

PEDIGREE ANALYSIS OF FLORIDA MANGO CULTIVARS

CECILE T. OLANO,¹ RAYMOND J. SCHNELL,^{1*}
WILBER E. QUINTANILLA¹ AND RICHARD J. CAMPBELL²

¹National Germplasm Repository, USDA ARS, Miami
13601 Old Cutler Road
Miami, FL 33158

²Fairchild Tropical Botanic Garden, Miami
10901 Old Cutler Road
Coral Gables, FL 33156

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Abstract. The Florida mango (*Mangifera indica* L.) cultivars were historically described as hybrids between Indian types (monoembryonic) and Southeast Asian types (predominantly polyembryonic). Early molecular data including isozyme and Randomly Amplified Polymorphic DNA analysis supported the hybrid origin. The Florida varieties are distinctive and combine characteristics of both Indian and Southeast Asian types. Although adapted to Florida conditions, they perform well across many different environments, and several, including 'Tommy Atkins', 'Keitt', 'Haden', 'Irwin', and 'Parvin' are produced commercially in tropical and sub-tropical countries. Using 25 microsatellite markers we analyzed 63 Florida varieties as well as cultivars from India, Asia and other locations, to construct likely pedigrees for each Florida cultivar. Parentage analysis was performed across four generations based on introduction dates of accessions into Florida and selection dates for Florida varieties. The cultivars were sampled from the accessions maintained at the National Germplasm Repository and by Fairchild Tropical Botanic Garden (FTBG). Results suggest that as few as four Indian cultivars, and the 'Turpentine' land race were involved in the early cultivar selections. Sixty-three of the 85 parents identified across the four generations were other Florida cultivars. Pedigree results are in agreement with the findings of a larger diversity analysis study that the Florida types are more closely related to Indian than to Southeast Asian types and that the Florida group is not more diverse than either of the originating parental groups. The Florida group is unique and a subset of the Florida types have proven to have an unusual level of production stability and environmental adaptability.

The mango (*Mangifera indica* L.) is native to southern Asia where it has been cultivated since ancient times. Movement of mango germplasm has been a consequence of its cultivation throughout tropical and subtropical regions of the world. Mango was introduced to Florida in the 19th century. The earliest introductions were from the West Indies and from India, followed by introductions of hundreds of accessions early in the 20th century from South Asia, India, and from secondary mango growing areas of the world. The result was the development of a Florida group of mangos (Knight and Schnell, 1994). The Florida mango cultivars are unique in that they are hybrids between Indian types (monoembryonic) and the Southeast Asian types (predominantly polyembryonic) selected under south Florida conditions. Florida selections are not

the result of formal breeding programs. Early Florida selections were made by growers and enthusiasts and historical information is often anecdotal. The Florida Mango Forum, established in 1938 for the advancement of mango production, endeavored to document historical information on the parentage of Florida varieties (Florida Mango Forum, 1947-1955; Florida Mango Forum, 1951). In addition to the USDA Germplasm Resources Information Network (GRIN) database, several sources compile information on Florida mango selections and introduction of accessions to Florida (Campbell, 1992; Singh, 1960).

The Florida selections are widely grown commercial cultivars affording production stability across many environments (Mukherjee, 1997). Understanding genetic relationships among the Florida cultivars and their relationship to both the Indian and Southeast Asian races is important for identification of genes involved with wide adaptation and for the efficient development of improved cultivars. Assessment of genetic relationships can be accomplished by means of molecular marker techniques. In mango, molecular markers have been used for analysis of genetic relationships and for pedigree analysis. A study using RAPD markers, supported the 'Haden' parentage of 'Eldon', 'Lippens', 'Tommy Atkins', and 'Zill'; however, the parentage of 'Glenn' and 'Os-teen' was questioned (Schnell et al., 1995). Using DFP markers Adato (1995) confirmed the pedigree of many of the 'Haden' seedlings. Most recently Viruel et al. (2005) used microsatellite markers to confirm pedigree information of the 'Haden' family which agreed with the previous analyses (Adato et al., 1995; Schnell et al., 1995) with one exception; their clone of 'Zill' was not resolved as a seedling of 'Haden'.

In this study we used 25 SSR markers to evaluate the genetic background of the Florida mangos. Pedigree estimation was accomplished by use of a parentage analysis program. Statistical evaluation of results of parentage tests allows for approximations of parental genotypes absent from the candidate pool. The quality of improved varieties has sustained interest in growing mango in Florida for well over a century. Development of improved cultivars can be greatly enhanced by knowledge of genotypes associated with desired phenotypes, relatedness of individuals and inheritance patterns of desired traits.

Materials and Methods

Leaf material was sampled from the USDA and Fairchild Tropical Botanic Garden (FTBG) *Mangifera* germplasm collections maintained at the National Germplasm Repository (NGR) in Miami, Florida. The collections included 63 Florida selections (Table 1) and 129 introduced cultivars.

DNA extraction was performed as described by Schnell et al. (2005). Twenty five microsatellite markers were used in this study (Table 2). Twelve were developed at the NRG-Miami (Schnell et al., 2005), 12 were reported by Viruel et al. (2005), one was provided from Cirad-flhor (Marie-France Duval, personal communication). PCR amplification and electrophoresis were carried out as described in Schnell et al. (2005), with the exception that BSA was not included in the

*Corresponding author; e-mail: rschnell@saa.ars.usda.gov

Table 1. Florida mango selections used as offspring in the four sets for parentage analysis, with parents reported in the literature and most-likely parents estimated from the analysis.

	Cultivar	Parent (from literature)	Estimated parent	
SET I: 1880-1910	Brooks	Sandersha	Sandersha	
	Haden	Mulgoba × Turpentine	Mulgoba × Turpentine	
SET II: 1910-1940	Anderson	Sandersha	Sandersha × Haden	
	Cushman		Haden × Amini	
	Edward	Haden × Carabao	Haden	
	Florigon	Saigon	Haden	
	Keitt	Mulgoba?	Brooks	
	Kent	Brooks	Brooks × Haden	
	Lippens	Haden	Haden	
	Palmer		Haden	
	S-01	Cambodiana	Haden	
	S-19	Cambodiana	Turpentine 10	
	Springfels	Haden (× Sandesha?)	Haden	
	Van Dyke		Haden	
	Zill	Haden	Haden × Bombay	
	SET III: 1940-1960	Carrie	Sophie Fry	Julie
Cogshall			Haden	
Earlygold			Haden	
Eldon		Haden	Cowasju Patel	
Glenn		Haden	Haden	
Golden Lippens		Lippens	Lippens	
Hodson			Haden	
Irwin		Lippens	Lippens × Haden	
Jacquelin			Haden × Bombay	
Osteen		Haden	Haden	
Pettigrew			Mulgoba	
Ruby			Haden	
Sensation			Haden × Brooks	
Sunset			Haden × Amini	
Tommy Atkins		Haden	Haden	
Valencia Pride		Haden	Haden	
SET IV: 1960-Present		Allen-King/Everbearing	Haden	Haden × Long
		Baileys Marvel		Haden × Bombay
	Becky	Sunset	Haden × Brooks	
	Becky ff		Lippens × Springfels	
	Beverly		Cushman	
	Dot	Carrie × (Paheri?)	Zill	
	Duncan	Saigon?	Nam Doc Mai	
	Dupuisse		Glenn	
	Eulogio		Cogshall	
	Ford		Tommy Atkins	
	Gary	Carrie	Carrie	
	Golden Nugget	Saigon?	Kent	
	Gootee		Brooks	
	Hatcher		Haden × Brooks	
	Iris		Irwin	
	Jakarta	Paheri	Zill × Kent	
	Jewel	Lippens	Lippens × Palmer	
	Joellen		Cambodiana	
	Jubilee		Sensation × Irwin	
	Lathrop		Brooks	
	Lily		Sensation × Springfels	
	Mapulehu		Step	
	Martin		Mulgoba	
	Merrit Island		Mulgoba	
	Rosigold		Ono	
	Saicra		Long	
	Southern Blush		Haden × Cushman	
	Spirit of 76	Zill (× Haden?)	Zill × Haden	
Torbet		Haden		
Winters	Ono	Ono × Haden		
Z-80		Zill × Kent		
Zilate		Keitt		

Table 2. Microsatellite loci used in the parentage analysis of Florida mango cultivars.

Locus	GeneBank accession number	Alleles	Size range
MiSHRS-1	AY942817	7	191-209
MiSHRS-4	AY942818	6	121-133
MiSHRS-18	AY942819	8	90-117
MiSHRS-26	AY942821	3	260-275
MiSHRS-29	AY942822	5	174-184
MiSHRS-30	AY942823	4	221-232
MiSHRS-32	AY942824	12	200-226
MiSHRS-33	AY942825	6	236-257
MiSHRS-34	AY942826	2	228-231
MiSHRS-37	AY942828	7	125-137
MiSHRS-39	AY942829	9	345-369
MiSHRS-44	AY942830	7	245-278
LMMA1	AY628373	10	195-215
LMMA4	AY628376	6	222-244
LMMA5	AY628377	3	278-282
LMMA7	AY628379	7	194-214
LMMA8	AY628380	9	254-270
LMMA9	AY628381	7	171-187
LMMA10	AY628382	12	150-188
LMMA11	AY628383	9	230-248
LMMA12	AY628384	7	198-206
LMMA14	AY628386	5	162-171
LMMA15	AY628387	7	207-221
LMMA16	AY628388	8	211-242
mMiCIR014	AJ635176	8	148-165

PCR reactions for the primers reported by Viruel et al. (2005). Data analysis was accomplished with GeneMapper 3.0 (Applied Biosystems, Inc.) for internal standard and fragment size determination and for allelic designations.

Parentage analysis. All Florida cultivars in our collections were included in the analyses as well as were mango cultivar introductions known to be in Florida at the time the Florida varieties were selected. Historical data was compiled concerning dates of introduction of each mango cultivar and dates of selection of Florida cultivars; four time periods were identified to be used in the parentage analysis. Sets of offspring and candidate parents were assembled corresponding to each time period. Each set included Florida selections (offspring; O) and cultivars known to be in Florida at the time the Florida varieties were selected (candidate parents; CP). Sets were additive such that each set included all members of the previous set (including Florida offspring) as candidate parents. Parentage analysis was performed in a multi-step process using the program CERVUS (Marshall et al., 1998; Slate et al., 2000). After generating an allele frequency dataset, this software uses a simulation program to generate log-likelihood scores and provides a confidence statistic for assigning paternity. Each offspring was compared locus by locus against each candidate parent, resulting in exclusion of candidate parents based on number of mismatches. When multiple parents remained non-excluded, preference was given to concurrence with historical data, or in the absence of historical data, the highest scoring estimation was selected.

Results

The parentage analysis, performed in a multistage process resulted in four parent-offspring sets. The possible pedigree

resulting from the parentage analysis is illustrated in Fig. 1 and listed in Table 1 for each offspring. Most-likely parents identified are summarized in Table 3.

The first set included 34 genotypes known to have existed in Florida between 1880 and 1910. These were the possible contributors to the first two named Florida cultivars, 'Haden' and 'Brooks'. The results of the analysis of Set I indicated that 'Haden' resulted from a 'Mulgoba' × 'Turpentine' hybridization. There were no mismatches at any loci between 'Haden' and 'Mulgoba', the known maternal parent. There were two 'Turpentine' genotypes with no O-CP mismatches: 'Turpentine 3' and 'Turpentine 10', but Offspring-Known Parent-Candidate Parent (O-KP-CP) mismatches were revealed for the cross of 'Mulgoba' with either of the 'Turpentine' genotypes. However, four other 'Turpentine' genotypes have alleles that would match at these loci, suggesting that the actual parental genotype is another 'Turpentine' not within the candidate parent pool. The results of the analysis of Set I also indicated 'Sandersha' is the most-likely parent of 'Brooks' with one O-CP mismatch (Table 3). A second parent was not identified for 'Brooks' due to mismatches with candidate parents at three or more loci.

Of the 26 possible parents for Set II, 17 most-likely parents were identified, represented by only four cultivars including 'Haden', 'Brooks', and two Indian cultivars ('Amini' and 'Bombay') that were in Florida after 1900 but did not contribute to 'Haden' or 'Brooks'. Four Florida selections from Set II ('Zill', 'Lippens', 'Springfels' and 'Edward') were expected to have 'Haden' as the maternal parent, and this was confirmed. 'Brooks' was confirmed to be a parent of 'Kent'. Several discrepancies were revealed between historical data and parentage estimation. 'Edward' was said to be the result of a 'Haden' × 'Carabao' hybridization, but there were mismatches at six loci with 'Carabao' and an additional mismatch occurring for the cross. 'Florigon' was reported to be a 'Saigon' seedling. None of the 'Saigon' genotypes were likely parents but 'Cambodiana', a putative 'Saigon' ancestor, had a single mismatch with 'Florigon'. Although 'S01' and 'S19' were reported as seedlings of 'Cambodiana', it was not found to be the most-likely parent having eight and seven O-CP mismatches with 'S01' and 'S19', respectively. Instead 'Haden' was identified as the most-likely parent of 'S01' and 'Turpentine 10' that of 'S19'. Allelic configurations indicate that a genotype of the 'Saigon' land race not in the current dataset can be expected as the other parent. The speculation that 'Mulgoba' was the parent of 'Keitt' was unsubstantiated as six O-CP mismatches were revealed, instead 'Brooks' was identified as the most-likely parent. With no genotyping errors allowed 'Brooks' was estimated to be the most-likely parent of 'Anderson'. However, with genotyping errors allowed 'Sandersha', the reported parent, was selected as the most-likely parent in spite of one locus mismatch.

Of the 32 possible parents for the 16 Florida selections in Set III, 20 were identified, consisting of eight cultivars: five were common to Set I and Set II, while 'Lippens' is a seedling of 'Haden', 'Julie' is from the West Indies and 'Covasju Patel' is an Indian variety. The reported parentage of five Florida selections was confirmed. The 'Haden' parentage of 'Eldon' was not confirmed due to two O-CP mismatches. Four candidates were found to have only one mismatch with 'Eldon', only one ('Covasju Patel') with 80% confidence. The reported parent of 'Carrie' ('Sophie Fry') was not included in the analysis and 'Julie' was selected as the most-likely candidate.

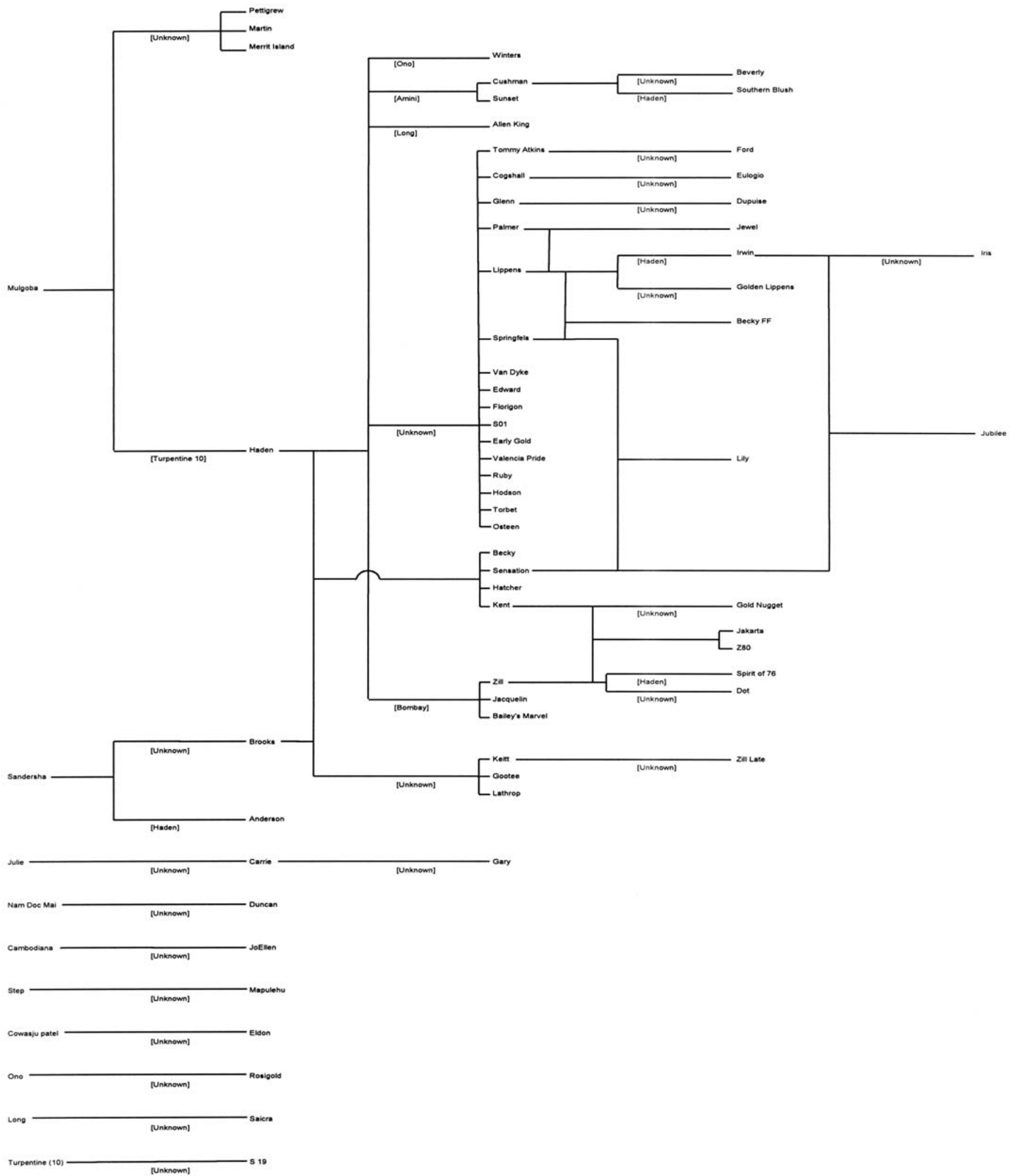


Fig. 1. Pedigree for 63 Florida mango selections estimated from CERVUS parentage analysis. Second parent indicated in brackets below line to progeny.

Table 3. Most-likely parents as identified in the CERVUS parentage analysis of Florida mango selections, across all time periods.

Cultivar	Parent	Origin	No. of times identified as a parent
Introduced cultivars			
Amini		India	2
Bombay (Paheri)		India	3
Cambodiana	Saigon*	Southeast Asia	1
Cowasju Patel		India	1
Julie		West Indies	1
Long		West Indies	2
Mulgoba		India	4
Nam Doc Mai		Southeast Asia	1
Ono	Paheri*	Hawaii	2
Sandersha		India	2
Step		Hawaii	1
Turpentine10		West Indies	2
Florida selections			
Haden	Mulgoba × Turpentine	Florida	31
Brooks	Sandersha	Florida	7
Lippens	Haden	Florida	4
Zill	Haden × Bombay	Florida	4
Cushman	Haden × Amini	Florida	2
Springfels	Haden	Florida	2
Palmer	Haden	Florida	1
Tommy Atkins	Haden	Florida	1
Irwin	Lippens × Haden	Florida	2
Cogshall	Haden	Florida	1
Glenn	Haden	Florida	1
Sensation	Haden × Brooks	Florida	2
Kent	Haden × Brooks	Florida	3
Keitt	Brooks	Florida	1
Carrie	Julie	Florida	1

*Parent from literature.

Set IV included 32 mango cultivars selected between 1960 and 2000. In total 45 of the 64 possible parents were identified, represented by 22 cultivars. Of these, five cultivars had been estimated as parents in previous generations and 17 were unique to Set IV. Only seven of the identified candidate parents did not have ‘Haden’ or ‘Brooks’ in their pedigrees: ‘Long’, ‘Bombay’, ‘Nam Doc Mai’, ‘Carrie’, ‘Cambodiana’, ‘Step’ and ‘Ono’. The remaining 15 cultivars (36 likely parents identified) were ‘Haden’, ‘Brooks’, ‘Mulgoba’ or a derivative of ‘Haden’ and/or ‘Brooks’. Reported parentage was confirmed for five Florida selections. ‘Winters’ had one O-CP mismatch with ‘Ono’, the reported parent and no mismatches with ‘Haden’. ‘Becky’ was reported to be a seedling of ‘Sunset’ although mismatches at four loci were detected and ‘Becky’ was estimated to be from a cross between ‘Haden’ and ‘Brooks’. ‘Duncan’ and ‘Gold Nugget’ were reported to be ‘Saigon’ seedlings but all ‘Saigon’ genotypes were excluded due to mismatches. ‘Jakarta’ is reported as a seedling of ‘Paheri’ but five O-CP mismatches were found between them. ‘Dot’ is reported to be from a ‘Carrie × Paheri’ cross but one mismatch was found with each putative parent and the more likely parent was identified as ‘Zill’ (Table 3).

Overall, among the 63 Florida cultivars evaluated across the four parent-offspring sets, two most-likely parents were identified for 22 cultivars and only one most-likely parent for the remaining 41 cultivars (all but one estimation with 80% confidence). Among the 126 possible parents, 85 (67%) were identified and 41 (33%) remain unknown. ‘Haden’ was identified as a most likely parent 31 times; ‘Brooks’ 7 times;

‘Haden’ and/or ‘Brooks’ derivatives 24 times; ‘Mulgoba’, the maternal parent of ‘Haden’, 4 times and ‘Sandersha’, the maternal parent of ‘Brooks’, 2 times. The remaining 25 most-likely parents were six Indian (three cultivars), five West Indian (three cultivars), two Southeast Asian (two cultivars), and one Florida selection of West Indian descent.

Discussion

The pedigree reconstruction depends on availability, accuracy, and completeness of the historical data as well as accuracy and robustness of the genetic data. The results of the parentage analysis yield the best approximation for the pedigree, given the data available. The parentage analysis consists of genetically comparing each offspring against many candidate parents to exclude candidates based on mismatches, within acceptable limits. Likelihood is used to statistically distinguish non-excluded candidate parents. Parentage is either assigned to the most-likely candidate parent with a pre-determined level of confidence, or is left unassigned. Since the statistics are based on allele frequencies, the genotypic composition of the pool of individuals placed in the parental candidate pool have an affect on the identification outcome.

Discrepancies between literature reports and current estimations may arise for a number of reasons, including record keeping inaccuracies, incomplete data (not all true parents were sampled) and propagation errors leading to misidentification. Molecular marker limitations such as null alleles and mutations produce mismatches, the impact of which is dra-

matically reduced by allowing for genotyping errors. For the Florida cultivars in this study, 20 of the reported parents in the literature were confirmed.

'Brooks' is a cultivar selected in Florida, reported to be a seedling of 'Sandersha' planted on the property of Mr. Brooks in Miami. All candidate parents were excluded for 'Brooks' when no genotyping error was allowed. However, with 0.01% error allowed 'Sandersha' was identified as the most-likely parent with one O-CP mismatch at locus MiSHRS30. This could result from the presence of a null allele in 'Sandersha' that was inherited by 'Brooks'. Both 'Sandersha' and 'Brooks' are homozygous for different alleles at MiSHRS30. The case of 'Anderson' is similar and the same reasoning applies. 'Anderson' is said to be grown from seed of 'Sandersha' sent from Jamaica. 'Sandersha' was identified as the most-likely parent with one O-CP mismatch at locus MiSHRS30, where both are homozygous for different alleles. However, when no genotyping errors are allowed, 'Brooks' is identified as the most-likely parent of 'Anderson' with no mismatches. It is a more likely scenario that seed form 'Sandersha' would have been sent from Jamaica at that time period. 'Sandersha' is recorded as having been sent to Jamaica by David Fairchild. The null allele hypothesis can also apply to the mismatch at locus MiSHRS32 existing between 'Winters' homozygous for allele 204 and its reported parent 'Ono' homozygous for allele 201. Several other discrepancies between reported parents and estimated parents involved greater numbers of mismatches and most of these cannot be explained by existence of null alleles. 'Mulgoba' was not identified as a likely parent for 'Keitt' due to six O-CP mismatches. Instead 'Brooks' was identified as 'Keitt's' most-likely parent, as it was found to have no mismatches. For the Florida selection 'Carrie', 'Julie' was identified as the most-likely parent in the absence of 'Sophie Fry', the reported parent. 'Julie' is the reported parent of 'Sophie Fry' and thus would actually be a grandparent to 'Carrie'. 'Dot' is reported as resulting from a 'Carrie' × 'Paheri' cross but with one mismatch for each, was estimated instead to have 'Zill' as a most-likely parent. In this study 'Paheri' and 'Bombay' were synonymous and 'Bombay' was found to be a most-likely parent of 'Zill'. The 'Haden' parentage of 'Glenn' and 'Osteen' was confirmed in this study, in contrast to the RAPD analysis where this parentage was questioned (Schnell, et al., 1995). However the RAPD study confirmed the 'Haden' parentage of 'Eldon' that is not confirmed here.

Among the Florida cultivars evaluated the genetic background was found to be based on as few as four Indian cultivars, and the polyembryonic race of 'Turpentine'. Two Indian cultivars, 'Mulgoba' and 'Sandersha' are in the background of most Florida types with 'Amini', 'Bombay', 'Cambodiana', 'Long' 'Julie' 'Nam Doc Mai', 'Ono' and 'Step' making lesser contributions. 'Haden' is reported as the maternal parent for 10 cultivars included in this analysis, but based on the parentage analysis we found 31 cultivars with 'Haden' as one of the most-likely parents. Likewise, the other early Florida cultivar

'Brooks' is estimated as the parent of seven other Florida selections. 'Haden' and seedlings of 'Haden' have contributed disproportionately to the Florida group as a whole.

Florida has been considered a secondary center of diversity for mango as many Indian and Southeast Asian types were imported and the Florida cultivars were developed from these imported types (Mukherjee, 1997; Knight and Schnell, 1994; Schnell et al., 1995). A substantial amount of genetic diversity exists in germplasm collections in south Florida; however, the Florida group of mangos as a whole, are not more diverse than the Indian or Southeast Asian types, and this is supported by the parentage analysis. The Florida types may not truly represent a secondary center for diversity of the species as has been previously reported. Our data suggest that The Florida group is genetically a more uniform group of cultivars selected under similar conditions. Selecting under south Florida conditions has led to a group of cultivars including 'Keitt', 'Tommy Atkins', 'Haden', 'Parvin', and 'Irwin' that produce dependably over a range of environmental conditions. This unusual stability found among the Florida types merits further investigation. Understanding this valuable genetic architecture, how it arose, and its mode of inheritance will be important for future mango breeding efforts and commercial production of mango worldwide.

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