Characterization of Polymorphic Chloroplast Microsatellites in *Prunus* Species and Maternal Lineages in Peach Genotypes

Chunxian Chen¹ and William R. Okie

U.S. Department of Agriculture, Agricultural Research Service, Southeastern Fruit and Tree Nut Research Laboratory, 21 Dunbar Road, Byron, GA 31008

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ABSTRACT. Several available *Prunus* chloroplast genomes have not been exploited to develop polymorphic chloroplast microsatellites that could be useful in *Prunus* phylogenetic analysis and maternal lineage group (MLG) categorization. In this study, using available bioinformatics tools, 80, 75, and 78 microsatellites were identified from the chloroplast genome of *P. persica* (CPpe), *P. kansuensis* (CPka), and *P. mume* (CPmu), respectively. The genome features and polymorphism status of these microsatellites were characterized. The genomic locations and motif types of most chloroplast microsatellites were conserved in CPpe, CPka, and CPmu. Of the 67 microsatellites with primer sequences and names, 57 were polymorphic for their in silico motif, amplicon lengths, or both among the three genomes. Based on the genotyping data of eight most polymorphic microsatellites, eight unique MLGs were found among the 736 peach materials in a breeding program. Most peach cultivars (111 of 161 genotyped) belong to MLG-1, the Chinese Cling-derived group reflecting the heavy use of this germplasm in early peach development. Forty-one cultivars belong to MLG-2, the European-derived group of peaches. MLG-3 consists of ornamental accessions. MLG-4 and MLG-5 contain only ‘Flordaking’ and ‘Reliance’, respectively. MLG-6 to MLG-8 consists of selections derived from *P. tangutica*, *P. davidiana*, and *P. mira*, respectively. These amplicons from the representative material for each MLG were sequenced, revealing additional single nucleotide polymorphisms (SNPs) within the amplicons. With the polymorphism status and amplification reliability validated, these new polymorphic chloroplast microsatellite markers may be useful in *Prunus* phylogenetic analysis.

Peach (*Prunus persica*) was domesticated several thousand years ago in China and subsequently introduced to Persia, the Mediterranean, the Americas, and elsewhere (Faust and Timon, 1995). Cultivars selected for cool dry climates generally do not perform well in hot humid climates or vice versa. The ability of peach to respond to both human as well as natural selection pressure has produced genotypes adapted to a very wide range of climates and cultural preferences (Aranzana et al., 2003; Okie, 1998). Before the introduction of ‘Chinese Cling’ from China in the 1850s, most of the peaches grown in the United States were of European origin (Myers et al., 1989). After the introduction of ‘Elberta’ (a ‘Chinese Cling’ seedling) in the 1870s followed by its many descendants, conventional breeding programs, particularly in the last century, became the primary generators of cultivar diversity (Aranzana et al., 2003; Cantin et al., 2010; Okie, 1998; Sitther et al., 2012). Accrued introgression and spontaneous mutations also played a role in peach evolution (Chen et al., 2016). Therefore, genetic knowledge of the maternal

origins and genetic diversity of peach cultivars and elite breeding materials is needed for additional guidance in optimizing the choice of parents, to increase the efficiency of conventional breeding (Scorza et al., 1985).

Chloroplasts in flowering plants generally are inherited maternally (Birky, 1995; Hansen et al., 2007), which allows tracking of the maternal origins of plant cultivars and breeding lines (Brundu et al., 2008; Gavrilenko et al., 2013; Molina-Cano et al., 2005; Riahi et al., 2011). Many microsatellite (also known as simple sequence repeat) markers have been developed from peach nuclear genomes (Chen et al., 2014; Testolin et al., 2000; Vendramin et al., 2007; Wang et al., 2002) and are widely used in peach cultivar identification, pedigree inference, diversity analysis, and genetic mapping (Aranzana et al., 2003; Dirlwanger et al., 2007; Sitther et al., 2012; Testolin et al., 2000). Likewise, there are many reports on the development and use of chloroplast microsatellite markers in plants (Brundu et al., 2008; Gavrilenko et al., 2013; Molina-Cano et al., 2005; Phumichai et al., 2015; Riahi et al., 2011). However, apparently no chloroplast microsatellite information has been reported in peach although four generic chloroplast microsatellite loci were used to determine the parentage lineage of three full-sib European plum (*Prunus domestica*) cultivars (Decroocq et al., 2004). In addition, chloroplast rpl16 intron sequences were also used in a phylogeny study of 207 accessions of seventeen North American plum taxa (Shaw and Small, 2005). Recently, 11 species in *Prunus* and within section *Prunocerasus* (mostly North American plum species) were phylogenetically studied using some nuclear, mitochondrial, and chloroplast DNA markers (Chavez et al., 2016). More chloroplast microsatellite markers of known amplification

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¹Corresponding author. E-mail: chunxian.chen@ars.usda.gov.

reliability and polymorphism status are needed to facilitate maternal inheritance and phylogenetic studies on peach and other *Prunus* species. Several chloroplast genome sequences from *Prunus* species are available in the GenBank, including NC_014697 from peach (Jansen et al., 2011), NC_023956 from *P. kansuensis*, and NC_023798 from *P. mume*. They are valuable resources to develop in silico polymorphic microsatellites as discrepant repeat motifs among the genomes that can be validated through sequence alignment. These in silico validated microsatellite polymorphisms greatly increase the likelihood of genotyping polymorphisms among the amplicons produced in peach and other *Prunus* materials (Chen et al., 2014, 2015).

This study includes the following objectives: to mine and characterize microsatellites in the three *Prunus* chloroplast genomes to determine their in silico polymorphism status; to genotype a collection of elite peach lines and cultivars using selected microsatellites validated in silico to be polymorphic among the three chloroplasts genomes; to categorize the maternal lineages among these peach materials; and lastly, to sequence and validate all the allelic amplicons from one representative genotype in each maternal group. An evolutionary and breeding perspective in these maternal groups, along with inconsistencies in expected maternal grouping of some peach materials, is discussed.

Materials and Methods

PEACH MATERIALS. A total of 736 peach accessions were genotyped, including 161 cultivars and 534 elite lines that include open-pollinated (OP) seedlings of some wild peach species. All were grafted on ‘Guardian’ or ‘Flordaguard’ rootstock. Each accession was also assigned a sample number (N001, N002, etc.) for brevity. Most accessions (713) are part of the peach breeding program collection managed at the U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS) Southeastern Fruit and Tree Nut Research Laboratory in Byron, GA. Of the remaining 23 materials, 21 were acquired from the National Clonal Germplasm Repository (NCGR), Davis, CA (‘Admiral Dewey’, ‘Belle of Georgia’, ‘Carmen’, ‘Clayton’, ‘Duke of York’, ‘Dwarf Elberta’, ‘Early Crawford’, ‘Elberta’, ‘George IV’, ‘Greensboro’, ‘Hiley’, ‘J.H. Hale’, ‘Late Crawford’, ‘Le Grand’, ‘Nectared 6’, ‘Redhaven’, ‘Reliance’, ‘Rio Oso Gem’, ‘Salway’, ‘Slappey’, and ‘Springtime’), whereas ‘Frank’ and ‘Sentinel’ were obtained from Bob Wells Nursery (Lindale, TX). The 736 samples contained 22 duplicates with the same names but from different trees or sources serving as controls to validate genotyping quality and compare these materials from different sources. Twelve of the duplicates are cultivars: Augustprince, Black Boy, Camden, Candy Cane (17 bud sports), Clayton, Elberta, Flameprince, GaLa, Junegold, Late Crawford, Q43183, and Redhaven. Genomic DNAs were isolated from 200 mg young leaves using a cetyltrimethylammonium bromide protocol (Doyle and Doyle, 1987), with in-house modifications to scale down to use 2-mL Eppendorf tubes for the isolation.

IDENTIFICATION OF POLYMORPHIC MICROSATELLITES IN THREE PRUNUS CHLOROPLAST GENOMES. Polymorphic microsatellites were mined from three *Prunus* chloroplast genome sequences that were retrieved from the National Center for Biotechnology Information (NCBI). The sequences, designated as CPpe, CPka, and CPmu, respectively, were NC_014697 from *P. persica* cv. Nemared (Jansen et al., 2011), NC_023956 from *P. kansuensis*

(submitted to the NCBI by S.Y. Michaud, S.B. Tittes, K.G. Keepers, and N. Kane), and NC_023798 from *P. mume* (submitted by S. Wang, C.-W. Gao, C. Shi, and L.-Z. Gao). Microsatellite discovery and primer design were performed in the three genome sequences separately, using MISA and Primer3 with modified running parameters (Rozen and Skaletsky, 2000; Thiel et al., 2003). The microsatellite motif length and minimum repeat number pairs were changed to 1-10 2-5 3-4 4-3 5-3 6-3. The interval between any adjacent microsatellites was set to 0, to ensure every microsatellite was found separately [i.e., to avoid any compound microsatellites (Chen et al., 2014; Thiel et al., 2003)]. The optimal primer length was set to 24 base pair (bp), and the expected microsatellite amplicon sizes were between 100 and 300 bp (Rozen and Skaletsky, 2000). Three individual tab-delimited text files were generated from CPpe, CPka, and CPmu, respectively, each containing all microsatellite motifs and primer information from the corresponding genome sequence and converted to Excel (Microsoft, Redmond, WA) files (Supplemental Table 1). An extra column was added to each of the Excel files and filled with CPpe, CPka, and CPmu, respectively, showing their chloroplast genome sources. The three files were merged into a single Excel file for comparison and identification of polymorphic microsatellites at the same loci. Polymorphic microsatellites were those with the same forward and reverse primer sequences and different motif repeat times, different amplicon lengths, or both among the three chloroplast genomes (Supplemental Table 1). Each primer was named with “CXcp,” and the start position of the forward primer and the predicted amplicon size in CPpe (the two values were connected by an underscore) and also given a serial short alias starting with “CP,” for brevity. For example, the values 36,648 and 290 in the primer CXcp36648_290 represent the start position of the forward primer and the predicted amplicon size in CPpe, respectively. CXcp36648_290 also had an alias, CP25. Sixteen well-distributed, in silico, polymorphic primers were selected to screen four peach accessions, three species (*P. mume*, *P. americana*, and *P. japonica*), and one plumcot (*P. salicina* × *P. armeniaca* ‘Spring Satin’), to validate the amplification reliability and polymorphism of these primers. The eight microsatellites with the most alleles detected were chosen to genotype all the materials.

MICROSATELLITE GENOTYPING, ALLELE SCORING, AND MATERNAL LINEAGE GROUPING. Primer synthesis, polymerase chain reaction (PCR) preparation, PCR program, genotyping protocol, and allele scoring method were previously described Chen et al. (2014), with a change of genotyping instrument to 3500 Genetic Analyzer (Applied Biosystems, Carlsbad, CA). Owing to a M13 tail (20 bp long) added to each forward primer, the detected amplicon sizes are ≈20 bp larger than the predicted product sizes, varying slightly with fluorescent dye. The microsatellite allele table and chromatograms were generated by GeneMarker (SoftGenetics, State College, PA). Maternal lineage groups of the genotyped materials were established based on the eight chloroplast alleles (sizes) detected by the eight microsatellites; materials sharing the same alleles (sizes) respectively from every of the eight microsatellites will be in the same group. The representative chosen in each group was a historically recognized cultivar (MLG-1 and MLG-2), a recently popular rootstock (MLG-3), one of the two hybrids (MLG-4), or the only material (MLG-5 to MLG-8).

SANGER SEQUENCING AND SEQUENCE ANALYSIS. The PCR products of the eight primers from one representative material selected in each maternal group were purified, using the DNA

Clean & Concentrator kit (Zymo Research, Irvine, CA). Sanger sequencing was performed at the University of Florida, Interdisciplinary Center for Biotechnology Research, Gainesville. After extraction and verification from the chromatograph files, sequence reads from the same primer and different representatives were aligned using Clustal Omega (Sievers and Higgins, 2014) and validated manually to determine whether the length of nucleotide discrepancies [insertion/deletion (Indel)] matched the size differences of the alleles. Single nucleotide polymorphisms were summarized, and these nucleotides were marked in each alignment.

Results and Discussion

MICROSATELLITE POLYMORPHISMS AND LOCATIONS IN THE THREE PRUNUS CHLOROPLAST GENOMES. A total of 80, 75, and 78 microsatellites were found in the sequence of CPpe, CPka, and CPmu, respectively. Of them, 74, 68, and 73 microsatellites were successful in primer design (Table 1; Supplemental Table 1). On average, there was about one microsatellite in every two kb of the chloroplast genomes, less frequent than that in peach nuclear genome (Chen et al., 2014). A and T repeats were predominant in the three genomes, accounting for 67.8% on average (Table 1; Supplemental Fig. 1). There is one set of three adjacent microsatellites and four sets of two adjacent microsatellites sharing the same forward and reverse primers, respectively. Each set of these adjacent microsatellites was treated as one locus because the same forward and reverse primer flanks the two or three microsatellites. With the factors considered, there were 67 microsatellites with primer names in the CPpe genome, and 57 of them were polymorphic for their in silico motif, amplicon lengths, or both among the three genomes (Supplemental Table 1). These

microsatellites with in silico polymorphism status and other mining details will be useful in any relevant genotyping and phylogenetic studies in *Prunus* and closely related taxa (Chavez et al., 2016; Decroocq et al., 2004; Phumichai et al., 2015; Shaw and Small, 2005).

The locations of most microsatellites are well conserved among CPpe, CPka, and CPmu (Fig. 1), as are the motifs (Supplemental Table 1). For example, 69 of the 80 CPpe microsatellites are in the long single copy (LSC) region, one in the inverted repeat A region, seven in the small single copy (SSC) region, and three in the inverted repeat B region (Fig. 1; Supplemental Fig. 2). The LSC and SSC regions play an important role in diversification of chloroplast genomes and genetic materials (Birky, 1995; Gavrilenko et al., 2013; Hansen et al., 2007; Molina-Cano et al., 2005). In another categorization, 49 of the 80 microsatellites are in noncoding sequences, 15 in exons, and 16 in introns (Supplemental Table 1).

MATERNAL LINEAGES OF PEACH MATERIALS BASED ON THE EIGHT MOST POLYMORPHIC MICROSATELLITES. Screening of 16 in silico polymorphic microsatellite primers generated three to seven alleles (amplicons of different sizes) (Table 2). Based on the numbers of alleles detected, the eight most polymorphic microsatellites were used to genotype and subsequently to classify the 736 materials into eight unique MLGs (Tables 3 and 4; Supplemental Table 2; Supplemental Fig. 3).

MLG-1 contained the majority of peach cultivars (111) and selections (354) genotyped (Table 4; Supplemental Table 2). ‘Chinese Cling’ is used as the representative because of its status as the progenitor clone for most of the cultivars in the group. The predominance is expected based on the heavy use of ‘Chinese Cling’ offspring such as ‘Elberta’ in early breeding work (Myers et al., 1989; Scorza et al., 1985). Almost all the cultivars in this group, for which pedigrees are available, descended from ‘Elberta’, ‘J.H. Hale’ (thought to be an ‘Elberta’ seedling), or ‘Late Crawford’ (an old American peach presumably of European ancestry, see below) via ‘Rio Oso Gem’ (Okie, 1998). Only two cultivars and several selections in this group were inconsistent with their pedigree-inferred maternal groups. ‘Majestic’ and several selections descended from it, supposedly traced back to ‘St. John’ (see MLG-2), an old American cultivar dating back to the 1860s. ‘Red Bird Cling’ was thought to be a seedling of ‘Heath Cling’ (MLG-2). One selection tracing back to ‘Admiral Dewey’ (MLG-2) via ‘LaPremiere’ and ‘Redglobe’ and another to ‘Sunprince’ (MLG-2) appeared in this group. Three other selections supposedly belonged to MLG-1 but fell in MLG-3.

MLG-2 consists of 41 cultivars and 152 selections (Table 4; Supplemental Table 2). The group includes ‘Heath Cling’ (used as the representative for this group because it is one of the oldest cultivars in the group) and most other old (i.e., pre-‘Elberta’) American cultivars and their European progenitors, which presumably trace back to peaches introduced into Europe over the last two millennia (Faust and Timon, 1995). Most cultivars with known pedigrees were descended from ‘Loring’, thought to be a seedling of ‘Frank’ [itself

Table 1. Number of microsatellites in the chloroplast genome of *Prunus persica* (CPpe), *P. kansuensis* (CPka), and *P. mume* (CPmu).

Repeat unit size (bp)	CPpe (no.)	CPka (no.)	CPmu (no.)
1	56	49	53
2	17	18	14
3	0	0	1
4	5	5	7
5	2	3	2
6	0	0	1
Total	80	75	78

Fig. 1. Locations of the microsatellites in the chloroplast genome of *Prunus persica* (CPpe), *P. kansuensis* (CPka), and *P. mume* (CPmu). Each vertical bar represents a microsatellite; LSC = long single copy (1–85,719 bp); IRA = inverted repeat A (85,720–112,215 bp); SSC = short single copy (112,216–131,371 bp); IRB = inverted repeat B (131,372–157,790 bp).

Table 2. Sixteen in silico polymorphic peach chloroplast microsatellites selected for screening and genotyping.

Primers names ^z	Alias	Forward/reverse primer sequences (5'–3')	Genomic locations ^y	Motif sizes (bp) ^x	Product sizes (bp) ^x	Detected alleles (no.)
CXcp01488_232	CP01	TTGAGGGCTTGTTATTCAACAGTA CAAGGAAATTATCTACTCCATCCG	psbAtrnK-UUU	(T)14-13-12	232-231-230	6
CXcp05368_299	CP06	CACCTTACGAAGATCTCTCCCTC TAAACTTGAGTTATGAGGGTGCAA	rps16 intron	(A)14-12-17	299-239-297 ^w	6
CXcp05874_266	CP07	ATCTTTAAATCGTTTCAAAATGGC ATTGATTAAATTCTCAAGGGCAAG	rps16 intron	(TTTGA)3-3-4	266-266-271	3
CXcp06764_253	CP08	TTTCGTATGCGTGTATTGAACT CAGAGCATACCTGACCCATAATAA	rps16trnQ-UUG	(A)11-12-11	253-254-249 ^w	5
CXcp12362_226	CP12	ACTTTATACATAGGCCGTCGATTC AGATGAGCATGGTATCTTCTA	atpF intron	(A)12-13-10	226-292-300	4
CXcp16527_214	CP15	CCCACACTTCTTTTCATCTCTTTT TCTTAAGAGCGAATTCATCCTTTT	rps2poC2	(A)11-10-16	214-210-289 ^w	4
CXcp30091_265	CP22	AAAGCAAAGAAATAGAAATTGGGAC AAATGAAATGGGCAGACTAGAATC	petNpsbM	(TA)6-6-5	265-259-261	3
CXcp36648_290	CP25	AAATGGATTCCACCAACCTAATA AACCGGTATAGTTCGAAACAAAG	psbCtrnS-UGA	(T)18-17-15	290-289-289	7
CXcp48374_176	CP31	TGTTATTAGAGGGTCTGCCCTAAG CCTATTCTATCCGTGCAATTTT	tmT-UGUtrnL-UAA	(A)15-13-12	176-171-170	3
CXcp58614_239	CP37	TCCCATCCTGTATATTGTCTCTTTT ACTTCTTGCCCCCTATTACATGA	rbcLaccD	(T)16-10-18	239-241-241	5
CXcp60541_140	CP38	CCAGGAAATCCCTATTAAACAAA AATAATACGACCCCAACAAAAGGA	accDpsal	(C)11-10-13	140-139-140	4
CXcp62273_244	CP40	CCAAAGCAAACGTTACGAAATAAT CAAATTAATAGTAGCGGATTGCG	ycf4cemA	(T)12-11-10	244-237-245 ^w	5
CXcp67171_228	CP46	TTTCCAAATTTATCCACAGTGA TCATCATTTGTGACATTTACCCAT	psbEpetL	(TA)5-5-6	228-228-229	3
CXcp68964_205	CP47	TCTGTAACTAATCCCTACCCCTTGC TATCTTGATGATATGTTGCCCC	psaJp133	(A)13-13-10	205-205-177	4
CXcp71903_234	CP52	TATCTCGCATAATGAAAGGTGAAG TTGAACTAATCCAAAAGAAAGGG	clpP intron	(AAAT)3-3-3	234-234-208	4
CXcp76664_272	CP57	ATAGAGATGGTTTACTTCGTCGG CTAAAGTCCCAAAATTCCTGAGTA	petB intron	(AT)5-5-5	272-274-277 ^w	5

^zThe eight primer names in bold are selected for genotyping all the 736 materials.

^yA caret mark ( ) between two chloroplast gene names or "intron" after a gene name indicates the microsatellite locating in the noncoding region between the two genes or in an intron of the gene, respectively.

^xThe three values represent the in silico motif sizes and product sizes in base pair (bp) among the chloroplast genome of *Prunus persica* (CPpe), *P. kansuensis* (CPka), and *P. mume* (CPmu), respectively. The microsatellites with different motif, product sizes, or both are in silico polymorphic.

^wThe primers for these amplicons flank one or two other microsatellite motifs, in addition to the one listed in the table.

a supposed seedling of ‘Elberta’ (see MLG-1)] (Okie, 1998). ‘Sunprince’ and its selections which trace back to ‘Redglobe’ and ‘Admiral Dewey’ landed in this group as would be expected based on the old origin of ‘Dewey’. Several cultivars in this group are ornamentals, including ‘MacDonald’, ‘Helen Borchers’, and ‘White English’, or have ornamental maternal ancestors such as ‘Flordaguard’ (via PI65821) and ‘Candy Cane’ (via ‘Shidaremomom’ from Japan). Several were selections from ‘Naturals’, feral seedlings found growing wild, which could trace back to pre-‘Chinese Cling’ peaches. Inconsistencies exist in one cultivar and a few selections in this group. ‘Saturn’, a flat peach, is reportedly descended from ‘J.H. Hale’ (MLG-1). Three selections with pedigrees tracing back to ‘J.H. Hale’ also

fell into this group, as did two selections tracing ‘Late Crawford’ via ‘Rio Oso Gem’ (MLG-1).

MLG-3 consists of only 30 accessions, mostly ‘Guardian’ and its seedlings (rootstock descended from S-37, an ornamental/rootstock selection from Stribling’s Nursery, CA) or other ‘S-37’ descendants (Okie et al., 1994), ‘Kikomo’ OP (Q36019e, ornamental from Japan) and ‘Hong Chui Zhi’ (Q43183, weeping ornamental from China); this group likely is the maternal source of some ornamental peaches. One selection tracing back to ‘Flameprince’ (MLG-1) also was in this group.

MLG-4 contains only ‘Flordaking’ and MLG-5 only ‘Reliance’, representing their unique maternal sources. ‘Flordaking’ descended from ‘Okinawa’, a low-chill clone of unknown origin

that was obtained from the island after which it was named (Andrews et al., 1979; Okie, 1998). ‘Reliance’ is a hybrid of ‘Minnesota PH-04559’ × ‘Meredith’ released in the New Hampshire Agriculture Experiment Station, which is extremely bud hardy and able to fruit in cold areas (Okie, 1998). PH-04559 probably descended from peaches growing at the northern limit of peach production, so it would be interesting to find the parentage of this special selection.

The next three groups, MLG-6 to MLG-8, consist of materials from three *Prunus* species. MLG-6 consists of two selections derived from *P. tangutica*, a species with dry-flesh, flattened fruit usually classified with almond (*P. dulcis*),

Table 3. The sizes of alleles detected in eight representative peach materials for eight different maternal lineage groups (MLG) by eight chloroplast (CP) primers.

MLG	Representative	Primers ^z							
		CP01	CP06	CP25	CP37	CP38	CP40	CP47	CP57
		(base pair)							
MLG-1	Chinese Cling	250	313	308	263	161	262	225	296
MLG-2	Heath Cling	255	316	310	264	158	264	225	295
MLG-3	Guardian	254	314	309	264	160	263	224	295
MLG-4	Flordaking	254	315	309	265	158	264	225	295
MLG-5	Reliance	255	310	310	264	158	264	225	295
MLG-6	<i>Prunus tangutica</i> ^y	253	310	301	254	142	267	220	298
MLG-7	<i>Prunus davidiana</i> ^x	255	317	303	262	160	260	222	295
MLG-8	<i>Prunus mira</i>	261	317	302	262	162	260	224	294

^zThe primer names for CP01, CP06, CP25, CP37, CP38, CP40, CP47, and CP57 are CXcp01488_232, CXcp05368_299, CXcp36648_290, CXcp58614_239, CXcp60541_140, CXcp62273_244, CXcp68964_205, and CXcp76664_272, respectively.

^yA hybrid of this species.

^xAn open-pollinated seedling of this species.

Table 4. Peach cultivars or lines belonging to each maternal lineage group (MLG). Cultivars tested in duplicate are underlined>.

MLG	Peach cultivars or lines
MLG-1	Chinese Cling, Arctic Belle, Arctic Gold, Arctic Sweet, Autumnprince, Belle of Georgia, Big Red, Bill Gross Indian, Blazeprince, Camden, Carmen, Carolina Gold, Carolina Red, Carored, Clayton, Compact Elberta, Clayton, Contender, Coronet, Cresthaven, Crimson Snow, Dixiland, Durbin, Dwarf Elberta, Eastern Glo, Elberta, Empress, Fackler, Fairtime, Fireprince, Flameprince, Flavorich, Flavrburst, Flordadawn, Flordaprince, Frank, GaLa, Glacier White, Greensboro, Hakuto, Hiland, Hiley, Honey Royale, Jefferson, Jerseyqueen, J.H. Hale, Joanna Sweet, Julyprince, Junegold, Juneprince, Juneprincess, Kawanakajima Hakutou, Late Crawford (N450), Le Grand, Lobo, Lobo Early, Majestic , O’Henry, Parade, PF10 Ball, PF11 Ball, PF17, PF19-007, PF23, PF24-007, PF24C, PF27A, PF36-007, PF5D Big, PF7A Freestone, PF8 Ball, PF9-007, PF-Late 24-007, PF-Late Large 23, Red Bird Cling , Redhaven, Rio Oso Gem, Roseprincess, Rubyprince, Sauzee King, Scarletpearl, Scarletprince, Sentinel, Slappey, Southern Pearl, Spice Zee, Springprince, Starlite, SummerFest, Summergold, Summerprince, Sunbrite, Sunland, Sureprince, Suwanee, Sweet Cap, Ta Qiao Yi Hao, TexPrince, Tri Lite, TruGold, Vulcan, White Cloud, White County, White Diamond, White Rock, Winblo, Zephre, Zin Dai Jiu Bao, 354 advanced selections
MLG-2	Heath Cling, Admiral Dewey, AR Late, Augustprince, Black Boy, Candy Cane, Cary Mac, Champion, Duke of York, Early Augustprince, Early Crawford, Flat Wonderful, Flordaguard, Frost, Galaxy, George IV, Goldprince, Grosse Mignonne, Halls Hardy, Helen Borchers, Karla Rose, Late Crawford (N721) , Loring, MacDonald, Nectared 6, NRL1, Oldmixon Free, Q26941, Q39622A, Q42535C, Q43148A1, Q43539, Salway, Saturn , Sentry, Springtime, St. John, Strawberry Free, Sunprince, TexKing, White English, White River, 152 advanced selections
MLG-3	Guardian, Q36019e (Kikomo op), Q43183 (Hong Chui Zhi), 26 other selections
MLG-4	Flordaking
MLG-5	Reliance
MLG-6	<i>Prunus tangutica</i> hybrid, two selections from it
MLG-7	<i>Prunus davidiana</i> hybrid
MLG-8	<i>Prunus mira</i>

Cultivars in bold appear inconsistent with their recorded pedigree (OP = open-pollinated).

and found in Sichuan, China. It is commonly named ‘Tang Gu Te Bian Tao’ or ‘Xi Kang Bian Tao’. MLG-7 consists of an OP seedling of *P. davidiana* (a wild Chinese peach species) which appears to be a hybrid with peach. MLG-8 is represented by a clone of *P. mira*, a peach species native to China and Nepal.

Among the duplicated samples, all the sets, including ‘Augustprince’, ‘Black Boy’, ‘Camden’, ‘Candy Cane’, ‘Clayton’, ‘GaLa’, ‘Elberta’, ‘Flameprince’, and ‘Redhaven’, yielded identical genotyping data among the eight primers, except for the two ‘Late Crawford’ samples. ‘Late Crawford’ is a very old freestone cultivar named in New Jersey in the early 1800s (Okie, 1998). ‘Late Crawford’ at Byron belongs to MLG-1, the ‘Chinese Cling’ group, whereas the clone from the NCRG belongs to MLG-2, the European peach group. One would expect ‘Late Crawford’ to fall into MLG-2 based on its origin, but it is odd that ‘Rio Oso Gem’ (a 1931 seedling thought to be from ‘Late Crawford’) and other cultivars that descended it, all fell into the MLG-1. In the case of historical cultivars such as Late Crawford, it is difficult to tell if a current clone is identical to that which existed over a century ago, although the clone at Byron matches the historical description based on flower and fruit characters. Sometimes seedlings of a cultivar retained the

parental name. In other cases, parentage was presumed or inferred from seedling performance. Cultivars grown at Byron appeared to fit the published descriptions, accounting for differences in climate, for ‘LaPremiere’ (MLG-1), ‘Majestic’ (MLG-1), ‘Red Bird Cling’ (MLG-1), and ‘Saturn’ (MLG-2), although based on their pedigree they were expected to group in MLG-1, MLG-2, MLG-2, and MLG-1, respectively. There are several other possible reasons for the inconsistent cases. Pedigree information, especially on old cultivars, may be anecdotal and inaccurate. Cross records for pedigrees can contain transcriptional errors, and trees used for crossing and propagation can be mislabeled. Bud failure results in rootstock growing as scion. For example, three selections expected to be in MLG-1 but falling in MLG-3 were proved to be ‘Guardian’ rootstock seedlings (data not shown). Genotyping of these inconsistent materials using nuclear microsatellite markers shall make it possible to infer their parentage, as reported in other studies (Aranzana et al., 2003; Chen and Okie, 2015; Decroocq et al., 2004; Sither et al., 2012; Testolin et al., 2000).

DISCREPANT NUCLEOTIDES IN MICROSATELLITE AMPLICONS AMONG THE MATERNAL LINEAGES. Discrepant nucleotides were revealed by sequencing and alignment of the microsatellite

Fig. 2. Alignment of amplicon sequences with primer CXcp36648_290 (CP25) in ‘Chinese Cling’ (N698), ‘Heath Cling’ (N697), ‘Guardian’ (N701), ‘Flordaking’ (N419), ‘Reliance’ (N704), *Prunus tangutica* hybrid (N057), *P. davidiana* open-pollinated (N046), and *P. mira* (N702) peaches. The sequences corresponding to the amplicons in CPpe (*P. persica*), CPka (*P. kansuensis*), and CPmu (*P. mume*) genomes, named as CP25_CPpe, CP25_CPka, and CP25_CPmu, were included in the alignment. The polymorphic microsatellite nucleotides were grayed, and the forward and reverse primer sequences were underlined. CP25_CPmu has the same amplicon size of 309 detected in genotyping, but only T₁₅ is mined because of an A (ungrayed) substitution in the microsatellite. In the asterisk line, asterisks represent consensus nucleotides, and spaces represent discrepant (polymorphic) nucleotides.

amplicons among the eight peach materials representing the eight MLGs (Fig. 2). The length differences among the microsatellite repeats matched well with those among the sizes of these alleles detected by the microsatellite genotyping (Table 3; Supplemental Fig. 3). Taking the primer CXcp36648_290 (CP25) as an example, 'Heath Cling' (CP25_N697) and 'Reliance' (CP25_N704), together with 'Nemared' (CP25_CPpe), have the T nucleotide repeating 18 times (T₁₈) at the microsatellite locus (Fig. 2) and yielding the largest allele size of 310 bp detected in genotyping (Table 2). The next are 'Guardian' (CP25_N701), 'Flordaking' (CP25_N419), and CP25_CPka, which have the repeat of T₁₇ and the amplicon size of 309. CP25_CPmu has the same amplicon size of 309, but only T₁₅ is mined because of one A substitution in the microsatellite. Likewise, 'Chinese Cling' (CP25_N698) has T₁₆ and 308; *P. davidiana* OP (CP25_N046) has T₁₁ and 303; *P. mira* (CP25_N702) has T₁₀ and 302; and *P. tangutica* hybrid (CP25_N057) has T₉ and 301. The T deletions/insertions at this satellite locus yield the length polymorphisms so do those at the other microsatellites. In addition, various numbers of SNPs were also found within the sequences of amplicons amplified by the eight primers (Fig. 2; Supplemental Table 3).

Cultivated peaches in the United States show low genetic diversity (Aranzana et al., 2003; Chen et al., 2014; Scorza et al., 1985; Sitther et al., 2012). Likewise, they may also have limited maternal origins, as this study has demonstrated. Peach is self-compatible, and conventional hybridization remains the main approach to breeding peach cultivars. The *in silico* chloroplast microsatellite polymorphisms and genotyping results in these peach materials provide insights into chloroplast genome variations and can guide combinations of parents with known maternal origins. In addition, the amplicon length polymorphisms only distinguished eight maternal groups; however, SNPs are common in these small amplicons. Further exploitation of these nucleotide polymorphisms will lead to more maternal subgroups and supply sufficient markers for a whole genome association study for chloroplast-controlled traits.

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Supplemental Fig. 1. Number of microsatellites with different repeat motifs found in the chloroplast genome of *Prunus persica* (CPpe), *P. kansuensis* (CPka), and *P. mume* (CPmu), respectively.

Supplemental Fig. 2. Peach chloroplast genome physical map generated by OrganellarGenomeDRAW (Lohse et al., 2013). All genes and the regions of long single copy (LSC), inverted repeat A (IRA), small single copy (SSC), and inverted repeat B (IRB) were proportionally displayed.

Supplemental Fig. 3. Microsatellite genotyping chromatographs of eight chloroplast markers. Columns present the eight *in silico* polymorphic chloroplast (cp) microsatellite markers (alias in the parentheses): CXcp01488_232 (CP01), CXcp05368_299 (CP06), CXcp36648_290 (CP25), CXcp58614_239 (CP37), CXcp60541_140 (CP38), CXcp62273_244 (CP40), CXcp68964_205 (CP47), and CXcp76664_272 (CP57). Rows present the eight peach materials representing the eight maternal lineage groups [MLGs (alias names in the parentheses)]: ‘Chinese Cling’ (N698), ‘Heath Cling’ (N697), ‘Guardian’ (N701), *P. tangutica* hybrid (N057), *P. davidiana* open-pollinated (N046), *P. mira* (N702), ‘Flordaking’ (N419), and ‘Reliance’ (N704), and two conflicting ‘Late Crawford’ (N450 and N721). These peaks are the chloroplast alleles among the materials detected by the microsatellite markers. The allele sizes are marked under the baseline of the peaks and displayed in Table 3.

Supplemental Table 1. In silico microsatellite polymorphisms and primers among the chloroplast genome of *Prunus persica* (CPpe), *P. kansuensis* (CPka), and *P. mume* (CPmu).^z

Source	SSR motif	Start (bp)	Genomic location	Primer name	Alias	Forward primer (5'-3')	Reverse primer (5'-3')	Size (bp)	Note
CPka	(TA) ₅	23							
CPka	(TA) ₅	54							
CPpe	(T) ₁₄	1,591	psbA ⁺ trnK-UUU	CXep01488_232	CP01	TTGAGGGCTTGTATTCAACAGTA	CAAGGAAATTATCTACTCCATCCG	232	
CPka	(T) ₁₃	1,663				TTGAGGGCTTGTATTCAACAGTA	CAAGGAAATTATCTACTCCATCCG	231	
CPmu	(T) ₁₂	1,595				TTGAGGGCTTGTATTCAACAGTA	CAAGGAAATTATCTACTCCATCCG	230	
CPpe	(A) ₁₁	2,905	matK		CP02	AAAGATCCCAAGAAGGTTAATTG	ACGCTCTCTTTGCATTATTAC	209	
CPka	(A) ₁₁	2,976				AAAGATCCCAAGAAGGTTAATTG	ACGCTCTCTTTGCATTATTAC	209	
CPmu	(A) ₁₁	2,907				TAGCAAGACTTCTTCTACGGGAT	ACGCTCTCTTTGCATTATTAC	267	
CPpe	(T) ₁₁	3,251	matK		CP03	CGCTCTAAGGAAGATACTAATCG	TTCTATACCCACTTATCTTTCGG	297	
CPka	(T) ₁₁	3,322				CGCTCTAAGGAAGATACTAATCG	TTCTATACCCACTTATCTTTCGG	297	
CPmu	(T) ₁₁	3,253				TCTGTGCTCTAAGGAAGATACG	ATCGTTTGTGGATAATGATG	237	
CPpe	(A) ₁₀	3,742	trnK-UUU		CP04	GGATACCTCGGAAACAAGTAAAGA	AAGACTTTTCCCATGAGTGATTA	262	
CPmu	(A) ₁₀	3,744				GGATACCTCGGAAACAAGTAAAGA	AAGACTTTTCCCATGAGTGATTA	262	
CPpe	(T) ₁₂	4,014	trnK-UUU		CP05	TTAGGGCTCTACCCATTATTTCAC	TCATCGGTAGAGTTTGTAAAGCCA	214	
CPka	(T) ₁₂	4,084				TTAGGGCTCTACCCATTATTTCAC	TCATCGGTAGAGTTTGTAAAGCCA	214	
CPmu	(T) ₁₀	4,016				TTAGGGCTCTACCCATTATTTCAC	TCATCGGTAGAGTTTGTAAAGCCA	212	
CPpe	(A) ₁₄	5,620	rps16 intron	CXep05368_299	CP06	CACTTACGAAGATCTCTTCCCTC	TAAACTTGAGTTATGAGGGTGCAA	299	3 loci
CPka	(A) ₁₂	5,667				GATAAATGGCTCAITGGGATAGAT	AAC TTGAGTTATGAGGGTGCAAA	239	
CPmu	(A) ₁₇	5,620				CAC TTACGAAGATCTCTTCCCTC	ATTCAA CTGAGTTATGAGGGTGTC	297	
CPpe	(AAAT) ₃	5,559	rps16 intron			CAC TTACGAAGATCTCTTCCCTC	TAAACTTGAGTTATGAGGGTGCAA	299	
CPka	(AAAT) ₃	5,606				GATAAATGGCTCAITGGGATAGAT	AAC TTGAGTTATGAGGGTGCAAA	239	
CPmu	(AAAT) ₃	5,559				CAC TTACGAAGATCTCTTCCCTC	ATTCAA CTGAGTTATGAGGGTGTC	297	
CPpe	(C) ₁₀	5,457	rps16 intron			CAC TTACGAAGATCTCTTCCCTC	ATTCAA CTGAGTTATGAGGGTGTC	297	
CPka	(C) ₁₀	5,504				GATAAATGGCTCAITGGGATAGAT	AAC TTGAGTTATGAGGGTGCAAA	239	
CPpe	(TTTGA) ₃	5,977	rps16 intron	CXep05874_266	CP07	ATCTTTAAATCGTTTCAAAATGGC	ATTGATTAATTTCTCAAAGGCAAG	266	
CPka	(TTTGA) ₃	6,022				ATCTTTAAATCGTTTCAAAATGGC	ATTGATTAATTTCTCAAAGGCAAG	266	
CPmu	(TTTGA) ₄	5,969				ATCTTTAAATCGTTTCAAAATGGC	ATTGATTAATTTCTCAAAGGCAAG	271	
CPpe	(A) ₁₁	6,827	rps16 ⁺ trnQ-UUG	CXep06764_253	CP08	TTTCGTATGCGTGTATTGAACT	CAGAGCATACCTGACCCATAATAA	253	2 loci
CPka	(A) ₁₂	6,872				TTTCGTATGCGTGTATTGAACT	CAGAGCATACCTGACCCATAATAA	254	
CPmu	(A) ₁₁	6,825				TTTCGTATGCGTGTATTGAACT	CAGAGCATACCTGACCCATAATAA	249	
CPpe	(AT) ₆	6,928	rps16 ⁺ trnQ-UUG			TTTCGTATGCGTGTATTGAACT	CAGAGCATACCTGACCCATAATAA	253	
CPka	(AT) ₆	6,974				TTTCGTATGCGTGTATTGAACT	CAGAGCATACCTGACCCATAATAA	254	
CPmu	(AT) ₅	6,926				TTTCGTATGCGTGTATTGAACT	CAGAGCATACCTGACCCATAATAA	249	
CPpe	(A) ₁₀	8,447	trnS-GCU ⁺ trnG-GCC		CP09	CTTGCTTGTATTACGATTGGAAA	GGAAATGGAATATTAATCAAACCGA	295	
CPka	(A) ₁₀	8,493				CTTGCTTGTATTACGATTGGAAA	GGAAATGGAATATTAATCAAACCGA	295	
CPmu	(A) ₁₀	7,685				TATGCCAGTAATACCCCTGTTTT	TTTTTACACCAAGAAATGAAGTGG	223	
CPpe	(T) ₁₂	9,637	trnR-UCU ⁺ atpA	CXep09539_158	CP10	AATGAAAAGCGTCCATTGTCTAAT	GC GTTTCTTATTCAAGTCATTCAA	158	
CPka	(T) ₁₂	9,683				AATGAAAAGCGTCCATTGTCTAAT	GC GTTTCTTATTCAAGTCATTCAA	158	
CPmu	(T) ₁₁	9,633				AATGAAAAGCGTCCATTGTCTAAT	GC GTTTCTTATTCAAGTCATTCAA	157	
CPpe	(TTAAT) ₄	9,710	trnR-UCU ⁺ atpA			AATGAAAAGCGTCCATTGTCTAAT	GC GTTTCTTATTCAAGTCATTCAA	157	
CPmu	(TTAAT) ₄	9,705				AATGAAAAGCGTCCATTGTCTAAT	GC GTTTCTTATTCAAGTCATTCAA	157	
CPka	(TTAAT) ₄	9,756				AATGAAAAGCGTCCATTGTCTAAT	GC GTTTCTTATTCAAGTCATTCAA	157	

Continued next page

Supplemental Table 1. Continued.

Source	SSR motif	Start (bp)	Genomic location	Primer name	Alias	Forward primer (5'-3')	Reverse primer (5'-3')	Size (bp)	Note
CPpe	(T)10	11,612	atpA ⁺ atpF	CXep11571_255	CP11	TAAATTTCATCTGCTCGAATGGTTA	CATTTCATTTTGAACAAACAAAGAGC	255	
CPmu	(T)10	11,606				TAAATTTCATCTGCTCGAATGGTTA	CATTTCATTTTGAACAAACAAAGAGC	254	
CPpe	(A)12	12,497	atpF intron	CXep12362_226	CP12	ACTTTATACATAGGCCGTCGATTC	AGATGCAGCATGGTATTCTTTCTA	226	
CPka	(A)13	12,542				GACCAACAAATAAATTGTCAGCAA	AGATGCAGCATGGTATTCTTTCTA	292	
CPmu	(A)10	12,490				GAGGACTCTTCTGACCAACAAAT	GATGCAGCATGGTATTCTTTCTAC	300	
CPpe	(AT)5	12,694	atpF intron		CP13	CTGAACCTTCAACCGGTTAGCTTT	GAGTGTGCGAGTTGTTTATTTC	230	
CPka	(AT)6	12,740				CTGAACCTTCAACCGGTTAGCTTT	GAGTGTGCGAGTTGTTTATTTC	230	
CPmu	(AT)6	12,685				CTGAACCTTCAACCGGTTAGCTTT	GAGTGTGCGAGTTGTTTATTTC	235	
CPka	(T)11	14,001				TTAGTTCTATCCACCCACGTAAT	TCCGTTTCATCTGTAATTGTGAAT	157	
CPmu	(T)11	13,946				TTCTATCCACGTAATTTTGTGA	TCCGTTTCATCTGTAATTGTGAAT	149	
CPpe	(T)10	14,670	atpH ⁺ atpI	CXep14625_291	CP14	GCCAAACAAAACCTTTAGGAAAAA	ATTCAAGCTCTTATTTTGAACC	291	
CPka	(T)10	14,722				GCCAAACAAAACCTTTAGGAAAAA	ATTCAAGCTCTTATTTTGAACC	291	
CPmu	(T)12	14,668				GCCAAACAAAACCTTTAGGAAAAA	ATTCAAGCTCTTATTTTGAACC	294	
CPpe	(T)12	16,548	rps2 ⁺ rpoC2	CXep16404_288	CP15	TACGTTTGCAGAGATATAAGGTG	AGAATAACGAATGGAAGAAATGC	288	2 loci
CPpe	(A)11	16,565	rps2 ⁺ rpoC2			CCCACACTCTTTTTCATCTCTTTT	TCTTAAGAGCGAATTCATCCTTTT	214	
CPka	(A)10	16,614				CCCACACTCTTTTTCATCTCTTTT	TCTTAAGAGCGAATTCATCCTTTT	210	
CPmu	(A)16	16,562				TACGTTTGCAGAGATATAAGGTG	AGAATAACGAATGGAAGAAATGC	289	
CPpe	(T)11	18,755	rpoC2	CXep18578_291	CP16	ATACTTTTCTTGGTGGGTGTGAT	GAGTCTTCTCCATAATGGTACGG	291	
CPmu	(T)11	18,757				ATACTTTTCTTGGTGGGTGTGAT	GAGTCTTCTCCATAATGGTACGG	291	
CPka	(T)11	18,803				ATACTTTTCTTGGTGGGTGTGAT	GAGTCTTCTCCATAATGGTACGG	291	
CPpe	(TA)5	20,127	rpoC2	CXep20039_256	CP17	AGATAATGGTTGTGTTTGAAGGGT	TGGATATCTTACACGCAGACTTGT	256	
CPka	(TA)5	20,175				AGATAATGGTTGTGTTTGAAGGGT	TGGATATCTTACACGCAGACTTGT	256	
CPmu	(TA)5	20,129				AGATAATGGTTGTGTTTGAAGGGT	TGGATATCTTACACGCAGACTTGT	256	
CPpe	(T)15	22,877	rpoC1 intron	CXep222822_259	CP18	TCCTTACTCAAGTTCCTCAAGAAAG	GCGAAGTAGAGACCTAAAAGATCG	259	
CPka	(T)13	22,925				TCCTTACTCAAGTTCCTCAAGAAAG	GCGAAGTAGAGACCTAAAAGATCG	257	
CPmu	(T)15	22,879				TCCTTACTCAAGTTCCTCAAGAAAG	GCGAAGTAGAGACCTAAAAGATCG	259	
CPpe	(T)10	26,483	rpoB	CXep26347_232	CP19	CCCTTCTTAATTCACATCTTTGTT	TCATCAGCTATGGGTTCAAATCTA	232	
CPka	(T)10	26,529				CCCTTCTTAATTCACATCTTTGTT	TCATCAGCTATGGGTTCAAATCTA	232	
CPmu	(T)10	26,485				CCCTTCTTAATTCACATCTTTGTT	TCATCAGCTATGGGTTCAAATCTA	232	
CPpe	(A)11	27,563	rpoB ⁺ trnC-GCA	CXep27477_274	CP20	TCAATGAGATAATGAGACAAAGGC	AGAGCTCTAATAAAGGGGAAAGCG	274	
CPka	(A)11	27,609				TCAATGAGATAATGAGACAAAGGC	AGAGCTCTAATAAAGGGGAAAGCG	274	
CPmu	(A)12	27,565				CTGTTCAATGGGATAATGAGACAA	AGAGCTCTAATAAAGGGGAAAGCG	279	
CPmu	(T)10	28,673				AGAAAGGCCGAAAGATACATTGTA	TGTCCTATGAACAATGGGAAGA	219	
CPpe	(T)11	29,253	petN ⁺ psbM	CXep29188_283	CP21	AGTATGGGGAAGAAAGTGACCTCA	TTTTACTTTCGAAAATACGAACACG	283	
CPka	(T)11	29,299				AGTATGGGGAAGAAAGTGACCTCA	TTTTACTTTCGAAAATACGAACACG	283	
CPmu	(AATA)3	29,397				AGTATGGGGAAGAAAGTGACCTCA	TTTTACTTTCGAAAATACGAACACG	285	
CPpe	(TA)6	30,150	petN ⁺ psbM	CXep30091_265	CP22	AAAGCAAGAAATAGAAATGGGAC	AAATGAAATGGGCAGACTAGAAATC	265	
CPka	(TA)6	30,190				AAAGCAAGAAATAGAAATGGGAC	AAATGAAATGGGCAGACTAGAAATC	259	
CPmu	(TA)5	30,155				GCAAAGAAATAGAAATGGGACATT	AAATGAAATGGGCAGACTAGAAATC	261	
CPpe	(TTTA)3	30,781	psbM ⁺ trmD-GUC	CXep30681_164	CP23	TCAAAATTGACTCATCGTCAAGAAAT	GATTGTACAAAAGAGGGCAGTAGGT	164	
CPka	(TTTA)3	30,821				TCAAAATTGACTCATCGTCAAGAAAT	GATTGTACAAAAGAGGGCAGTAGGT	164	
CPmu	(TTTA)3	30,784				TCAAAATTGACTCATCGTCAAGAAAT	GATTGTACAAAAGAGGGCAGTAGGT	164	
CPka	(AAAAG)3	31,644				AATCCACATATGCTCTCTCTCCCAT	ACTTGGGATTGTAGTTCAATTGGT	280	

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Source	SSR motif	Start (bp)	Genomic location	Primer name	Alias	Forward primer (5'-3')	Reverse primer (5'-3')	Size (bp)	Note
CPpe	(C)10	33,810	trnT-GGU ^ψ psbD	CXep33652_210	CP24	GATGCGAGAAGGATACTTTTCATTT	CCTCTCGTTCCATCAACTAATCTT	210	
CPpe	(T)18	36,743	psbC ^ψ trnS-UGA	CXep36648_290	CP25	AAATGGATTCCACCAAACCTAATA	AAACCGGTATAGTTCGAAACAAAAG	290	
CPka	(T)17	36,782				AAATGGATTCCACCAAACCTAATA	AAACCGGTATAGTTCGAAACAAAAG	289	
CPmu	(T)15	36,743				AAATTGATTCCACCAAACCTAATA	AAACCGGTATAGTTCGAAACAAAAG	289	
CPpe	(TA)5	37,085	trnS-UGA ^ψ psbZ	CXep37000_226	CP26	CCCAATAGAACATGGACATATGAG	GGATTTTACCATGAAATCCATCATT	226	
CPka	(TA)5	37,123				CCCAATAGAACATGGACATATGAG	GGATTTTACCATGAAATCCATCATT	226	
CPmu	(TA)5	37,082				CCCAATAGAACATGGACATATGAG	GGATTTTACCATGAAATCCATCATT	226	
CPpe	(T)10	37,239	trnS-UGA ^ψ psbZ	CXep37202_282	CP27	AATGATGGATTTCATGGTAAAATCC	ATACCCACAGAAAGACTAATCCA	282	
CPka	(T)10	37,277				AATGATGGATTTCATGGTAAAATCC	ATACCCACAGAAAGACTAATCCA	282	
CPmu	(T)10	37,236				AATGATGGATTTCATGGTAAAATCC	ATACCCACAGAAAGACTAATCCA	282	
CPpe	(T)10	44,171	ycf3 intron	CXep44056_215	CP28	ACTTGAGAGTTTCCGACCTCATAC	CTGAAGGTGGGAGAAAAGATAAAA	215	
CPka	(T)10	44,205				ACTTGAGAGTTTCCGACCTCATAC	CTGAAGGTGGGAGAAAAGATAAAA	215	
CPmu	(T)10	44,160				ACTTGAGAGTTTCCGACCTCATAC	CTGAAGGTGGGAGAAAAGATAAAA	208	
CPpe	(TTTC)3	44,739	ycf3 intron	CXep44498_286	CP29	AAGCAGTCTTATTGTATCGGACC	CGTGGGACTATCTCCACTATAGAA	286	
CPka	(TTTC)3	44,773				AAGCAGTCTTATTGTATCGGACC	GATCTGTCAATTACGTGGGACTATC	298	
CPmu	(TTTC)3	44,734				CTTATTGTATCGGACCAAAACCTC	GATCTGTCAATTACGTGGGACTATC	296	
CPpe	(A)13	45,714	ycf3 intron	CXep45652_261	CP30	TTCTATCCCGAATAATGTTGTCT	GTTTCTCACGAAAGGGAATTA	261	
CPmu	(AATA)3	48,017							
CPpe	(AT)6	48,038	trnT-UGU ^ψ trnL-UAA						
CPmu	(AT)5	48,035							
CPka	(AT)6	48,071							
CPpe	(A)15	48,492	trnT-UGU ^ψ trnL-UAA	CXep48374_176	CP31	TGTTATTAGAGGGTCTGCCCTAAG	CCTATTCTATCCGTGCAATTTT	176	
CPka	(A)13	48,525				TGTTATTAGAGGGTCTGCCCTAAG	ATTCTATCCGTGCAATTTTGAAT	171	
CPmu	(A)12	48,472				TGTTATTAGAGGGTCTGCCCTAAG	ATTCTATCCGTGCAATTTTGAAT	170	
CPpe	(TA)5	48,575	trnT-UGU ^ψ trnL-UAA	CXep48516_188	CP32	TTCAAAGTATTAAAAATTGCACGGA	CTTTGATCTCTATCGGAGACCAGT	188	
CPka	(TA)5	48,606				GAAAAGGCGAAAAGCTAATATGAAA	CTTTGATCTCTATCGGAGACCAGT	232	
CPpe	(AT)5	50,069	trnF-GAA ^ψ ndhJ	CXep49901_256	CP33	TTCTGGCACATGAGTAATTTT	GAGAGGAGACACAGTATGGACAAA	256	
CPka	(AT)5	50,100				TTCTGGCACATGAGTAATTTT	GAGAGGAGACACAGTATGGACAAA	268	
CPpe	(TA)5	50,320	trnF-GAA ^ψ ndhJ	CXep50133_299	CP34	TTTGTCACATCTGTCTCTCTCTC	GAAACTAAAAGAGTCCGCATCAT	299	
CPka	(TA)7	50,363				TACTGTCTCTCTCTCTCTCTCTC	ATGAACTAAAAGAGTCCGCATCAT	299	
CPmu	(TA)7	50,296				TTTGTCACATCTGTCTCTCTCTCTC	GAAACTAAAAGAGTCCGCATCAT	299	
CPpe	(T)10	51,949	ndhK ^ψ ndhC	CXep51752_291	CP35	AAGTCGAATCGTGAGCCTATTAAAC	TGCTTATCTTAATTGTGGTTCAA	291	
CPka	(T)10	51,998				AAGTCGAATCGTGAGCCTATTAAAC	TGCTTATCTTAATTGTGGTTCAA	291	
CPmu	(T)10	51,932				ATTGAAAAGGTCGCTTGATGATGAT	TGATGTTGAAAACGGTTTTTCTTTA	285	
CPpe	(AT)5	52,871	ndhC ^ψ trnV-UAC						
CPpe	(AT)5	52,892	ndhC ^ψ trnV-UAC						
CPka	(AT)5	52,920							
CPmu	(AT)5	52,739							
CPmu	(AT)5	52,851							
CPpe	(T)10	56,026	atpB	CXep55938_189	CP36	ACCAGAGCGTTGTAAATATTAGGC	TTTTTGATTTTCAGTAATTTTCGCA	189	

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Supplemental Table 1. Continued.

Source	SSR motif	Start (bp)	Genomic location	Primer name	Alias	Forward primer (5'-3')	Reverse primer (5'-3')	Size (bp)	Note
CPka	(T)10	56,038				ACCAGAGCGTTGTAATAATTAGGC	TTTTTGATTTTCAGTAATTTTCGCA	189	
CPmu	(T)10	55,997				ACCAGAGCGTTGTAATAATTAGGC	TTTTTGATTTTCAGTAATTTTCGCA	189	
CPpe	(T)16	58,725	rbcl ⁺ accD	CXcp58614_239	CP37	TCCCATCTGTATATGTGCTTTT	ACTTCTTGCCCTATTTACATGA	239	
CPka	(T)10	58,737				TCCCATCTGTATATGTGCTTTT	ACTTCTTGCCCTATTTACATGA	241	
CPmu	(T)18	58,696				TCCCATCTGTATATGTGCTTTT	ACTTCTTGCCCTATTTACATGA	241	
CPpe	(C)11	60,639	accD ⁺ psal	CXcp60541_140	CP38	CCAGGAAATCCCTATTAAACAAA	AATAATACGACCCCAACAAAAGGA	140	
CPka	(C)10	60,653				CCAGGAAATCCCTATTAAACAAA	AATAATACGACCCCAACAAAAGGA	139	
CPmu	(C)13	60,610				CCAGGAAATCCCTATTAAACAAA	AATAATACGACCCCAACAAAAGGA	140	
CPpe	(T)13	61,220	psal ⁺ ycf4	CXcp61150_298	CP39	ATTGCAATGGCTTCTTTATCTCTT	GTAAATCCCTTTTCACATGATTC	298	2 loci
CPka	(T)13	61,233				ATTGCAATGGCTTCTTTATCTCTT	GTAAATCCCTTTTCACATGATTC	298	
CPmu	(T)12	61,200				ATTGCAATGGCTTCTTTATCTCTT	GTAAATCCCTTTTCACATGATTC	295	
CPmu	(AT)5	61,385				ATAACTATTTTCAAAATGGATCGGG	GTGAACGTTTAGGGTAATTCATC	145	
CPpe	(T)12	62,326	ycf4 ⁺ cemA	CXcp62273_244	CP40	CCAAAGCAAAACGTTACGAAATAAT	CAAAATTAATAGTAGTCGGATTGCG	244	2 loci
CPka	(T)11	62,339				CCAAAGCAAAACGTTACGAAATAAT	TATTACAAAGTTATCGAGCCCATGA	237	
CPmu	(T)10	62,422				AAGTTCTTCAGAACGCTTATTG	GAAAGAACTCCGTTCTATCAAAA	245	
CPpe	(T)11	62,449	ycf4 ⁺ cemA			CCAAAGCAAAACGTTACGAAATAAT	CAAAATTAATAGTAGTCGGATTGCG	244	
CPpe	(A)10	62,588	ycf4 ⁺ cemA	CXcp62509_124	CP41	TTAATTGAAGTGGCTTTTCTT	TATTACAAAGTTATCGAGCCCATGA	124	
CPka	(A)10	62,478				TTAATTGAAGTGGCTTTTCTT	TATTACAAAGTTATCGAGCCCATGA	124	
CPka	(TC)5	62,680		CXcp62499_228	CP42	TCATGGCTCGATAACTTGTAAATA	ACCAATTAGTAACCCAAAGATTCCA	228	
CPpe	(TC)5	62,790	cemA			TCATGGCTCGATAACTTGTAAATA	ACCAATTAGTAACCCAAAGATTCCA	228	
CPmu	(TC)5	62,769				TCATGGCTCGATAACTTGTAAATA	ACCAATTAGTAACCCAAAGATTCCA	228	
CPpe	(A)12	65,165	petA ⁺ psbJ	CXcp64998_289	CP43	TTAATAATGATTCGATCCCGTT	TTTACTCTTTTGGATGACTCACAA	289	
CPka	(A)12	65,055				TTAATAATGATTCGATCCCGTT	TTTACTCTTTTGGATGACTCACAA	289	
CPmu	(A)11	65,142				AATTAATAATGATTCGATCCCG	TTTACTCTTTTGGATGACTCACAA	289	
CPmu	(A)10	65,929				AGATGTGACGATTCAATTCAACA	CCGACAAAGGAATCCATTAATAAC	280	
CPpe	(G)10	66,324	psbE ⁺ petL	CXcp66113_284	CP44	GTCGGCTTCTGTAAATAACTCGT	CGATCTCACAAAGGTTTATTTCAT	284	
CPmu	(G)10	66,302				CGGCTTCTGTAAATAACTCGT	CCCTGGTACAATAATTGACGATCT	300	
CPpe	(A)12	67,119	psbE ⁺ petL	CXcp66942_253	CP45	CTAATTACAAAGGTGGGCTTCTA	TCACTGTGGAATAAAATTGGAAAA	253	
CPka	(A)11	67,008				CTAATTACAAAGGTGGGCTTCTA	TCACTGTGGAATAAAATTGGAAAA	252	
CPmu	(A)11	67,095				ACAAGAGTGGGCTTCTAATATGG	TCACTGTGGAATAAAATTGGAAAA	247	
CPpe	(TA)5	67,288	psbE ⁺ petL	CXcp67171_228	CP46	TTTTCCAATTTTATCCACAGTGA	TCATCATTTGTGACATTTACCCAT	228	
CPka	(TA)5	67,176				TTTTCCAATTTTATCCACAGTGA	TCATCATTTGTGACATTTACCCAT	228	
CPmu	(TA)6	67,262				TTTTCCAATTTTATCCACAGTGA	TCATCATTTGTGACATTTACCCAT	229	
CPpe	(A)13	69,031	psaJ ⁺ rpl33	CXcp68964_205	CP47	TCTGTAACATAATCCCTACCCCTGC	TATCTGTATGATATGTTGCCCC	205	
CPka	(A)13	68,919				TCTGTAACATAATCCCTACCCCTGC	TATCTGTATGATATGTTGCCCC	205	
CPmu	(A)10	69,004				TCTGTAACATAATCCCTACCCCTGC	CAACCATCACATTTTATTTCCAC	177	
CPpe	(T)11	69,005	psaJ ⁺ rpl33	CXcp68765_297	CP48	GAAAGAGGAGTTTAAATGCGA	CGAAACCATTTTCTTTGTTTTT	297	
CPka	(T)11	68,893				GAAAGAGGAGTTTAAATGCGA	CGAAACCATTTTCTTTGTTTTT	297	
CPmu	(T)10	68,979				TCTGTAACATAATCCCTACCCCTGC	CAACCATCACATTTTATTTCCAC	177	
CPpe	(T)15	69,301	psaJ ⁺ rpl33	CXcp69274_142	CP49	ATTGATTCGAGTTACCTGCTTC	CTGTTACTCGGACATCTTACCCCT	142	
CPka	(T)15	69,189				ATTGATTCGAGTTACCTGCTTC	CTGTTACTCGGACATCTTACCCCT	142	
CPmu	(T)12	69,270				ATTGATTCGAGTTACCTGCTTC	ATGTTTGTAAACAACAGGACAGAA	282	
CPpe	(A)13	70,035	rps18	CXcp69999_174	CP50	AGAAACAAATTTGAAAGAAAGCGAGT	TAGTTTAAATGGAGTTCCTGGAG	174	

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Source	SSR motif	Start (bp)	Genomic location	Primer name	Alias	Forward primer (5'-3')	Reverse primer (5'-3')	Size (bp)	Note
CPka	(A)13	69,923				AGAAACAAATTTGAAAGAAAGCGAGT	TAGTTAAATGGAGTTCCCTGGAG	174	
CPmu	(A)13	70,001				AGAAACAAATTTGAAAGAAAGCGAGT	TAGTTAAATGGAGTTCCCTGGAG	174	
CPpe	(A)12	70,738	rp120 ⁺ rps12	CXep70633_265	CP51	ACAAAACGGATTCTTCCAATGTAT	TGATTAAATGAAAAGAAAAGAGGC	265	
CPka	(A)11	70,626				ACAAAACGGATTCTTCCAATGTAT	TGATTAAATGAAAAGAAAAGAGGC	264	
CPpe	(AAAT)3	72,059	clpP intron	CXep71903_234	CP52	TATCTCGCATAAATGAAAAGGTGAAG	TTTGAACATAATCCAAAAGAAAAGGG	234	
CPka	(TAAA)3	71,945				TATCTCGCATAAATGAAAAGGTGAAG	TTTGAACATAATCCAAAAGAAAAGGG	234	
CPmu	(AAAT)3	72,025				TATCTCGCATAAATGAAAAGGTGAAG	ATTGGATCATTTACCAACCTGAGT	208	
CPmu	(T)11	71,983				TATCTCGCATAAATGAAAAGGTGAAG	ATTGGATCATTTACCAACCTGAGT	208	
CPmu	(A)10	72,337				TAGACATCCCGATAGTTCGTATAA	TTCAATGGATCTTTTATTTCTGGT	267	
CPpe	(A)11	72,859	clpP intron	CXep72773_189	CP53	ATAAAGTCGGTTGATTGGGATAAA	CCCTTCTTGA AAAATGAAAAGAAAA	189	
CPka	(A)11	72,746				ATAAAGTCGGTTGATTGGGATAAA	CCCTTCTTGA AAAATGAAAAGAAAA	189	
CPpe	(A)11	72,991	clpP intron	CXep72938_152	CP54	TTTCTTTCATTTTTC AAGAAAGGG	TTCAAGGAACAAGAAAATACATCG	152	
CPka	(A)11	72,878				TTTCTTTCATTTTTC AAGAAAGGG	TTCAAGGAACAAGAAAATACATCG	152	
CPpe	(T)17	73,207	clpP intron	CXep73060_203	CP55	AAATCGCATGTAATTTTCTTGT	GAAATGGGTGAATAATTTGTTGTA	203	
CPka	(T)18	73,094				AAATCGCATGTAATTTTCTTGT	GAAATGGGTGAATAATTTGTTGTA	204	
CPmu	(T)13	73,170				CGGATGTAATTTTCTTGTACTG	GAAATGGGTGAATAATTTGTTGTA	195	
CPpe	(AT)6	73,862	clpP ⁺ psbB	CXep73700_253	CP56	CTCACTTGTGTAAGAAAGCGTAAC	AGAAGCAGATCTATTCTACACCG	253	
CPka	(AT)6	73,750				CTCACTTGTGTAAGAAAGCGTAAC	AGAAGCAGATCTATTCTACACCG	253	
CPmu	(AT)6	73,815				CTCACTTGTGTAAGAAAGCGTAAC	AGAAGCAGATCTATTCTACACCG	253	
CPpe	(AT)5	76,717	petB intron	CXep76664_272	CP57	ATAGAGATGGTTTACTTCGTCGG	CTAAGTCCCAAAAATTCCTGAGTA	272	2 loci
CPka	(AT)5	76,605				ATAGAGATGGTTTACTTCGTCGG	CTAAGTCCCAAAAATTCCTGAGTA	274	
CPmu	(AT)5	76,671				ATAGAGATGGTTTACTTCGTCGG	CTAAGTCCCAAAAATTCCTGAGTA	277	
CPpe	(T)10	76,798	petB intron			ATAGAGATGGTTTACTTCGTCGG	CTAAGTCCCAAAAATTCCTGAGTA	272	
CPka	(T)13	76,685				ATAGAGATGGTTTACTTCGTCGG	CTAAGTCCCAAAAATTCCTGAGTA	274	
CPmu	(T)16	76,751				ATAGAGATGGTTTACTTCGTCGG	CTAAGTCCCAAAAATTCCTGAGTA	277	
CPmu	(A)10	79,412				ACAACCGTCTTTTGGATTGGTACT	AATTCACTTTGAAGCGACTATTCC	172	
CPmu	(A)10	79,573				CAATTAATTTTGGACCTATTCCG	CAGGTCTATAATTGCCCTCAAAAG	280	
CPmu	(ATCTAT)3	83,454				ACGGTGAATAATATACGGAATCGT	AATGTTGAAAAGATAATATTCGCC	257	
CPpe	(T)11	84,415	rp116 ⁺ rps3	CXep84331_227	CP58	AAAAACCAACGAGTCACACACTAA	ATCTATGGGTATTAGGCATCAAA	227	
CPka	(T)13	84,285				AAAAACCAACGAGTCACACACTAA	ATCTATGGGTATTAGGCATCAAA	229	
CPmu	(T)10	84,378				AAAAACCAACGAGTCACACACTAA	ATCTATGGGTATTAGGCATCAAA	226	
CPpe	(T)10	85,211	rps3 ⁺ rpl22	CXep85184_295	CP59	ATCGTGAAACGTCATATCTGTTTG	ATTAGTAAAGCCGAAGTCAACGAG	295	
CPka	(T)10	85,083				ATCGTGAAACGTCATATCTGTTTG	ATTAGTAAAGCCGAAGTCAACGAG	295	
CPmu	(T)10	85,173				ATCGTGAAACGTCATATCTGTTTG	ATTAGTAAAGCCGAAGTCAACGAG	295	
CPpe	(G)10	95,920	ycf2 ⁺ tmL-CAA	CXep95716_257	CP60	GTCTATTATTGAATTCACCCGACC	TATCTAAATGAATGGGGAGTCTGCT	257	
CPka	(G)10	95,798				GTCTATTATTGAATTCACCCGACC	TATCTAAATGAATGGGGAGTCTGCT	257	
CPmu	(G)10	95,882				GTCTATTATTGAATTCACCCGACC	TATCTAAATGAATGGGGAGTCTGCT	257	
CPpe	(A)10	112,256	ndhF	CXep112116_276	CP61	AAGAGATACGAGTGAATGGAAAAGG	AATTGATGGAATACGAATGGAGT	276	
CPka	(A)10	112,134				AAGAGATACGAGTGAATGGAAAAGG	AATTGATGGAATACGAATGGAGT	276	
CPmu	(A)10	114,445				CAATTAACATTGGAACCTGGAAGTG	ACCCCTTCAACACAGAAAACAAATA	144	
CPmu	(A)14	114,831				ATGAATCATAAAAGCGCATAACTTG	CGAAGAAAAGTACACCCAACTTT	300	
CPpe	(T)10	115,324	ndhF ⁺ rpl32	CXep115232_281	CP62	ATTTTATGCGAATTTTACACACCC	AGA ACTCCCGGTAGAAAAGAGATT	281	
CPka	(T)11	116,214				GGTAGTTATATGACGAATGTGCCA	GGGTTTTTCGTTGTGTAGAGATT	263	

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Supplemental Table 1. Continued.

Source	SSR motif	Start (bp)	Genomic location	Primer name	Alias	Forward primer (5'-3')	Reverse primer (5'-3')	Size (bp)	Note
CPmu	(TAA) ₄	117,041				GAAATAGGTAGACACGCTGCTCTT	CTTTTCTGACGAATCATATAGCCC	292	
CPka	(TTGA) ₃	120,429				TGCGATAGAACCAAGATACTTTGA	GATAGTCGCCAATTAAAAGGAAAT	278	
CPmu	(TTGA) ₃	120,461				TGCGATAGAACCAAGATACTTTGA	GATAGTCGCCAATTAAAAGGAAAT	278	
CPpe	(TTGA) ₃	120,88	ndhE			TGCGATAGAACCAAGATACTTTGA	GATAGTCGCCAATTAAAAGGAAAT	278	
CPpe	(A) ₁₀	120,853	ndhE ^Δ ndhG	CXep120779_255	CP63	CCAACCTCTTATCAAATTTCGATTC	TCTATAGAAATTGGCAACAGCAATC	255	
CPmu	(T) ₁₂	121,951				TATTGACCAACAAACGAAAGAA	GGTCTGTTTACCAATGTCAGTAAT	208	
CPpe	(T) ₁₀	129,489	ycf1	CXep129376_280	CP64	GAATGCTTTTAATGATCCGTTTTT	CACAGATCTAAATTACAAGCCCT	280	
CPka	(T) ₁₀	129,429				GAATGCTTTTAATGATCCGTTTTT	CACAGATCTAAATTACAAGCCCT	280	
CPmu	(T) ₁₀	129,451				AAACAGAAATGCTTTTAAATGATCCA	CACAGATCTAAACTACAAGCCCT	285	
CPpe	(A) ₁₃	130,098	ycf1	CXep130045_225	CP65	TGTTTTACGCATCCAAATTTCTAA	AAACAGCCGAAATGCTTTAATAC	225	
CPka	(A) ₁₅	130,038				TGTTTTACGCATCCAAATTTCTAA	AAACAGCCGAAATGCTTTAATAC	225	
CPmu	(A) ₁₃	130,060				TAAATACTATCCCAGGCTTCTGC	TCAAAGGCGTAAACAGTTACTTG	262	
CPpe	(T) ₁₀	130,482	ycf1	CXep130399_241	CP66	CTCCCTCTTGTGTCTCTAAATCGT	TTGGCTGTAAATCAACATCAAGT	241	
CPka	(T) ₁₀	130,422				CTCCCTCTTGTGTCTCTAAATCGT	TTGGCTGTAAATCAACATCAAGT	241	
CPmu	(T) ₁₀	130,444				GTGATTAATTTGTATGACACCGA	TTGGCTGTAAATCAACATCAAGT	218	
CPpe	(C) ₁₀	147,658	tmL-CAA ^Δ ycf2	CXep147613_257	CP67	TATCTAATGAATGGGGAGTCTGCT	GTCTATTATTGAATTCACCCGACC	257	
CPka	(C) ₁₀	147,598				TATCTAATGAATGGGGAGTCTGCT	GTCTATTATTGAATTCACCCGACC	257	
CPmu	(C) ₁₀	147,620				TATCTAATGAATGGGGAGTCTGCT	GTCTATTATTGAATTCACCCGACC	257	
CPka	(TA) ₅	157,682							
CPka	(TA) ₅	157,713							
CPpe	(TA) ₅	157,736	rps19 ^Δ tmH-GUG						
CPpe	(TA) ₅	157,767	rps19 ^Δ tmH-GUG						

SSR = simple sequence repeat.

*The 16 primer names in bold are selected for screening, and eight of them are highlighted in green for genotyping. The polymorphic microsatellite motifs and product sizes are in red font.

Supplemental Table 2. Peach cultivars and selections belonging to each maternal lineage group (MLG), along with first maternal ancestor and progenitor ancestor cultivar.

MLG ^z	Cultivar ^y	Selections (no.)	First maternal ancestor cultivar	Progenitor cultivar or type
1	Arctic Belle		Arctic Queen	JH Hale
1	Arctic Gold		Ruby Gold	JH Hale
1	Arctic Sweet		White Lady	JH Hale
1		3	ARS-Kearneysville selections	Unknown
1		48	ARS-Parlier selections	Unknown
1	Autumnprince	4	O'Henry	JH Hale
1	Belle of Georgia		Chinese Cling	Chinese Cling
1	Big Red=CVN#3		Unknown	Unknown
1	Bill Gross Indian		Unknown	Unknown
1		8	Biscoe	JH Hale
1	Blazeprince	10	O'Henry	JH Hale
1	Camden		Hiley	JH Hale
1	Carman		Elberta	Chinese Cling
1	Carolina Gold		Biscoe	JH Hale
1	Carolina Red		Nectared 4	JH Hale
1	Carored		Springbrite	JH Hale
1		4	Challenger	JH Hale
1	Chinese Cling		Unknown	Chinese Cling
1	Clayton		Pekin	JH Hale
1	Compact Elberta		Fay Elberta	Chinese Cling
1	Contender	5	Winblo	JH Hale
1	Coronet IR		Halehaven	JH Hale
1	Cresthaven		Kalhaven	JH Hale
1	Cresthaven IR		Kalhaven	JH Hale
1	Crimson Snow	3	Sungrand	JH Hale
1	Dixiland		Halehaven	JH Hale
1	Durbin		Flaming Gold	JH Hale
1	Dwarf Elberta		Elberta	Chinese Cling
1	Eastern Glo		Red Grand	JH Hale
1	Elberta		Chinese Cling	Chinese Cling
1	Elberta		Chinese Cling	Chinese Cling
1	Empress		Unknown	Unknown
1	Fackler		Unknown	Unknown
1	Fairtime		Rodeo	Late Crawford
1		1	Fire Pearl	Unknown
1	Fireprince	1	Halberta Giant	JH Hale
1	Flameprince	29	Summerset	Late Crawford
1	Flameprince Ham		Summerset	Late Crawford
1	Flameprince Pearson		Summerset	Late Crawford
1	Flavorich		May Grand	JH Hale
1		11	Flavortop	Late Crawford
1	Flavrburst		Blazeprince	JH Hale
1	Flordadawn		Flordagold	Unknown
1	Flordaprince		Southland	JH Hale
1	Frank		Elberta	Chinese Cling
1	GaLa		Harvester	JH Hale
1	Glacier White		Unknown	Unknown
1	Greensboro		Connett	Historic
1		1	Hakuho	Chinese Cling
1	Hakuto	1	Chinese Cling	Chinese Cling
1	Hiland		Southland	JH Hale
1	Hiley		Belle of Georgia	Chinese Cling
1	Honey Royale		Unknown	Unknown
1	J.H.Hale		Elberta?	Chinese Cling
1	Jefferson		JH Hale	JH Hale
1	Jerseyqueen		Candoka	JH Hale

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Supplemental Table 2. Continued.

MLG ²	Cultivar ^y	Selections (no.)	First maternal ancestor cultivar	Progenitor cultivar or type
1	Joanna Sweet		O'Henry	JH Hale
1	Julyprince	1	Unknown	Unknown
1	Julyprince Pearson		Unknown	Unknown
1	Junegold		Flamingo	Late Crawford
1	Juneprince	13	Sunhigh	JH Hale
1	Juneprincess		Flamekist	JH Hale
1	K.Hakuto		Unknown	Chinese Cling
1		1	Khodjent Kostokos	P.ferganensis
1*		1	LaPremiere	Admiral Dewey
1	Late Crawford		Unknown	Late Crawford
1	Le Grand		Elberta	Chinese Cling
1	Lobo		Unknown	Unknown
1	Lobo - early		Unknown	Unknown
1		22	LSU selections	Unknown
1*	Majestic	2	Meadow Lark	St John
1		12	Natural selections	Unknown
1	O'Henry	80	Merrill Bonanza	JH Hale
1		1	Ouachita Gold	JH Hale
1	Parade		Unknown	Unknown
1		15	Pekin	JH Hale
1	PF10 Ball		Unknown	Unknown
1	PF11 Ball		Unknown	Unknown
1		3	PF12A	Unknown
1	PF17		Unknown	Unknown
1	PF19-007	3	Unknown	Unknown
1	PF23		Unknown	Unknown
1	PF24-007		Unknown	Unknown
1	PF24C		Unknown	Unknown
1	PF27A		Unknown	Unknown
1	PF36-007		Unknown	Unknown
1	PF5D Big		Unknown	Unknown
1	PF7A Freestone		Unknown	Unknown
1	PF8 Ball		Unknown	Unknown
1	PF9-007		Unknown	Unknown
1	PF-Late 24-007		Unknown	Unknown
1	PF-Late Large 23		Unknown	Unknown
1*	Red Bird Cling		Unknown	Heath Cling
1		1	Red Diamond	JH Hale
1	Redhaven		Halehaven	JH Hale
1		5	Redgold	JH Hale
1	Rio Oso Gem		Late Crawford?	Historic
1	Roseprincess	1	Red King	JH Hale
1	Rubyprince	1	Fireprince	JH Hale
1*		1	S37 op	S-37
1	Sauzee King		Honeykist	JH Hale
1	Scarletpearl		Biscoe	JH Hale
1	Scarletprince	8	O'Henry	JH Hale
1	Sentinel		Halehaven	JH Hale
1	Slappey		Unknown	Historic
1	Southern Pearl	3	Roseprincess	JH Hale
1	Spice Zee		May Grand	JH Hale
1		1	Spicula	Unknown
1	Springprince	4	Springcrest	JH Hale
1	Starlite		Hiley	JH Hale
1	SummerFest		Bounty	JH Hale
1	Summergold		JH Hale	JH Hale
1	Summerprince		Summerset	Late Crawford

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Supplemental Table 2. Continued.

MLG ²	Cultivar ^y	Selections (no.)	First maternal ancestor cultivar	Progenitor cultivar or type
1		17	Summerset	Late Crawford
1	Sunbrite		Sunhigh	JH Hale
1	Sunland		Newday	JH Hale
1		1	Sunprince	Loring
1	Sureprince		Dixiland	JH Hale
1	Suwanee		JH Hale	JH Hale
1	Sweet Cap		xxxxx	Unknown
1	Ta Qiao		Unknown	Unknown
1		3	Texas A&M selections	Unknown
1	Texprince		Firebrite	Late Crawford
1	Tri Lite		O'Henry	Unknown
1	TruGold		Redhaven	JH Hale
1	Vulcan		Veecling	JH Hale
1	White Cloud		Allgold	JH Hale
1	White County		Unknown	Unknown
1	White Diamond		Unknown	Unknown
1		6	White Lady	JH Hale
1	White Rock		Unknown	Unknown
1		2	White Snow	Unknown
1	Winblo	3	Redskin	JH Hale
1		5	Yumyeong	Chinese Cling
1	Zephyr		Unknown	Unknown
1	Zin Dai Jiu Bao	9	Unknown	Unknown
2	Admiral Dewey		Alexander	Historic
2		4	Arkansas Late	Unknown
2		6	ARS-Parlier selections	Unknown
2		1	Augustprince	Loring
2	Candy Cane	3	Shidaremomo	Ornamental
2	Cary Mac		Loring	Loring
2	Champion		Unknown	Historic
2	Duke of York		Early Rivers	Historic
2	Early Augustprince		Sunprince	Loring
2	Early Crawford		Unknown	Historic
2*		1	Flameprince	JH Hale
2	Flat Wonderful		Unknown	Unknown
2*		1	Flavortop	JH Hale
2	Flordaguard		PI65821	PI65821
2	Frost		Unknown	Unknown
2		4	GA107	Natural
2	Galaxy		Armking	Goldmine nect
2	George IV		Unknown	Historic
2	Goldprince		Loring	Loring
2	Grosse Mignonne		Unknown	Historic
2	Halls Hardy		Unknown	Unknown
2		1	Hardired	Lippiatt nect
2		3	Harrow Blood	Natural
2	Heath Cling	4	Unknown	Historic
2	Helen Borchers	9	Unknown	Ornamental
2		1	Indian Blood	Natural
2		2	Isom Late	Unknown
2		2	Jerseypink	Ornamental
2	Karla Rose		Unknown	Unknown
2	Late Crawford		Unknown	Historic
2	Loring		Frank	Loring
2	MacDonald	3	Unknown	Ornamental
2		16	Natural selections	Unknown
2*	Nectared 6	2	Elberta	Chinese Cling

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Supplemental Table 2. Continued.

MLG ^z	Cultivar ^y	Selections (no.)	First maternal ancestor cultivar	Progenitor cultivar or type
2	NRL1		Unknown	Natural
2	Oldmixon Free		Unknown	Historic
2		3	Pillar	Ornamental
2	Q26941 = Criollo		Unknown	Unknown
2	Q39622A = Pingbaizi		Unknown	Unknown
2	Q42535C = Sanguine de Chateauneuf		Unknown	Historic
2	Q43148A1 = IC 20841		Unknown	Unknown
2	Q43539 = Clone 60		Unknown	Unknown
2		3	Red English	Natural
2		1	Redglobe	Admiral Dewey
2*		1	Roseprincess	JH Hale
2		1	RRL-2N	Natural
2	Salway		Unknown	Historic
2*	Saturn		Golden Globe	JH Hale
2	Sentry		Loring	Loring
2		4	Shidaremomo	Ornamental
2		2	SN45/3	Unknown
2	Springtime		Luken's Honey	Unknown
2	St. John		Unknown	Historic
2	Strawberry Free		Unknown	Unknown
2*		2	Summerset	Late Crawford
2	Sunprince	74	Redglobe	Loring
2	Texking		Goldprince	Loring
2	White English		Unknown	Ornamental
2	White River		Loring	Loring
3*		1	Flameprince	Chinese Cling
3	Guardian	4	S-37	S-37
3*		1	Juneprince	JH Hale
3	Q36019e = Kikomo op	6	Kikomo	Kikomo
3	Q43183 = Hong Chui Zhi		Unknown	Unknown
3		14	S37 op	S-37
4	Flordaking		Okinawa	Okinawa
5	Reliance		Unknown	Unknown
6	P.tangutica hybrid	2	Unknown	P.tangutica
7	P.davidiana op		Unknown	P.davidiana
8	P.mira		Unknown	P.mira

^zAsterisks indicate entries in a different group than expected based on known pedigree.^yWhere no cultivar is listed the parent was not tested, but the indicated number of selections were maternally derived from the first parent.

Supplemental Table 3. Number of single nucleotide polymorphisms (SNPs) in the chloroplast amplicon sequences from the eight representative materials amplified by the eight primers.

Primer name	<u>A/G</u>	<u>C/T</u>	<u>A/C</u>	<u>G/T</u>	<u>A/T</u>	<u>C/G</u>	<u>Others</u>	<u>Total</u>
	SNPs (no.)							
CXcp01488_232	1				1			2
CXcp05368_299								0
CXcp36648_290	10	3	2	2	6	1	1	25
CXcp58614_239	7	2	1	4	5		2	21
CXcp60541_140								0
CXcp62273_244	1				1			2
CXcp68964_205	3	1	1	3	4	1		13
CXcp76664_272	1			1	1			3