR loci on 20 coconuts, then used eight of these on a larger sample. Teulat et al. (2000) used 37 of the SSR loci developed by Rivera et al. (1999) to measure genetic diversity in 31 individual plants representing 14 populations of diverse geographic origin. Perera et al. (2000) used eight loci across 130 individuals representing 94 different varieties. In all cases, SSRs were very successful in distinguishing between coconut genotypes.

In this paper we used 15 SSR primer pairs (see Table 2) developed by Perera et al. (1999, 2000, and unpublished) to investigate the genetic diversity of coconut germplasm collections in south Florida, with particular application to distinguishing a "true" genotype for the 'Fiji Dwarf' cultivar in Florida. A major hypothesis that we hoped to address is whether a LY-resistant 'Fiji Dwarf' genotype can be distinguished and if this genotype can be phenotypically characterized as well. These data were used in a parentage analysis of the 'Fiji Dwarf' progeny at both locations. We were interested in answering several questions. Is susceptibility to LY in Florida 'Fiji Dwarf' coconuts restricted to recognizable off-phenotypes (presumably out-crossed)? What can parentage analysis tell us about breeding behavior in a small stand of open-pollinated 'Fiji Dwarf' individuals (the UF five) surrounded by a diverse assemblage of potential pollen parents, and how can this information then be adapted to managing a seed production grove of 'Fiji Dwarf' coconuts to supply the tropical landscape plant industry? Finally, we wished to ascertain if 'Malayan Dwarf' off-types, recognized phenotypically by trunk and fruit characteristics, could also be identified using SSR markers.

Materials and methods

Sampling and DNA protocols

Sampling

Fifty six individual coconuts samples were sampled from the USDA-ARS-SHRS in Miami, Florida, representing five populations of five varieties (Table 1). Fifty four individuals were sampled from the University of Florida Fort Lauderdale Research and Education Center, representing ten populations of nine varieties or hybrids (Table 1).

DNA extraction

DNA was extracted from either 200 mg of fresh or 30 mg of silica gel dried leaf tissue collected from the most recently fully expanded leaf. Young leaves were collected and cut into about 1.0 cm^2 pieces that were extracted using the FastDNA Kit (BIO 101 Inc., Carlsbad, Calif.) according to the manufacturer's protocols with a FP 120 FastPrep cell disrupter (Savant Instruments Inc., Holbrook, New York). Samples were quantified with a GeneQuant pro RNA/DNA calculator (Amersham Pharmacia Biotech Inc., Piscatawy, N.J.).

DNA amplification

Polymerase chain reactions (PCR) were performed as described by Perera et al. (1999, 2000) with the following modifications: 200 µM of dNTPs, 1 U of *Taq* polymerase, and 10 pmol of fluorescent 5′end-labelled forward primers. Reactions were performed using an ABI 877 Integrated Thermocycler (Applied Biosystems, Inc., Foster City, Calif.).

Electrophoresis

Capillary electrophoresis was performed using Performance Optimized Polymer 4 (POP4, Applied Biosystems, Inc., Foster City, Calif.). Samples were prepared immediately prior to electrophoresis by adding 1 µl of PCR product to 12 µl of deionized formamide and 0.5 µl of GeneScan 500 ROX size standard (Applied Biosystems, Inc., Foster City, Calif.), then denatured at 95 °C for 5 min, followed by 5 min on ice. Samples were electrophoresed on an ABI 310 genetic analyzer (Applied Biosystems Inc., Foster City, Calif.). Samples were injected electrokinetically at 15 kV for 10 s and were run at 15 kV and 60 °C for 24 min. Resulting data were imported into GeneScan 3.1 (Applied Biosystems Inc., Foster City, Calif.) for internal standard and fragment-size deter-

Table 1 Cultivar populations, sample size, labels used in analyses and original seed source of *C. nucifera* employed in the SSR analyses

Name	Sample size (number used in population analyses if lower)	Label (*A* = letter, *n* = number)	Seed source
'Fiji Dwarf' (UF)	5(4)	FD*n*UF*n*	Coconut Industry Board (CIB), Jamaica
'Fiji Dwarf' progeny(UF)	15(12)	FD×*An*	Open pollination at UF
'Fiji Dwarf' (USDA)	1	Fdtrue	CIB, Jamaica
'Fiji Dwarf' progeny (USDA)	15(10)	FD*n*	Open pollination at UF
'Green Malayan Dwarf' (UF)	9	GrMD*n*	CIB, Jamaica
'Green Malayan Dwarf' off-type (UF)	2(0)	GrM*n*ot, otGrM*n*	CIB, Jamaica
'Red Malayan Dwarf'-1 (USDA)	10	RMD*n*	CIB, Jamaica
'Red Malayan Dwarf' (UF)	4	GOMD*n*	CIB, Jamaica
'Red Malayan Dwarf' off-type (UF)	1(0)	GOMD*n*ot, otGOMD*n*	CIB, Jamaica
'Red Malayan Dwarf'-2 (USDA)	5	RM1-5	Local, bulk pollinated
'Yellow Malayan Dwarf (USDA)	5	YM6-10	Local, bulk pollinated
'Atlantic (Jamaican) Tall' (UF)	9(7)	JT*n*	Local, open pollinated
'Panama Tall' (UF)	6	PT*n*	CIB, Jamaica
'Maypan' (UF)	1(0)	M*n*	CIB, Jamaica
'Green Niño' (USDA)	10	N*n*	CIB, Jamaica
'Red Spicata' (USDA)	10(7)	RS*n*	Local, open pollinated
'Red Spicata' (UF)	1	Rs*n*UF*n*	CIB, Jamaica
Undetermined tall (UF)	1(0)	TALL?	?

mination. Preliminary analysis of raw microsatellite data (sorting of fragments) was performed using Genotyper 2.1 (Applied Biosystems Inc., Foster City, Calif.).

Data analysis

Overall genetic variation

Gene diversity values for each locus (see Table 2) and averages across all loci for each cultivar group (see Table 6) were calculated using Nei's (1987) unbiased estimate $\hat{H}$:

$$\hat{H} = n\left(1-\sum p_i^2\right)/n-1,$$

where n = number of individuals sampled, p_i is the frequency of the ith allele. The variance of this statistic was calculated as:

$$V(\hat{H}) = 2/\left[(n-2)\left[\left(\sum p_i^3\right)-\left(\sum p_i^2\right)^2\right]+\sum p_i^2-\left(\sum p_i^2\right)^2\right].$$

While related to measures of heterozygosity (Nei 1987), this statistic is more appropriate for artificial populations where estimates of Hardy-Weinberg equilibrium are inappropriate.

All samples were scored for all allele frequencies and subjected to cluster analyses with the neighbor joining method (NJ, Saitou and Nei 1987) using Wright's (1978) modified Roger's distance with the program NTSYSpc version 2.10t (Exeter Software, Inc., Setauket, N.Y.). Principal coordinate analyses (PCA; Sokal and Rohlf 1995) were performed on a subset of the data using the SAS System for Windows v. 8.0 (SAS Institute, Cary, N.C.), also with modified Rogers' distance (Wright 1978). For the PCA, when several individuals of the same cultivar group had the same genotype, only one was included. Since many of the individual trees sampled were of uncertain origin, the purpose of the overall analyses was to identify phenetic groups based on overall allele diversity that could then be treated as distinct populations with a varietal class. The phenetic analyses were also useful in identifying sporadic outlying individuals within a varietal population that most likely were of hybrid origin. Likewise, 'Fiji Dwarf' progeny, identified by the parentage analysis as the likely result of out-crossing with a different variety, were removed from later analyses.

Parentage analysis of the 'Fiji Dwarf' variety

Parentage analysis was performed on the 'Fiji Dwarf' progeny (Table 1) using the program CERVUS (Marshall et al. 1998; Slate et al. 2000). The maternal parent of the 15 UF progeny was known. The exact maternal parent of the 15 USDA progeny was not known, but could only be any of the original UF 'Fiji Dwarf' plants, including the surviving five (FD1UF–FD5UF). The parentage analysis is conducted by first constructing an allele frequency data set from the genotypes of all progeny and the known and potential parents. The pool of potential parents was restricted to all of the palms sampled at UF since all maternal parents were located there. Using these results, a simulation of parentage analysis is run to evaluate the confidence in parentage of the most-likely candidate parent [the one with the largest likelihood ratio (Edwards 1972)]. The simulation estimates both the resolving power of the SSR loci and the critical values of the log-likelihood statistic "Delta" [defined as the difference in the log (to base e) of the product of the likelihood ratios at each locus (LOD) scores between the most-likely candidate parent and the second most-likely candidate parent] so that the results of parentage tests carried out using the parentage analysis module of the program can be evaluated statistically. Ten thousand simulation cycles were run with the following parameters: 70 candidate parents, 0.8 proportion of the candidate parents sampled, proportion of loci typed = 1, and a 1% rate of typing error. The relaxed confidence level was set at 80%, strict at 95%.

For the USDA 'Fiji Dwarf' progeny for which neither parent was known, a two-stage analysis was conducted in which the most-likely parents identified by the first run are used as known parents in the second run. A similar two-stage analysis was also run on the UF progeny to compare the accuracy of the identification of the "true" maternal parent with inferred parentage.

Varietal population genetic variation

After off-type or putative hybrid individuals, as determined by the parentage and overall cluster analysis, and known hybrids ('Maypan') were removed from the sample; 92 individual genotypes were sorted into nine cultivar populations ('Atlantic Tall', 'Fiji Dwarf', 'Green Nino', 'Green Malayan Dwarf' 'Panama Tall', 'Red Malayan Dwarf'-1 and -2, 'Red Spicata' and 'Yellow Malayan Dwarf') for the generation of estimates of genetic variation and

Table 2 Primers, repeat size and type, number of alleles recovered, and gene diversity for SSR loci used in the analysis of Florida *C. nucifera* cultivars

Locus	Repeat	Primers (5′-3′)	No. alleles	Size range (bp)	Diversity $[\hat{H} \pm \sqrt{V(\hat{H})}]$
CAC4	$(CA)_{19}(AG)_{17}$	CCCCTATGCATCAAAACAAG CTCAGTGTCCGTCTTTGTCC	5	185–207	0.634 ± 0.187
CAC6	$(AG)_{14}(CA)_{9}$	TGTACCATGTTTTTTGCCCAA CGATGTAGCTACCTTCCCC	7	146–164	0.698 ± 0.192
CAC8	$(AG)_{10}(CA)_{9}$	ATCACCCCAATACAAGGACA AATTCTATGGTCCACCCACA	6	186–210	0.667 ± 0.187
CAC11	$(CA)_{n}$ $(TA)_{n}$ (complex)	GATCTTCGGCGTTCCTCA TCTCCTCAACAATCTGAAGC	2	144–147	0.503 ± 0.192
CAC13	$(CA)_{9}(TA)_{5}A(TA)_{4}(CA)_{6}$	GGGTTTTTTAGATCTTCGGC CTCAACAATCTGAAGCATCG	2	151–153	0.505 ± 0.192
CAC20	$(CA)_{19}$	CTCATGAACCAAACGTTAGA CATCATATACATACATGCAACA	5	124–133	0.676 ± 0.200
CAC21	$(CA)_{11}$	AATTGTGTGACACGTAGCC GCATAACTCTTTCATAAGGA	2	149–151	0.479 ± 0.187
CAC23	$(CA)_{8}$	TGAAAACAAAAGATAGATGTCAG GAAGATGCTTTGATATGGAAC	3	170–179	0.593 ± 0.190
CAC52	$(CA)_{19}$	TTATTTTCTCCACTTCTGTGG ATATTACCCATGCACAGTACG	6	142–160	0.710 ± 0.198
CAC56	$(CA)_{14}$	ATTCTTTTGGCTTAAAACATG TGATTTTACAGTTACAAGTTTGG	8	138–162	0.778 ± 0.226
CAC65	$(AC)_{15}$	GAAAAGGATGTAATAAGCTGG TTTGTCCCCAAATATAGGTAG	5	150–173	0.634 ± 0.184
CAC68	$(CA)_{13}$	AATTATTTTCTTGTTACATGCATC AACAGCCTCTAGCAATCATAG	4	130–146	0.482 ± 0.176
CAC71	$(CA)_{17}$	ATAGCTCAAGTTGTTGCTAGG ATATTGTCATGATTGAGCCTC	5	172–183	0.425 ± 0.170
CAC72	$(CA)_{18}$	TCACATTATCAAATAAGTCTCACA GCTCTCTTTCTCATGCACA	4	124–132	0.599 ± 0.190
CAC84	$(CA)_{13}$	TTGGTTTTTGTATGGAACTCT AAATGCTAACATCTCAACAGC	3	150–163	0.223 ± 0.198

both modified Roger's distance and Nei's (1978) unbiased genetic identity and distance, using the software programs Genetic Data Analysis (GDA; Lewis and Zaykin 2001) and Tools for Population Genetic Analysis (TFPGA; Miller 1997). The unweighted pairgroup method (UPGMA, Sokal and Rohlf 1995) was used to generate phenograms using the distance coefficients with 1,000 replications of bootstrapping (Felsenstein 1985) to test support at the branch nodes. Nodes with bootstrap values below 50% are considered unsupported.

Results

Levels of polymorphism

A total of 67 alleles were detected across all 110 coconut individuals sampled, ranging from eight alleles for CAC56 to two for CAC11, CAC13 and CAC21 (Table 2). The average was 4.5 ± 1.8. Gene diversity $(\hat{H})$ ranged from 0.778 for CAC56 to 0.223 for CAC84, with a mean of 0.574 ± 0.140 (Table 2). No locus was monomorphic across the entire sample population. Of the 110 individuals, the 15 SSR loci successfully differentiate all but the 'Red Malayan Dwarf'-1 (RMD1-10, GOMD1-3), two of the 'Red Malayan Dwarf'-2 (RM2 and 5), three of the 'Green Malayan Dwarf' (GrMD5, 7 and 9), four of the 'Red Spicata' (RS1UF35, RS7, 9 and 10) and five of the 'Green Niño' (N1, 4-6, 10) genotypes (see Fig. 1).

Parentage analysis

Thirteen of the 15 loci were used in the parentage analysis, due to a sizable number of loci mismatches between the known parent and the UF 'Fiji Dwarf' progeny with CAC8 and CAC20, with the latter partially accountable by missing data. Two analyses were performed on the UF progeny, one in which the known maternal parents were provided (Table 3); and a second, 2-stage analysis in which both parents were treated as unknown (Table 4). In the former, there were four instances of offspring (O) - known parent (KP) mismatches, two at one locus and two at two loci (Table 3). In two of these cases (FD×H5 and FD×I2), the first part of the two-stage analysis chose a different candidate parent with 0 loci mismatches (Table 4). Ten of the 15 results of the 2-stage analysis were in agreement with the putative known parent of the progeny. With known maternal parents designated, 13 of the 15 progeny most-likely had a 'Fiji Dwarf' pollen parent, 4 at the 95% confidence level, 8 at the 80% confidence level and 1 at less than 80% (Table 3). Of these 13 UF progeny, 5 (33% of the population) were putatively inbred. When known parents are not designated, confidence levels for a 'Fiji Dwarf' first parent are 5 at 95%, 6 at 80% and 4 at less than 80% confidence (Table 4). With the first parents in the 2-stage analysis designated as the "known parents", the second most-likely parents are exactly the same as in the analy-

Table 3 Parentage analysis of UF 'Fiji dwarf' coconuts based on 13 SSR loci with known parents included

Offspring	Known parent	No. of offspring-known parent locus mismatches	Candidate parent	No. of offspring-candidate parent locus mismatches	LOD[a]	Delta	Confidence[b]
FD×A5	FD1UF17	1	FD1UF17	1	4.49	4.49	*
FD×B5	FD1UF17	0	FD2UF15	0	5.42	0.30	–
FD×D7	FD3UF16	2	FD5UF18	2	3.91	2.20	+
FD×E5	FD2UF15	0	FD1UF17	1	4.64	4.64	*
FD×E7	FD1UF17	0	M1	1	1.12	0.78	+
FD×F7	FD3UF16	0	JT7	0	2.14	0.67	+
FD×G7	FD2UF15	0	FD1UF17	1	4.38	1.29	+
FD×G8	FD1UF17	0	FD3UF16	0	7.70	4.03	*
FD×H5	FD2UF15	2	FD3UF16	0	6.02	1.53	+
FD×H7	FD1UF17	0	FD1UF17	0	6.81	6.81	*
FD×H8	FD3UF16	0	FD3UF16	0	6.81	5.21	*
FD×I2	FD3UF16	1	FD1UF17	1	4.93	2.41	*
FD×I5	FD2UF15	0	FD1UF17	1	2.54	2.54	*
FD×I7	FD2UF15	0	FD2UF15	0	8.36	2.67	*
FD×J2	FD3UF16	0	FD3UF16	0	6.68	1.35	+

[a] Sum of the log-likelihood ratios at each locus
[b] + = 95%, * = 80%, – = most likely (<80%)

Table 4 Two-stage parentage analysis of UF 'Fiji dwarf' coconuts based on 13 SSR loci without known parents included

Offspring	Known parent	No. of offspring-known parent locus mismatches	Candidate parent	No. of offspring-candidate parent locus mismatches	LOD[a]	Delta	Confidence[b]
Estimate of first parent							
FD×A5	–	–	FD1UF17	1	4.42	4.42	*
FD×B5	–	–	FD1UF17	0	6.00	1.69	+
FD×D7	–	–	FD5UF18	2	4.09	1.98	+
FD×E5	–	–	FD4UF14	1	4.83	0.76	–
FD×E7	–	–	JT5	0	4.48	0.91	–
FD×F7	–	–	FD3UF16	0	3.03	1.21	–
FD×G7	–	–	FD2UF15	0	5.58	1.23	+
FD×G8	–	–	FD1UF17	0	7.01	1.34	+
FD×H5	–	–	FD3UF16	0	6.14	3.36	*
FD×H7	–	–	FD1UF17	0	7.88	7.19	*
FD×H8	–	–	FD3UF16	0	6.59	4.33	*
FD×I2	–	–	FD2UF15	0	6.69	2.58	+
FD×I5	–	–	FD2UF15	0	4.60	0.92	–
FD×I7	–	–	FD2UF15	0	8.82	4.13	*
FD×J2	–	–	FD3UF16	0	7.18	3.12	*
Estimate of second parent							
FD×A5	FD1UF17	1	FD1UF17	1	4.49	4.49	*
FD×B5	FD1UF17	0	FD2UF15	0	5.42	0.30	–
FD×D7	FD5UF18	2	FD5UF18	2	3.34	0.58	+
FD×E5	FD4UF14	1	FD1UF17	1	4.87	4.87	*
FD×E7	JT5	0	FD3UF16	1	1.78	1.78	+
FD×F7	FD3UF16	0	JT7	0	2.14	0.67	+
FD×G7	FD2UF15	0	FD1UF17	1	4.38	1.29	+
FD×G8	FD1UF17	0	FD3UF16	0	7.70	4.03	*
FD×H5	FD3UF16	0	FD3UF16	0	6.54	4.71	*
FD×H7	FD1UF17	0	FD1UF17	0	6.81	6.81	*
FD×H8	FD3UF16	0	FD3UF16	0	6.81	5.21	*
FD×I2	FD2UF15	0	FD1UF17	1	5.26	2.82	*
FD×I5	FD2UF15	0	FD1UF17	1	2.54	2.54	*
FD×I7	FD2UF15	0	FD2UF15	0	8.36	2.67	*
FD×J2	FD3UF16	0	FD3UF16	0	6.68	1.35	+

[a] Sum of the log-likelihood ratios at each locus
[b] + = 95%, * = 80%, – = most likely (<80%)

Table 5 Two-stage parentage analysis of USDA ‘Fiji Dwarf’ coconut progeny based on 13 SSR loci

Offspring	Known parent	No. of offspring-known parent locus mismatches	Candidate parent	No. of offspring-candidate parent locus mismatches	LOD[a]	Delta	Confidence[b]
Estimate of first parent							
FD1	–	–	FD1UF17	0	7.72	7.43	*
FD2	–	–	FD3UF16	0	6.26	3.99	*
FD3	–	–	FD4UF14	0	6.92	4.84	*
FD4	–	–	FD1UF17	1	5.30	4.06	*
FD5	–	–	FD1UF17	0	7.65	3.90	*
FD6	–	–	GOMD1ot	0	4.61	1.67	+
FD7	–	–	FD1UF17	0	7.71	3.89	*
FD8	–	–	FD1UF17	0	7.91	6.05	*
FD9	–	–	FD2UF15	3	2.62	0.89	–
FD10	–	–	FD5UF18	0	3.83	2.23	+
FD11	–	–	FD3UF16	0	5.31	1.34	+
FD12	–	–	RS1UF35	2	3.88	1.65	+
FD13	–	–	FD3UF16	0	4.13	1.06	–
FD14	–	–	RS1UF35	3	3.88	0.30	–
FD15	–	–	FD1UF17	0	7.60	5.40	*
Estimate of second parent							
FD1	FD1UF17	0	FD1UF17	0	6.28	6.28	*
FD2	FD3UF16	0	FD3UF16	0	6.86	5.29	*
FD3	FD4UF14	0	FD1UF17	1	3.93	3.03	*
FD4	FD1UF17	1	FD1UF17	1	5.89	4.36	*
FD5	FD1UF17	0	FD1UF17	0	5.99	3.67	*
FD6	GOMD1ot	0	FD1UF17	0	7.44	0.19	–
FD7	FD1UF17	0	FD1UF17	0	5.50	1.42	+
FD8	FD1UF17	0	FD1UF17	0	6.56	5.36	*
FD9	FD2UF15	3	FD2UF15	3	3.51	2.44	*
FD10	FD5UF18	0	FD1UF17	1	0.32	0.32	–
FD11	FD3UF16	0	FD5UF18	0	5.51	4.62	*
FD12	RS1UF35	2	PT3	2	–0.31	0.00	
FD13	FD3UF16	0	JT5	1	0.84	0.12	–
FD14	RS1UF35	3	FD4UF14	2	1.26	1.26	+
FD15	FD1UF17	0	FD1UF17	0	6.05	5.89	*

[a] Sum of the log-likelihood ratios at each locus

[b] + = 95%, * = 80%, – = most likely (<80%)

sis with the “true” known parents (Table 3) for all progeny except FD×E7. The two-stage analysis identifies seven of the progeny as the result of self-pollination (47%).

The most-likely first candidate parents for 12 of the 15 USDA progeny were identified as ‘Fiji Dwarf’ individuals (Table 5), 2 at the 95% confidence, 8 at 80% and 2 at less than 80%. Offspring-candidate parent mismatches were recorded for four of the progeny, two at three loci (FD9 and FD 14), one at two loci (FD12) and one at one locus (FD4). Three of the first candidate parents were identified as other varieties. With the results of first analysis used as known parents, ‘Fiji Dwarf’ second parents were identified for 13 of the 15 offspring, 2 with 95% confidence, 9 at 80% and 2 at less than 80%. One of the assignments of a different variety as second parent (PT3 for FD12) had a negative LOD score, thus there is no confidence in that parentage assignment. Based on the 2-stage parentage analysis, eight of the 15 progeny (53%) were estimated to be inbred. Two of the USDA ‘Fiji Dwarf’ estimated to be out-crossed with other varieties (FD12 and FD14) have 2 to 3 loci mismatches with both putative parents, and FD12 had a negative LOD score for the second parent. As both were evaluated as “true” ‘Fiji Dwarf’ phenotypes, it is likely that the true parents of these two palms are not represented in the pool of candidate parents.

Overall cluster analysis

When 17 putative hybrid and off-type individuals are removed from the analysis, NJ with modified Roger’s distance unites all of the ‘Malayan Dwarf’ individuals in a single cluster (Fig. 1) flanked successively by the single F_1 hybrid (‘Malayan Dwarf’ × ‘Panama Tall’) ‘Maypan’ (M1) included in the analysis, a cluster consisting of two ‘Atlantic Tall’ (JT2, JT4) and an undetermined tall (TALL?), and one out-lying ‘Panama Tall’ individual (PT6). The five USDA ‘Red Malayan Dwarf’-2 individuals (RM1-5) that are red phenotypes form a sister cluster to the other USDA population [which also includes the four ‘Red Malayan Dwarf’ from UF (GOMD1-4)], while five yellow ‘Malayan Dwarf’ phenotypes (YM6-10) from the same population are distributed within the predominantly ‘Green Malayan Dwarf’ cluster.

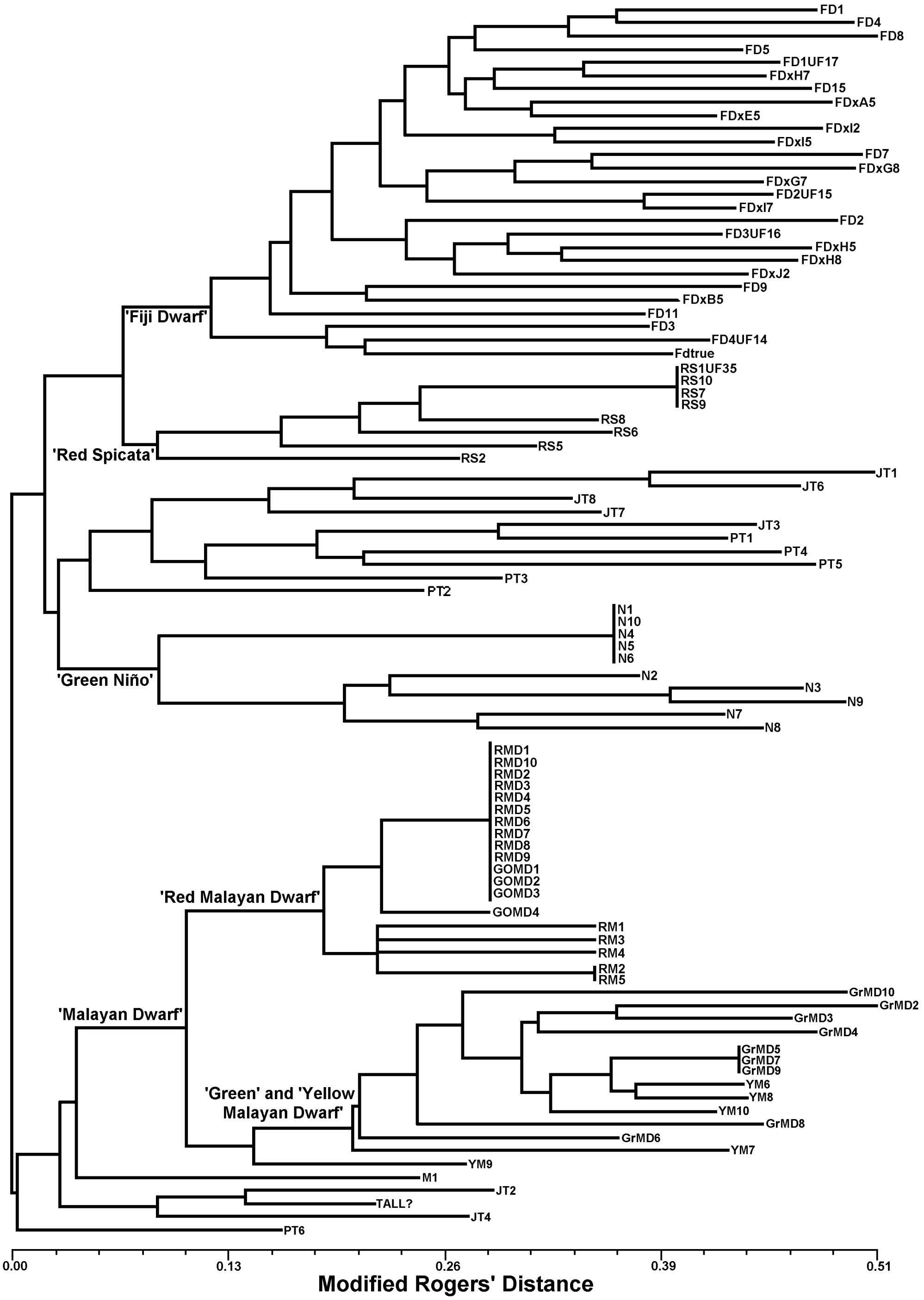

Fig. 1 Neighbor joining tree using modified Rogers distance (Wright 1978) based on overall allele frequencies across 93 coconut genotypes (17 putative hybrids and outliers removed)

Genetic variation within cultivar groups

The highest gene diversity was found in the tall cultivars (Table 6), with $\hat{H} = 0.583$ cumulatively, and 0.679 for the 'Panama Tall'. The lowest diversity was found in the 'Red Malayan Dwarf'-1 population, a mixture of USDA and UF palms, which were monomorphic across all loci. The cumulative $\hat{H}$ for the 'Malayan Dwarf' individuals was 0.202. After the tall coconuts, the 'Fiji Dwarf' were most genetically diverse, with $\hat{H} = 0.436$. Observed heterozygosity levels are mostly commensurate with the gene diversity values, but both 'Fiji Dwarf' and 'Green Niño' are more allelically diverse than heterozygous. Levels of heterozygosity in the 'Fiji Dwarf' population may be artificially lowered by the restricted number of 'Fiji Dwarf' parents, and the relatively sizable number of inbred individuals according to the parentage analysis (33–53%).

Table 6 Genetic variation within the nine *C. nucifera* cultivar populations analyzed across 15 SSR loci

Cultivar	n[a]	P[b]	A[c]	Ap[d]	Ho[e]	Gene diversity
‘Atlantic Tall’	7.0	1.00	2.80	2.80	0.467	0.583 ± 0.153
‘Panama Tall’	5.8	1.00	3.00	3.00	0.618	0.679 ± 0.152
All ‘Tall’	12.8	1.00	3.20	3.20	0.535	0.583 ± 0.182
‘Red Malayan Dwarf’1	13.0	0.00	1.00	***	0.000	0.000
‘Red Malayan Dwarf’2	6.0	0.13	1.33	3.50	0.011	0.074 ± 0.240
‘Yellow Malayan Dwarf’	5.0	0.53	1.60	2.13	0.160	0.210 ± 0.221
‘Green Malayan Dwarf’	9.0	0.67	1.80	2.20	0.089	0.190 ± 0.170
All ‘Malayan Dwarf’	33.0	0.73	2.27	2.73	0.051	0.202 ± 0.210
‘Fiji Dwarf’	26.8	0.87	2.73	3.00	0.281	0.436 ± 0.267
‘Green Niño’	9.9	0.73	1.80	2.09	0.167	0.392 ± 0.215
‘Red Spicata’	9.0	0.80	1.93	2.17	0.215	0.254 ± 0.152

[a] Average sample size
[b] Proportion polymorphic loci
[c] Alleles per locus
[d] Alleles per polymorphic locus
[e] Observed heterozygosity

Table 7 Unique alleles within the nine *C. nucifera* cultivar populations analyzed

Locus	Allele (bp)	Frequency	Cultivar
CAC6	148	0.037	‘Fiji Dwarf’
CAC20	127	0.429	‘Atlantic Tall’
CAC20	129	0.292	‘Fiji Dwarf’
CAC52	145	0.352	‘Fiji Dwarf’
CAC52	160	0.093	‘Fiji Dwarf’
CAC56	139	0.143	‘Atlantic Tall’
CAC56	145	0.574	‘Fiji Dwarf’
CAC56	159	0.019	‘Fiji Dwarf’
CAC71	173	0.037	‘Fiji Dwarf’
CAC84	150	0.300	‘Green Niño’

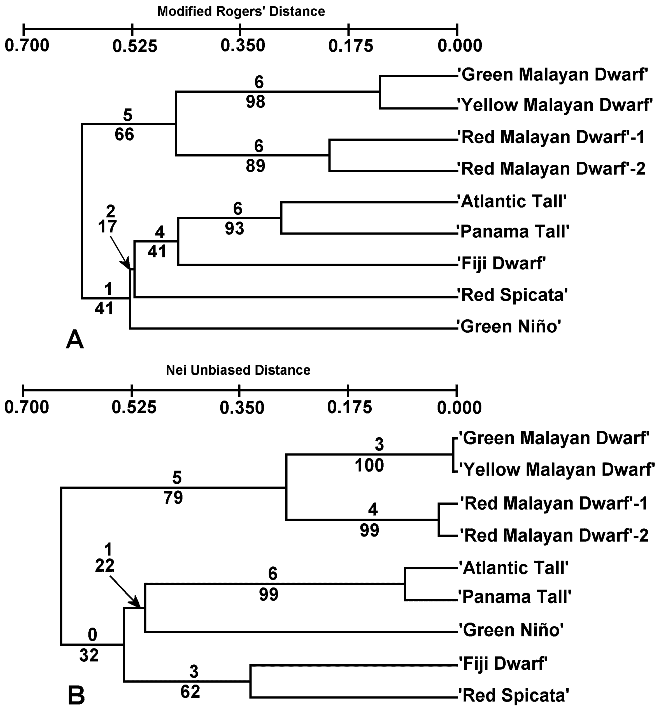

Fig. 2A, B UPGMA dendrograms based on genetic distances between coconut varietal populations. **A** Modified Rogers’ distance (Wright 1978). **B** Nei’s (1978) unbiased distance. *Numbers* above branches are the number of loci supporting that node; *numbers* below are bootstrap percentages

Unique alleles are found in only three cultivars (Table 7), seven in the ‘Fiji Dwarf’, two in the ‘Atlantic Tall’ and one in ‘Green Niño’. The greatest genetic identity (Table 8) between varieties is 0.995 between ‘Yellow Malayan Dwarf’ and ‘Green Malayan Dwarf’, the lowest is between the ‘Red Malayan Dwarf’ and the ‘Fiji Dwarf’ (average of ‘Red Malayan Dwarf’-1 and 2 = 0.405). Overall, average pairwise identities among the three ‘Malayan Dwarf’ phenotypes is 0.839. The highest identity is 0.920 between the ‘Atlantic Tall’ and ‘Panama Tall’. The highest identity values of the ‘Fiji Dwarf’ are with the ‘Panama Tall’ (I = 0.716) and ‘Red Spicata’ (I = 0.717) cultivars.

Using both modified Rogers’ distance (Wright 1978) and Nei’s (1978) unbiased genetic distance coefficients to generate UPGMA phenograms generates two trees of similar topology. Highest bootstrap support is for the clusters that join the two tall cultivars and all of the ‘Malayan Dwarf’ phenotypes, respectively (Fig. 2). Weakest bootstrap support in both trees is for the nodes that resolve the genetic relationships of the tall cultivars with ‘Green Niño’, ‘Red Spicata’ and ‘Fiji Dwarf’. Modified Roger’s distance (Fig. 2A) resolves ‘Fiji Dwarf’ closest to the talls, with 41% bootstrap support, with ‘Red Spicata’ and ‘Green Niño’ as successive outliers with no support (17% and 41%, respectively). Nei’s (1978) unbiased distance (Fig. 2B) places ‘Green Niño’ in a cluster with the talls with no support (22%). ‘Red Spicata’ and ‘Fiji Dwarf’ cluster together with 62% bootstrap support, and are joined to the former with a bootstrap of 32%.

Principal coordinate analysis

PCA was performed on the talls and ‘Malayan Dwarf’ phenotypes, including out-lying and off-type individuals (Fig. 3). The talls and ‘Malayan Dwarf’ phenotypes are clearly distinguishable in the PCA scattergram (Fig. 3),

Table 8 Pairwise identities and distances between nine *C. nucifera* varietal populations in Florida analyzed across 15 SSR loci. Above diagonal, Nei's (1978) unbiased identity; below diagonal, modified Rogers' distance (Wright 1978)

Cultivar	FD	RMD1	GrMD	JT	N	PT	RS	RMD2	YMD[a]
FD	****	0.395	0.414	0.598	0.506	0.716	0.717	0.415	0.414
RMD1	0.701	****	0.739	0.606	0.548	0.590	0.645	0.972	0.798
GrMD	0.644	0.494	****	0.575	0.485	0.570	0.462	0.736	0.995
JT	0.482	0.582	0.552	****	0.562	0.920	0.513	0.604	0.600
N	0.563	0.627	0.624	0.526	****	0.651	0.605	0.544	0.529
PT	0.417	0.594	0.555	0.284	0.479	****	0.597	0.594	0.573
RS	0.452	0.570	0.657	0.577	0.544	0.533	****	0.599	0.510
RMD2	0.668	0.174	0.488	0.568	0.616	0.576	0.593	****	0.769
YMD	0.651	0.443	0.137	0.546	0.605	0.561	0.634	0.464	****

[a] FD = 'Fiji Dwarf', RMD1 = 'Red Malayan Dwarf'-1, GrMD = 'Green Malayan Dwarf', JT = 'Atlantic Tall', N = 'Green Niño', PT = 'Panama Tall', RS = 'Red Spicata', YMD = 'Yellow Malayan Dwarf', RMD2 = 'Red Malayan Dwarf'-2

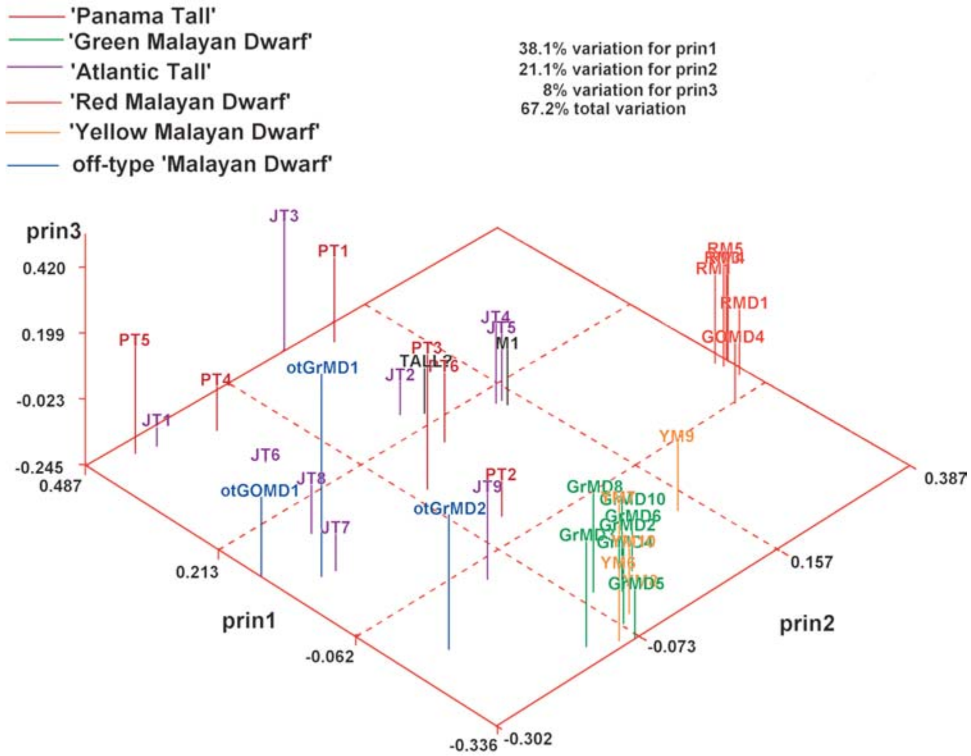

Fig. 3 Three-dimensional principal coordinate analysis plot of tall and dwarf coconut genotypes using modified Rogers' distance (Wright 1978) based on overall allele frequencies in 15 SSR loci

which captures 67.2% of the total variation in the three coordinates. There is no distinction between the 'Atlantic Tall' and 'Panama Tall' varieties. They are all broadly dispersed but, with the exception of a single 'Atlantic Tall' (JT9), are restricted to four of the plot squares defined by coordinates 1 and 2 within which no dwarfs appear. Only three palms appear in the intervening plot space between the talls and dwarfs, one 'Panama Tall' (PT2) and one 'Atlantic Tall' (JT9). JT9 also clustered with the dwarfs in the overall NJ tree (data not shown), where PT2 is distantly joined with a putative 'Fiji Dwarf' × 'Atlantic Tall' hybrid (FD×F7). In the reduced data set NJ tree from which JT9 was dropped (Fig. 1), PT2 resolves as an outlier to the larger tall cluster. One 'Green Malayan Dwarf' off-type resolves between the talls and dwarfs along coordinate 1, but is closer to one subgroup of talls along coordinate 2. The other dwarf off-types resolve closer to the talls than to any of the dwarf clusters. The 'Malayan Dwarf' palms form two distinct and fairly tight clusters, one comprising all 'Red Malayan Dwarf' phenotypes at both UF and USDA, and the other a combination of the 'Green' and 'Yellow' phenotypes.

Discussion

Relationships among cultivar groups

Previous studies of SSR variation in coconuts (Perrera et al. 1999, 2000; Rivera et al. 1999; Teulat et al. 2000) have all concluded that tall varieties and dwarf varieties can be distinguished by the higher heterozygosity and gene diversity of the talls compared to dwarfs. This dichotomy reflects the difference in breeding habit between tall coconuts and dwarfs; talls are primarily outcrossing, while dwarfs are autogamous (Liyanage 1949). Talls also do not express the diversity of color phenotype that characterizes many dwarf cultivars (Harries 1978). Dwarfs readily interbreed with each other, but also with talls when a minority of dwarfs are situated near numerous tall types (Harries 1978).

Our analysis encompasses fewer cultivars groups than those of Perrera et al. (2000) or Teulat et al. (2000), but a similar conclusion can be drawn from the NJ (Fig. 1) and UPGMA (Fig. 2) analyses of our data. The tall varieties are much more genetically diverse than the 'Malayan Dwarf' populations (Tables 6 and 8). However, our larger population sampling of the 'Malayan Dwarf' phenotypes indicates that the 'Red Malayan Dwarf' shows significant genetic distance from the 'Green' and 'Yellow Malayan Dwarf' phenotypes. Moreover, the 'Yellow Malayan Dwarf' phenotypes are not distinct from the 'Green Malayan' (Figs. 1, 3). Yellow was the predominant color form when the 'Malayan Dwarf', thought to have been introduced from Java about 1890 (Harries 1978), was first popularized (Jack and Sands 1922). While green phenotypes now dominate in Jamaica (Harries 1978), red and yellow phenotypes are the most common in Malaysia (Whitehead 1966b). Our data would suggest that yellow and green 'Malayan Dwarf' phenotypes are derived from a common gene pool, while the red phenotypes represent a distinct, albeit related genotype. It is possible, however, that the introduction of these dwarf phenotypes in Jamaica, from which our populations were derived, may not have included the full extent of genetic variation within these varieties throughout their broad cultivated range.

Harries (1978) recognized three basic dwarf types in *C. nucifera*. Two are synonymous to the *nana* and *javanica* varietal groups proposed by Narayana and John (1949). The 'Green Niño' of our study is a *nana* (coconiño or baby coconut) type; the 'Malayan Dwarf' belongs to the *javanica* class. The third dwarf class recognized by Harries (1978) is the 'Niu leka' or 'Fiji Dwarf'.

The 'Fiji Dwarf' coconut is most easily characterized by its very short internodes that result in a stout trunk, dense canopy and broad leaves. Harries (1978) notes that in all respects other than height, the 'Fiji Dwarf' resembles a tall: swollen trunk base, out-crossing reproductive behavior, lack of bright red- and yellow-fruited phenotypes, and large fruit size. The isolated distribution of this phenotype, until recent decades, across a few islands of the South Pacific, led Harries (1978) to suggest that it represented a completely distinct domestication event relative to other dwarf classes. Our data support this conclusion insofar as the cultivar shows the second highest gene diversity after the talls (Table 6), and the largest number of unique alleles of any cultivar group within our study (Table 7). Moreover, the lowest genetic identities of the 'Fiji Dwarf' are with the 'Malayan Dwarf' phenotypes (Table 8).

What are the genetic affinities of the 'Fiji Dwarf' cultivar? The NJ phenogram using Nei (1978) genetic identity (Fig. 2B) posits 'Fiji Dwarf' as closest to 'Red Spicata' with weak bootstrap support (62%). Modified Rogers distance (Fig. 2A) places the 'Fiji Dwarf' with the talls, but with only 41% support. The highest genetic identities of this variety are with 'Panama Tall' (I = 0.716) and 'Red Spicata' (I = 0.717), two varieties that have only an I of 0.597 between them. Moreover, the identity between the 'Atlantic Tall' and the 'Fiji Dwarf' is only I = 0.598, while that between the 'Atlantic' and 'Panama Tall' is 0.920. This at first seems puzzling, but is supportive of an hypothesis of coconut dissemination (Harries 1977). It is generally accepted that introduction of coconuts to the Atlantic coasts of Africa and tropical America (including the Caribbean) date to no more than 500 years BP (Purseglove 1985), and that the gene pool for these introductions was derived from germplasm in India and Sri Lanka that was first brought to Mozambique (Harries 1977). The SSR studies of Perera et al. (2000) and Teulat et al. (2000), and the RFLP study of Lebrun et al. (1998), both of which heavily sampled African and south Asian genotypes, support this dispersal theory. The 'Panama Tall', however, originated on the Pacific coast of America, an introduction which Whitehead (1976) hypothesized as a culmination of an eastward migration from Southeast Asia to the south Pacific and finally, to the west coast of America. The high identity between the 'Fiji Dwarf' and 'Panama Tall' would be consistent with this hypothesis. 'Panama Tall' coconuts were similarly allied with Polynesian coconuts in Rohde et al.'s (1995) study using ISTRs. The high identity between the 'Panama Tall' and the 'Atlantic Tall', and our inability to distinguish discrete clusters of each cultivar (Figs. 1, 3), may indicate that hybridization between these two tall varieties occurred in the Jamaican source population of our 'Panama Tall'.

'Green Niño', as the sole representative of the *nana* cultivar group in our analyses, also has its highest genetic identity with the 'Panama Tall' (0.651), and one unique allele. This adds additional support to Whitehead's (1976) hypothesis of an eastward, trans-Pacific movement of Southeast Asian coconuts, and also indicates that the *nana* and *javanica* genotypes probably arose independently in the process of human-mediated selection of coconuts. In gross morphology, the 'Green Niño' appears intermediate between the talls and dwarfs, which is reflected genetically as well ($\hat{H}$= 0.392, Table 6).

'Spicata' coconuts are anomalous for the absence of inflorescence branches and the predominance of female flowers. They were first described from India (Jacob 1941). Like the *javanica* dwarfs, 'Spicata' coconuts have strongly pigmented red and yellow forms. Unlike the 'Malayan Dwarf' color phenotypes, reds and yellows did not segregate with our data. The highest genetic identity of 'Red Spicata is with the 'Fiji Dwarf', which would contradict the biogeographic affinity hypothesis of Harries (1977). One characteristic that both varieties share is a large number of female flowers on the inflorescences (Jacob 1941; Whitehead 1966a). However, introgression of other varieties into the 'Red Spicata' population that we sampled cannot be ruled out, as most of the germplasm at USDA was hastily regenerated from local seed after Hurricane Andrew struck in 1992. Perera et al. (2000) included one sample labeled “Philippine Tall (Spicata)” in their analysis, and it was resolved within a mixed cluster of mostly tall phenotypes from southeast Asia. However, the *spicata* characteristic may have originated several times within diverse genotypes. In fruit size and shape, ‘Red Spicata’ is similar to the *nana* cultivar group to which ‘Green Niño’ belongs.

Off-type ‘Malayan Dwarf’ phenotypes

When Hurricane Gilbert devastated the coconut seed orchards of the Jamaican CIB in 1988, the main source of certified ‘Malayan Dwarf’ seed for Florida coconut growers was interrupted. Many growers began collecting seed from ‘Malayan Dwarf’ individuals in the local landscapes, which had much higher potential for hybridization with surviving talls. As these hybrids often exhibited heterosis in production, they were sometimes preferentially selected for their vigor by growers. As these palms appeared in the landscape, we noted characteristics of intermediacy, and coined the term “off-type” to describe these putative hybrids. The main characteristics in these palms that depart from the ‘Malayan Dwarf’ phenotype are a (1) a significant basal trunk bole (versus little swelling at the base) and (2) a more 3-sided fruit (versus round). The three putative ‘Malayan Dwarf’ off-types included in this study do in fact fail to cluster with the true dwarfs in the PCA analysis (Fig. 3), and appear as outliers in the initial NJ tree of all 110 individuals (data not shown). SSR loci could thus be used to rogue putative hybrids from large seedling populations of ‘Malayan Dwarf’ seedlings.

Phenotypic variation in the ‘Fiji Dwarf’

Whitehead (1966a, p 31) described the ‘Niu Leka’ or ‘Fiji Dwarf’ coconut as follows: “The crown of the palm is commonly heavy and dense with short leaves, heavy mid-ribs and broad attachment to the trunk. There are often large numbers of branches and female flowers in the inflorescence.” Whitehead (1966a) also noted some degree of variation in fruit color. Harries (1978) noted how the expression of dwarfness in the ‘Fiji Dwarf’ (i.e., reduced internode length) can be so extreme as to inhibit flowering and fruiting. This is evident in two of the coconuts sampled in this study; both FD4UF14 and FD2 exhibit this characteristic. Harries (1978) also remarked on segregation for dwarfness, which he attributed to both out-crossing with talls and the inherent heterozygosity of the variety. Again, this is evident in the USDA progeny (the UF progeny are not yet sufficiently mature to evaluate). The 15 loci that we used in our analyses do not uncover any obvious associations between phenotype and genotype in the ‘Fiji Dwarf’. Without controlled crosses between known parents and/or a segregating F_2 generation it is difficult to assess how color and overall phenotype are inherited.

SSR markers and LY resistance

Lethal yellowing killed 42% of 122 ‘Fiji Dwarf’ coconuts that were planted at five different sites in resistance trials in Jamaica by 1979 (CIB, 1979). Fifty percent of the original phenotypically off-type ‘Fiji Dwarf’ imports from Jamaica to the University of Florida have died from LY, while none of the true types have been lost after 20 years of exposure. It has been suggested that individual ‘Fiji Dwarf’ palms that express the phenotype characterized by Whitehead (1966a) are more resistant, while off-types are more susceptible (Broschat et al. 2002). While we cannot yet adequately address the hypothesis of a truly LY-resistant ‘Fiji Dwarf’ phenotype/genotype, the genetic characterization of the germplasm of this cultivar at both UF and USDA will allow us to associate loss to LY with particular SSR genotypes in the future.

Our genetic characterization of the talls at UF will be equally if not even more valuable for the future tracking of relative LY resistance, since the ‘Atlantic Tall’ population at UF was derived from surviving individuals in the landscape during the first catastrophic epidemic of LY in the 1970s and 80s. In that regard, the clustering of some of these palms with the dwarfs in the NJ tree (Fig. 1), may ultimately reflect variance in LY resistance among the ‘Atlantic Tall’ coconuts.

Inferences from parentage analysis of ‘Fiji Dwarf’ coconuts

From a practical standpoint, it is useful to consider what the parentage analysis of the ‘Fiji Dwarf’ population tells us about the reproductive behavior of this variety in the context of varietal seed-production. Four of the original UF parents are approximately 6 to 8 m apart in the grove, surrounded by various other varieties. Despite these demographics, which would seem to increase the likelihood of out-crossing, our parentage analyses (Tables 3–5) suggest that only 20% of the progeny were out-crossed to other varieties, while 40–46% were possible selfs. This bodes well for maintaining varietal purity in a larger stand of ‘Fiji Dwarf’ coconuts maintained at a reasonable distance from any other variety, conditions which characterize the ‘Fiji Dwarf’ orchard at USDA. However, if LY resistance is indeed linked to the dwarf phenotype, as hypothesized by Broschat et al. (2002), then the high levels of heterozygosity in the ‘Fiji Dwarf’ will necessitate identification of homozygous individuals exhibiting this phenotype for propagation purposes.

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References

Ashburner GR, Thompson WK, Halloran GM (1997) RAPD analysis of South Pacific coconut palm populations. Crop Sci 37:992–997

Beck HT, Balick MJ (1990) Useful palms of the world. Columbia University Press, New York

Broschat TK, Harrison NA, Donselman H (2002) Losses to lethal yellowing cast doubt on coconut cultivar resistance. Palms 46: (in press)

Carpio CB (1982) Biochemical studies of *Cocos nucifera* L. Kalikasan Philipp. J Biol 11:319–338

Coconut Industry Board (CIB) (1979) Annual report of research. Kingston, Jamaica

Edwards AWF (1972) Likelihood. Cambridge University Press, Cambridge, UK

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Harries HC (1970) The 'Malayan Dwarf' supercedes the 'Jamaica Tall' coconut. I. Reputation and performance. Oléagineux 25:527–531

Harries HC (1973) Selection and breeding of coconuts for resistance to diseases such as lethal yellowing. Oléagineux 28:395–398

Harries HC (1977) The Cape Verde region (1499–1549); the key to coconut culture in the Western hemisphere? Turrialba 27:227–231

Harries HC (1978) The evolution, dissemination and classification of *Cocos nucifera* L. Bot Rev 44:265–320

Harries HC (1995) Coconut (*Cocos nucifera* L.). In: Smartt J, Simmond NW (eds) Evolution of crop plants, 2nd edn. Longman, London New York, pp 389–394

Harries HC, Romney DH (1974) Maypan: an F_1 hybrid coconut variety for commercial production in Jamaica. World Crops 26:110–111

Howard FW, Barrant CI (1989) Questions and answers about Lethal Yellowing disease. Principes 33:163–171

Jack HW, Sands WN (1922) The dwarf coconut in Malaya. Malayan Agric J 10:140–165

Jacob KC (1941) A new variety of coconut palm (*Cocos nucifera* L. var. *spicata* K. C. Jacob). J Bombay Nat Hist Soc 41:906–907

LeBrun P, N'Cho YP, Seguin M, Grivet L, Baudouin L (1998) Genetic diversity in coconut (*Cocos nucifera* L.) revealed by restriction fragment length polymorphism (RFLP) markers. Euphytica 101:103–108

Lewis PO, Zaykin D (2001) Genetic data analysis: computer program for the analysis of allelic data. Version 1.0 (d16c). Free program distributed by the authors over the internet from http://lewis.eeb.uconn.edu/lewishome/software.html

Liyanage DV (1949) Preliminary studies on the floral biology of the coconut palm. Trop Agric 105:171–175

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol 7:639–655

McCoy RE, Howard FW, Tsai JH, Donselman HM, Thomas DL, Basham HG, Atilano RA, Eskafi FM, Britt L, Collins ME (1983) Lethal yellowing of palms. University of Florida Agricultural Experiment Station Bulletin 834, Gainesville, Florida

Meerow AW (1992) Betrock's guide to landscape palms. Betrock Information Systems, Hollywood, Florida

Miller MP (1997) Tools for population genetic analyses (TFPGA) 1.3: a Windows program for the analysis of allozyme and molecular population genetic data. Computer software distributed by the author

Narayana GV, John CM (1949) Varieties and forms of the coconut (*Cocos nucifera* Linn.). Madras Agric J 8:349–368

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

Perera L, Russell JR, Provan J, McNichol JW, Powell W (1998) Evaluating genetic relationships between indigenous coconut (*Cocos nucifera* L.) accessions from Sri Lanka by means of AFLP profiling. Theor Appl Genet 96:545–550

Perera L, Russell JR, Provan J, Powell W (1999) Identification and characterization of microsatellite loci in coconut (*Cocos nucifera* L.) and the analysis of coconut populations in Sri Lanka. Mol Ecol 8:344–346

Perera L, Russell JR, Provan J, Powell W (2000) Use of microsatellite DNA markers to investigate the level of genetic diversity and population genetic structure of coconut (*Cocos nucifera* L.). Genome 43:15–21

Pursglove JW (1985) Tropical crops: monocotyledons, 5th edn. Longman, London

Rohde W, Kullaya A, Rodriguez J, Ritter E (1995) Genome analysis of *Cocos nucifera* L. by PCR amplification of spacer sequences separating a subset of *Copia*-like *Eco*R1 repetitive elements. J Genet Breed 49:179–186

Rivera R, Edwards KJ, Barker JHA, Arnold GM, Ayad G, Hodgkin T, Karp A (1999) Isolation and characterization of polymorphic microsatellites in *Cocos nucifera* L. Genome 42:668–675

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Slate J, Marshall TC, Pemberton JM (2000) A retrospective assessment of the accuracy of the paternity inference program Cervus. Mol Ecol 9:801–808

Sokal R, Rohlf FJ (1995) Biometry, 3rd edn. W.H. Freeman, New York

Teulat B, Aldam C, Trehin R, Lebrun P, Barker JHA, Arnold GM, Karp A, Baudouin L, Rognon F (2000) An analysis of genetic diversity in coconut (*Cocos nucifera*) populations from across the geographic range using sequence-tageed microsatellites (SSRs) and AFLPs. Theor Appl Genet 100:764–771

Whitehead RA (1966a) Sample survey and collection of coconut germplasm in the Pacific Islands (30 May–5 September 1964). Ministry of Overseas Development, HMSO, London

Whitehead RA (1966b) Some notes on dwarf coconut palms in Jamaica. Trop Agric Trinidad 43:277–298

Whitehead RA (1976) Coconut. In: Simonds NW (ed) Evolution of crop plants. Longman, London, pp 221–225

Wright S (1978) Evolution and the genetics of populations, vol. 4. Variability within and among natural populations. University of Chicago Press, Chicago