Theor Appl Genet (2003) 106:1341–1352
DOI 10.1007/s00122-002-1128-5

M. J. Aranzana · J. Carbó · P. Arús

Microsatellite variability in peach [*Prunus persica* (L.) Batsch]: cultivar identification, marker mutation, pedigree inferences and population structure

Received: 27 May 2002 / Accepted: 8 August 2002 / Published online: 22 October 2002

Abstract A collection of 212 peach and nectarine cultivars covering a wide variation of the species were studied with 16 polymorphic single-locus microsatellite, or simple-sequence repeat (SSR), markers. The average number of alleles per locus was 7.3, 35% of the cultivar × locus combinations analyzed were heterozygous and 87% of the cultivars studied could be individually identified. Most of the groups where two or more cultivars had the same SSR fingerprint included known peach mutants or possible synonymies. Pedigree information was tested with the SSR data. Five unexpected genotypes, due to a mutation at five SSR loci were found when comparing the SSR fingerprint of 14 known mutant cultivars and putative synonymous cultivars. The pedigree data were not consistent with the observed data in 11 out of 38 cases that could be analyzed. The group of non-melting fruit flesh cultivars, generally used by the canning industry, was more variable and genetically distant than the rest of the cultivars tested. Based on their level of homozygosity it was possible to separate those cultivars that were obtained by modern breeding technologies from those that were selected from traditional orchards after generations of seed propagation. The former had a distribution of genotypic frequencies close to a random mating model while the latter had a higher level of homozygosity. The implications of these data for the use of SSR fingerprints in breeder's rights protection and peach breeding are discussed.

Keywords Simple sequence repeats · SSRs · Variability · Molecular markers · Breeder's rights

Communicated by H. Nybom

M.J. Aranzana · P. Arús (✉)
IRTA, Department de Genètica Vegetal, Carretera,
de Cabrils s/n; 08348 Cabrils, Barcelona, Spain
e-mail: pere.arus@irta.es
Tel.: +34-93-750-75-11, Fax: +34-93-753-39-54

J. Carbó
IRTA-Fundació Mas Badia, Carretera de la Tallada s/n,
17134 La Tallada d'Empordà, Girona, Spain

Introduction

Peaches are grown in temperate and subtropical zones worldwide, being the second most important fruit crop in these regions, after apple. A large number of varieties are commercialized, and the breeding industry, mainly private, is particularly dynamic with many new cultivars being released yearly (Fideghelli et al. 1997). Peach is self-compatible and tolerant to inbreeding, which makes possible the breeding of new cultivars either by outcrossing or by inbreeding (Hesse 1975). In addition, most modern cultivars have originated from those produced by the US breeding programs of the early twentieth century using a very limited number of parents. All this has drastically eroded the genetic variability of this crop (Scorza et al. 1985, 1988). Results on comparative isozyme variation of *Prunus* species have corroborated this fact, indicating that peach is the least variable crop of the genus (Arulsekar et al. 1986; Byrne 1990).

Modern peach cultivars are vegetatively propagated, which allows the maintenance of their genetic information, but it also makes breeder's rights more difficult to protect. The high number of existing cultivars and their important economic value has encouraged the development of fast and reliable techniques for peach molecular fingerprinting. Of the possible alternatives, isozymes are not sufficiently variable due to the low polymorphism of the species (Arulsekar et al. 1986; Messeguer et al. 1987). RAPDs have also been assayed (Warburton and Bliss 1996), but problems with amplification reproducibility make them inappropriate for this objective. Other more robust markers such as RFLPs require complex and time-consuming methods, and AFLPs are based on a patented method that requires licensing for its commercial use.

The widespread presence of microsatellites in the eukaryotic genome, their high level of polymorphism, co-dominant Mendelian inheritance and easy detection by PCR and electrophoresis methods make them high quality markers for genetic analysis (Morgante and Olivieri 1993). Until recently, their use was limited by the

high cost associated with the development of the markers, but new library enrichment and automatic sequencing procedures has meant that a high number can be effeciently obtained. SSR markers are now broadly used, replacing other molecular markers in cultivar identification and other applications. More than 100 microsatellites have recently been isolated and characterized in peach (Cipriani et al. 1999; Sosinski et al. 2000; Testolin et al. 2000; Aranzana et al. 2002a; Dirlewanger et al. 2002), and the position on the *Prunus* map of most of them has been determined (Aranzana et al. 2002b).

We present our results on the variability of SSRs in a large collection of commercial peach cultivars. The detailed genetic knowledge of these markers (codominance, inheritance and map position) allowed us to determine not only their efficiency for cultivar identification and variability analysis, but also to assess the importance of certain factors for the accurate use of SSRs in the defense of breeder's rights. These factors include the choice of markers on the basis of their variability and map position, the occurrence of SSR mutations or the effect of the genetic characteristics of the cultivar population from which the cultivars to be studied are sampled on the estimation of the probability that two cultivars have the same marker fingerprint by chance.

Materials and methods

A set of 212 *Prunus persica* cultivars including 89 melting flesh peaches, 80 melting flesh nectarines, both used for fresh consumption, and 43 non-melting flesh cultivars (all peaches with the exception of the nectarine NJN-76), that are generally used for canning although they are also consumed fresh in Spain, was used for this study. Cultivars were obtained from different sources (Table 1); most of them (173) were obtained from the peach germplasm collection of IRTA-Fundacio Mas Badia (Girona, Spain). Of the rest, 11 were provided by M. L. Badenes (IVIA, València, Spain), ten by I. Batlle (IRTA-Mas Bové, Tarragona, Spain) and 18 were from the Spanish company 'Selección Plantas Sevilla S.L.'.

Genomic DNA was extracted as described in Viruel et al. (1995) and was analyzed with 16 published SSRs, all of them based on dinucleotide repeat microsatellites, shown in Table 2. SSRs were selected in two steps. A first group of seven SSRs (pchgms1, pchgms2, pchgms3, pchgms6, PceGA34, PS12e2 and PS9f8) are among the first obtained in *Prunus* and were kindly provided by their developers [B. Sosinsky and A. Abbott from Clemson University (USA), A.F. Iezzoni from Michigan State University (USA) and G.J. King from HRI, Wellesbourne (UK)] without prior knowledge of their level of variability in peach. The nine remaining (all CPPCT and BPPCT markers) were later added to the first group based on their high polymorphism in peach (Aranzana et al. 2002a; Dirlewanger et al. 2002). The map position of these SSRs was unknown when they were selected for this study with the exception of pchgmsl, pchgms3, PS12e2 and PS9f8 (Joobeur et al. 2000). PCR products were obtained in a total volume of 10 µl with 20 ng of DNA, 0.2 µM of both primers, 200 µM of each dNTP and 1 U of *Taq* polymerase in 10 mM Tris-HC1 pH 8.3, 1.5 mM $MgC1_2$, 50 mM KC1 and 0.001% gelatin. The amplification was performed in a PE9700 Thermal Cycler (PE/Applied Biosystems, Foster City, Calif., USA) under the following conditions: 1 min at 95 °C, 30 cycles of 15 s at 95 °C, 15 s at the appropriate annealing temperature, and 1 min at 72 °C, followed by a 5-min extension at 72 °C. Products were analyzed by capillary electrophoresis with the automatic sequencer ABI/Prism 310 (PE/Applied Biosystems). For multifluorophore fragment analysis, forward primers where labelled with 6-carboxyfluorescein (6-FAM), or 6-carboxy-l,4-dichloro-2′,4′,5′,7′-tetra-chlorofluorescein (HEX), or 7-trichloro-5-carboxyfluorescein (NED).

At least two independent SSR reactions were performed for each DNA sample until two data points were available for each SSR × cultivar combination. The parameters used to evaluate the information given by the 16 SSRs studied were: the number of alleles (A) and the effective number of alleles (A_e) per locus ($A_e = 1/\Sigma p_i^2$, where p_i is the frequency of the ith allele), the observed (H_0 = number of heterozygous individuals/number of individuals scored) Y and expected ($H_e = 1 - \Sigma p_i^2$) heterozygosity, and Wright's fixation index ($F = 1 - H_0/H_e$) (Wright 1951). The ability of a marker to discriminate between two random cultivars was estimated for each locus with the 'power of discrimination' ($PD = 1 - \Sigma g_i^2$, where g_i is the frequency of the ith genotype) (Kloosterman et al. 1993). PD values were used to calculate the probability of confusion [$C = \Pi(1 - PD_i)$, where PD_i is the PD value of the ith locus], i.e. the probability that any two cultivars had identical SSR genotypes by chance alone, considering all loci.

Based on the genotypic frequencies of each locus, the expected frequency of pairs of cultivars that differed for a certain number of markers was calculated. This frequency is equal to the probability of confusion (C) for pairs of cultivars with an identical SSR genotype, and equal to ΠPD_i for pairs of cultivars that are different at all loci. The algorithm to calculate the frequencies of the remaining classes can be calculated sequentially for each new locus added, using the information produced by the previous ones. A general formula is:

$$C_n^k = C_{0n} \cdot C_{n-1}^k + C_{Tn} \cdot C_{n-1}^{k-1},$$

where C_n^k is the probability that two genotypes differ at k loci when n are studied, $C_{0n} = 1 - C_{Tn} = \Sigma g_i^2$ for locus n, and C_{n-1}^k and C_{n-1}^{k-1} are the probabilities of finding k and k–1 loci respectively different between two genotypes from the n – 1 studied.

The genetic distance between cultivars was analysed with Nei's parameter (Nei 1972) implemented by the SimQual procedure of NTSYSpc V. 2.1 program (Rohlf 1994). A dendrogram was constructed from a 0/0.5/1 (absence/allele in heterozygosity/allele in homozygosity) matrix using the unweighted pair group method average (UPGMA) clustering and drawn with the Molecular Evolutionary Genetics Analysis (MEGA) program, V. 2.1 (Kumar et al. 1993).

Results

Polymorphism detected by SSRs

Two hundred and twelve peach cultivars were analyzed with 16 polymorphic SSRs. Alleles were clearly differentiated using the capillary electrophoresis sequencer and no discrepancies were found in the banding pattern of the duplicate analysis of each DNA sample. Allele sizes differed by two or more nucleotides with the exception of two alleles of pchgmsl with 188 and 189 bp. Heterozygotes for these two alleles could not be clearly distinguished from the homozygotes. To avoid ambiguous data we scored both alleles as one, resulting in the underestimation of the information given by this marker.

Size differences detected between alleles at a locus ranged from 2 to 61 bp (Table 2). Differences between consecutive alleles ranged from 2 to 30 bp, being 2 bp in 61% of the cases. Null alleles were detected in only one marker, PS12e2 (developed from *Prunus cerasus*), where

Table 1 Cultivars studied and their main characteristics

Cultivar	Pedigree[a,b]	Breeder – Country	Fruit traits[c]
Alexandra	Fayette × Royal Gold	Zaiger's Genetics (Zaiger) – USA	PWM
Aline	O'Henry × Giant Babcock+(1)	Zaiger – USA	PWM
Andross	Dix 5A-1 × Fortuna	University of California, Davis (UCD) – USA	PYN
Armking	(Palomar × Springtime) × (Palomar × Springtime)	Armstrong Nurseries (Armstrong) – USA	NYM
Armking 2	–	Armstrong – USA	NYM
Armking 3	–	Armstrong – USA	NYM
Auberge	–	Traditional cultivar (TC) – Spain	PYN
Auberge Blanc	–	TC – Spain	PYN
Auberge Ferran	–	TC – Spain	PYN
Auberge Marino	–	TC – Spain	PYN
August Orebrad	(Red Diamond × Autumn Free) F_2+(1)	Bradford Farms (Bradford) – USA	NYN
August Queen	Stark Redgold × Snow Queen+(2)	IPSA, Faenza (IPSA) – Italy	NWM
August Grand	Aurelio Grand op.	Bradford – USA	NYM
Autumn Free	Sun Grand × Gold King	Bradford – USA	NYM
Babygold 8	PI35201 × Ambergem	Rutgers University and New Jersey A.E.S., New Brunswick (RU-NJ)-USA	PYN
Babygold 7	(Lemon Free × PI35201) × NJ196	RU-NJ-USA	PYN
Betty	Redwing × W6-120	Zaiger – USA	PWM
Big Top	–	Zaiger – USA	NYM
Bigsun	–	Maillard – France	PYFM
Binaced	–	Servicio de Investigación Agraria, Zaragoza – Spain	PWN
Bolero	Cresthaven × Flamecrest+(1)	University of Bologna, ICA-CMVF, Bologna (ICA-CMVF) – Italy	PYM
Calabacero	–	TC – Spain	PYN
Calante	–	TC – Spain	PYN
Canongi	–	TC – Spain	PYN
Carolina	Fla.3-4N × Sunred	University of Florida, Gainesville (UFG) – USA	NYM
Carson	Maxine × Leader	U.S.D.A. Palo Alto, California – USA	PYN
Casaboi	–	University of Florida/Selección Plantas Sevilla (UFG-SPS) – USA/Spain	PWM
Casarob	–	UFG-SPS – USA/Spain	PWM
Casasil	–	UFG-SPS – USA/Spain	NWM
Catherina	NJC95 × D42-13w	RU-NJ-USA	PYN
Cofrentes-3	–	TC – Spain	PYN
Cofrentes-5	–	TC – Spain	PYN
Cofrentes-6	–	TC – Spain	PYN
Corine	–	Escande – France	PYM
Cotigua	–	TC – Spain	
Cresthaven	Kalhaven × SH309	Michigan State University, East Lansing (MSU) – USA	PYM
Crimson Lady	(Red Diamond × Springcrest) × seedling	Bradford – USA	PYN
Cristalrose	NB570 × Favols	Escande – France	NYM
Daisy	Rhone Gold × Redwing	Zaiger – USA	PWM
Delice	–	Maillard – France	PYM
Dellys	–	Escande – France	NWM
Dolores	O'Henry × Early Babcock*	Zaiger – USA	PWM
Douceur	–	Maillard – France	PWM
Duchessa d'Este	(Mayflower × Amsden) op.	Scanavini – Italy	PWM
Early Giant	–	Zaiger-USA	NYM
Early Maycrest	Maycrest mutation	Toeus, Ridley, California-USA	PYM
Early O'Henry	O'Henry op.*	Grant Merrill (Merrill) – USA	PYM
Early Orebrad	–	Bradford – USA	NYM
Early Sun Grand	Sun Grand op.	Bradford – USA	NYM
Elegent Lady	Early O'Henry × July Lady + (2)	Merrill – USA	PYM
Escarolita	–	TC – Spain	PYN
Escarolita Ferran	–	TC – Spain	PYN
Etoile	–	Maillard – France	PYM
Evaisa	–	TC – Spain	PYN
Fairlane	P60-38 × Fantasia*	U.S.D.A. Fresno, California (Fresno) – USA	NYM
Fantasia	Gold King × P101-24	Fresno – USA	NYM
Fantasie	–	Maillard – France	PYM
Federica	NJC11 × (NJ13232 × Cherryred)	RU-NJ/ISF – USA/Italy	PYN
Festina	–	Escande – France	NWM
Fidelia	(O'Henry × Giant Babcock) × (May Grand × Sam Houston)	Zaiger – USA	PWM
Fidelia Ruth	Fidelia mutation	IRTA-Fundació Mas Badia, Girona (IRTA) TC – Spain	NWM

Table 1 (continued)

Cultivar	Pedigree[a,b]	Breeder – Country	Fruit traits[c]
Fire Gold	–	Zaiger – USA	NYM
Fire Red	B8-12 self.	UCD – USA	PYM
Fireking	–	Armstrong – USA	NYM
Flaminia	Fayette × Fairtime	ISF – Italy	PYM
Flavor Crest	P53-68 × FV89-14	Fresno – USA	PYM
Flavor Gold	Rhone Gold × Royal Gold	Zaiger – USA	NYM
Flavortop	Fairtime op.	Fresno – USA	NYM
Flavor Giant	–	Zaiger – USA	NWM
Flordaking	Fla.9-67 × Early Amber	UFG – USA	PYM
Flordastar	Flordagold × EarliGrande	UFG – USA	PYM
Glady's	–	Zaiger – USA	PWM
Glenna	–	Zaiger – USA	PWM
Gorga	–	TC – Spain	PYN
Gratia	–	Zaiger – USA	PWM
Hardired	Lexington × NJN32	Ontario, Agriculture Canada, Harrow – Canada	NYM
Hermione	–	Zaiger – USA	PWM
Independence	Red King op.	Fresno – USA	NYM
Isabella D'Este	–	Lodi, Ferrara – Italy	PWM
Jeronimo	–	TC – Spain	PYN
Jesca	Sel. from Amarillos Tardios de Calanda	TC – Spain	PYN
John Henry	O'Henry mutation	California – USA	PYM
July Lady	J.H.Hale X × Merril Gem F_2	Merrill – USA	PYM
June Crest	Fayette × Mexican Sdlg.	Zaiger – USA	PYM
June Lady	Fortynine × Gemfree	Merrill – USA	PYM
Kay Grand	Red Grand × Stark Sunglo	Bradford – USA	NYM
Lamone	Babygold 6 × Shasta	ICA-CMVF – Italy	PYN
Large White	–	USDA Baton Rouge, Louisiana – USA	PWM
Lisbeth	Fortyniner × Gemfree	Merrill – USA	PYM
Lucie	–	Bradford – USA	PYM
Maria Aurelia	Stark Redgold self.*	University of Florence, DOFI, Florence (DOFI) – Italy	NYM
Maria Bianca	Honey Dew Hale × Michelini	DOFI – Italy	PWM
Maria Delizia	Cesarini op.	DOFI – Italy	PWM
Maria Emilia	May Grand self.	DOFI – Italy	NYM
Maria Laura	Flavortop self.*	DOFI – Italy	NYM
Maruja	–	TC – Spain	PYN
Maybelle	Armking mutation	Parnagian, Fowler, California – USA	NYM
Maycrest	Springcrest mutation	Minami, Reedley, California – USA	PYM
MB-3	–	IRTA – Spain	PWN
Melodie	Early O'Henry op.*	Maillard – Italy	PYM
Mechelini	–	Michelini, Savona – Italy	PYM
Mid Silver	–	Zaiger – USA	NWM
Moon Grand	(Red Grand × Early Sun Grand) F_2	Bradford – USA	NYM
Morsiani 51	Sirio × Superstar + (2)	P.L.Morsiana, Ravenna – Italy	NYM
N-1534/70	–	Zaiger – USA	NYM
Nectaross	Stark Redgold × Le Grand + (1)	ISF, Rome (ISF) – Italy	NYM
Niagara	Red Grand × Early Sun Grand*	Bradford – USA	NYM
NJC-97	–	RU-NJ – USA	PYN
NJN-76	–	RU-NJ – USA	NYN
O'Henry	Merrill Bonanza op.	Merrill – USA	PYM
P-1319	–	Zaiger – USA	PYM
P-86-124	–	Zaiger – USA	PWM
P-88-206	–	UFG-SPS – USA/Spain	PWM
Pacific Star	May Grand op.	Weinberger, Fresno, California – USA	NYM
Paraguayo Delfin	–	TC – Spain	FPN
Peret	–	TC – Spain	PYN
Peret Ferran	–	TC – Spain	PYN
Peret Marino	–	TC – Spain	PYN
Perfect Delight	Firebrite × Stark Redgold*	Zanzi, Ferrara – Italy	NYM
Queencrest	Maycrest mutation	Balakian, Reedley, California – USA	PYM
Queen Giant	–	Zaiger – USA	NWM
Queen Lady	July Lady × 59-125 + (1)	Merrill – USA	PYM
Queen Ruby	–	Zaiger – USA	NWM
Red Coast	–	C.L.V. Ferrara – Italy	PYM
Red Delight	6W120 op.	Zaiger – USA	NYM
Red Diamond	Red Grand × Early Sun Grand*	Bradford – USA	NYM
Red Fair	–	Zaiger – USA	NYM
Red Moon	Flamecrest op.	C.I.V. Ferrara – Italy	PYM
Red Pearl	–	Zaiger – USA	NYM

Table 1 (continued)

Cultivar	Pedigree[a,b]	Breeder – Country	Fruit traits[c]
Red Silver	–	Zaiger – USA	NWM
Redtop	Sunhigh × July Elberta	Fresno – USA	PYM
Red Valley	Elegant Lady op.*	C.I.V. Ferrara – Italy	PYM
Redhaven	Halehaven × Kalhaven	MSU – USA	PYM
Redwing	Babcock × Stensgaard July Elberta	Armstrong – USA	PWN
Rich Lady	Amparo op.	Zaiger – USA	PYM
Rich May	[(May Grand × Peach) × Sam Houston] × (Tasty Gold × Maycrest)	Zaiger – USA	PYM
Rome Star	(Fayette × Stark Redgold) op.	ISF – Italy	PYM
Romea	Catherina op.*	ISF – Italy	PYN
Rose Diamond	Early Diamond × Dwarf Peach	Bradford – USA	NYM
Rosired 3	Southland self.	ICA-CMVF – Italy	PYM
Royal Gem	–	Zaiger – USA	PYM
Royal Giant	Red Grand × Sdlg.	Zaiger – USA	NYM
Royal Glory	May Grand op.	Zaiger – USA	PYM
Royal Moon	–	Zaiger – USA	PYM
Royal Prince	–	Zaiger – USA	PYM
Ruby Gem	–	Zaiger – USA	NWM
Ruby Rich	–	Zaiger – USA	PYM
Sarah	–	Maillard – France	PWM
San Lorenzo	–	TC – Spain	PYN
Seduction	–	Maillard – France	PYM
Sensation	–	Maillard – France	PYM
September Orebrad	(Red Free × Tom Grand) × Sparkling Red	Bradford – USA	NYM
September Queen	Stark Redgold × Snow Queen + (2)	IPSA – Italy	NWM
Sibelle	–	Maillard – France	PYM
Silver Belle	–	Zaiger – USA	NWM
Silver Gem	May Grand × Chance sdlg.	Zaiger – USA	NWM
Silver King	Armking mutation	Prim – France	NWM
Silver Late	–	Zaiger – USA	NWM
Silver Ray	(Stark Redgold × Snow Queen) op.*	V.Ossani, FaViFrut, Faenza (FaViFrut) – Italy	NWM
Silver Rome	Stark Redgold × Snow Queen*	FaViFrut – Italy	NWM
Silver Star	(August Queen) op.*	FaViFrut – Italy	NWM
Silvery	–	Zaiger – USA	NWM
Sirio	Flamekist × Fantasia*	ISF – Italy	NYM
Snow Queen	–	Armstrong – USA	NWM
Snowred	–	Escande – France	NWM
Snowflame	(Nectar op.) op.	James F. Doyle – USA	PYM
Spring Bright	May Diamond × Sdlg.	Bradford – USA	NYM
Spring Lady	–	Merrill – USA	PYM
Spring Red	Summer Grand op.*	Bradford – USA	NYM
Springbelle	–	Batistini – Italy	PYM
Springold	FV89-14 × Springtime	U.S.D.A. Byron and Fort Valley, Georgia (Byron) – USA	PYM
Starcrest	Springcrest mutation	Chapus and Beauvy – France	PYM
Stark Redgold	Sun Grand op.	Bradford – USA	NYM
Starlite	FV89-14 × Springtime	Byron – USA	PWM
Summer Grand	Late Le Grand × Early Sun Grand*	Bradford – USA	NYM
Summer Lady	O'Henry mutation	California – USA	PYM
Summer Rich	–	Zaiger – USA	PYM
Suncrest	Alamar × Gold Dust	Fresno – USA	PYM
Super Crimson Gold	Zee Gold × Early Sun Grand + (3)	Zaiger – USA	NYM
Superqueen	Stark Redgold × Snow Queen + (3)	IPSA – Italy	NWM
Superstar	Summer Grand op.*	Sun World International – USA	NYM
Sweet Cap	–	Maillard – France	FWM
Sweet Lady	Stark Redgold op.*	CO.VI.CO, Faenza – Italy	NYM
Sweet Red	Stark Redgold op.*	CO.VI.CP, Faenza – Italy	NYM
Symphonie	Early O'Henry op.*	Maillard – France	PYM
Tardibelle	–	Maillard – France	PYM
Tasty Free	Red Free × Autumn Gold	Bradford – USA	NYM
Tendresse	–	Maillard – France	PWM
Tirennia	Vivian × Federica	ISF – Italy	PYN
Top Lady	–	Merrill – USA	PYM
Topcrest	Fayette × Mexican Sdlg	Zaiger – USA	PYM
Venus	Stark Redgold × Flamekist*	ISF – Italy	NYM
Villa Ada	Catherina op.*	ISF – Italy	PYN
Villa Doria	Catherina op.*	ISF – Italy	PYN
Villa Guilia	Catherina op.*	ISF – Italy	PYN

Table 1 (continued)

Cultivar	Pedigree[a,b]	Breeder – Country	Fruit traits[c]
Vistarich	Rich Lady op.*	Zaiger – USA	PYM
Voluptia	–	ISF – Italy	PWM
Weinberger	F100-62 × Red June	ISF – Italy	NYM
Weinberger 5199	–	Italy	PYM
White Lady	(O'Henry × Giant Babcock) × (May Grand × Sam Houston)	Zaiger – USA	PWM
Zee Glo	Red Grand op. × (Sun Grand × Merill Gem)	Zaiger – USA	NYN
Zee Lady	O'Henry × June Lady*	Zaiger – USA	PYM
Zincal 5	–	Zaiger – USA	NYM
Zinebel	–	Zaiger – USA	NYM
Zinege	–	Zaiger – USA	NWM
Zinepre	–	Zaiger – USA	NYM
Zisearl	–	Zaiger – USA	NYM
Zisecan	–	Zaiger – USA	PYM
Zisemay	–	Zaiger – USA	PYM
Zisesil	–	Zaiger – USA	NWM
Zisesun	–	Zaiger – USA	NYM
86-6	–	UFG-USA	NYN

[a] Pedigree data were obtained from Okie (1998)
[b] – Unknown; * pedigree confirmed with SSRs; + pedigree not confirmed. In paranthesis after +: number of SSRs with a discrepant genotype
[c] First letter: P = peach, N = nectarine, F = flat peach. Second letter: W = white, Y = yellow. Third letter: N = nonmelting flesh, M = melting flesh

Table 2 Characteristics of the 16 SSRs studied

Locus	Allele length – bp	Ta[a](°C)	Origin	LG[b]	Reference
BPPCT001	128, 144, 151, 153, 155, 158, 160, 164, 168	57	*P. persica*	G2 (15)	Dirlewanger et al. (2002)
BPPCT006	111, 113, 115, 117, 125, 127, 129, 131, 133, 135, 137	57	*P. persica*	G8 (7)	Dirlewanger et al. (2002)
BPPCT007	124, 129, 139, 141, 143, 145, 147	57	*P. persica*	G3 (8)	Dirlewanger et al. (2002)
BPPCT008	99, 127, 133, 135, 137, 145, 147, 154, 156, 158, 160	57	*P. persica*	G6 (28)	Dirlewanger et al. (2002)
CPPCT002	74, 98, 100	52	*P. persica*	G3 (32)	Aranzana et al. (2002a)
CPPCT005	149, 151, 153, 158, 169, 172, 174, 178	52	*P. persica*	G4 (11)	Aranzana et al. (2002a)
CPPCT022	249, 251, 261, 279, 281, 284, 291, 293, 295, 297	50	*P. persica*	G7 (16)	Aranzana et al. (2002a)
CPPCT029	170, 174, 180, 186, 188, 190, 192, 194	55	*P. persica*	G1 (69)	Aranzana et al. (2002a)
CPPCT030	170, 172, 188, 190, 192, 196, 198, 200	50	*P. persica*	G6 (84)	Aranzana et al. (2002a)
PceGA34	140, 144, 146, 148	50	*P. cerasus*	G2 (43)	Downey and Iezzoni (2000)
pchgms1	187, 200	55	*P. persica*	G2 (34)	Sosinski et al. (2000)
pchgms2	155, 157, 163	60	*P. persica*	G4 (7)	Sosinski et al. (2000)
pchgms3	174, 180, 202, 204, 206, 208	60	*P. persica*	G1 (41)	Sosinski et al. (2000)
pchgms6	186, 203, 211, 215, 217, 219, 221, 223	58	*P. persica*	G7 (17)	Aranzana et al. (2002b)
PSI2e2	162, 164, 166, 178, 208, 210, null	56	*P. cerasus*	G4 (61)	Joobeur et al. (2000)
PS9f8	155, 157, 160, 162, 164, 166, 168, 171	48	*P. cerasus*	G1 (71)	Joobeur et al. (2000)

[a] Ta: annealing temperature
[b] LG: linkage group, distance in centimorgans from the top of the linkage group as in the general *Prunus* map, Aranzana et al. (2000b)
[c] Approximate position base on the map of Joobeur et al. (2000)

absence of amplification or very low amplification intensity (lower than 10% of the intensity of amplified alleles) was observed in homozygotes. For this reason, this locus was not used for the calculation of the *F* value.

The SSRs studied amplified 113 alleles, an average of 7.3 alleles per locus. More than half of these alleles (59) were present in frequencies lower than 5%, but only one was nearly fixed (frequency > 95%). Considering all the loci but PS12e2, we found $H_0 = 0.35$ and $H_e = 0.50$. Consequently, the *F* values were positive, with a mean of 0.23 for all loci. Using the PD values, we calculated the probability of confusion to be 2.32×10^{-9}. Additional details on the variability parameters are shown in Table 3.

P. persica SSR diversity

The 16 microsatellites studied distinguished 195 different genotypes of the 212 possible. One hundred and eighty five cultivars (87%) had a unique SSR pattern and the 27 remaining were distributed into seven groups: one of seven cultivars, two of three and seven of two cultivars each.

Table 3 Variability parameters calculated for 16 SSR markers in 212 peach cultivars

SSR/cv group	A	Ae	Ho	He	F	# Genotypes	PD
BPPCT001	9	3.4	0.57	0.70	0.20	25	0.87
BPPCT006	11	2.8	0.51	0.64	0.19	19	0.81
BPPCT007	7	2.4	0.47	0.59	0.20	12	0.75
BPPCT008	11	1.6	0.18	0.37	0.51	22	0.46
CPPCT002	3	2.1	0.30	0.52	0.43	6	0.67
CPPCT005	8	2.5	0.44	0.59	0.26	15	0.77
CPPCT022	10	5.2	0.56	0.81	0.30	26	0.93
CPPCT029	8	2.0	0.44	0.50	0.12	11	0.68
CPPCT030	8	2.7	0.51	0.62	0.18	14	0.80
PCeGA34	4	1.8	0.27	0.44	0.38	6	0.60
pchgms1	2	1.0	0.00	0.00	0.00	2	0.01
pchgms2	3	1.2	0.14	0.16	0.09	4	0.27
pchgms3	6	1.3	0.21	0.26	0.19	10	0.40
pchgms6	8	3.4	0.52	0.71	0.26	15	0.86
PSI2e2	7	3.0	0.08	0.67	–	10	0.71
PS9f8	8	2.1	0.44	0.51	0.15	13	0.72
Average	7.3	2.4	0.35	0.50	0.23	13	0.64
All loci	113	38.3	–	–	–	210	1.00
All melting peaches	73	33.5	0.37	0.44	0.03	7	0.59
All melting nectarines	84	33.8	0.36	0.44	0.13	8	0.57
All non-melting cultivars	76	40.7	0.26	0.46	0.37	8	0.56

A dendrogram constructed from the SSR data divided the cultivars into three major clusters (Fig. 1). Two of them (2 and 3) contain only cultivars with non-melting flesh, and the largest cluster (1) has the remaining cultivars with the exception of the yellow melting peach 'Rich May' that was separate from the rest. In cluster 1, there are two subclusters (1.1 and 1.2) that include most of the 89 melting flesh peaches (80%) and some of the 43 non-melting flesh peaches (5%) plus a few of the 80 melting flesh nectarines (5%). The rest of the melting flesh nectarines (95%) and a limited number of peaches (19%) were located in the remaining subclusters of cluster 1.

The parameters of variability were calculated separately for melting peaches (P), nectarines (N) and non-melting cultivars (NM) (Table 3). Differences were found in the number and distribution of alleles: the P cultivars had 73 alleles, eight of them specific to this group; the N cultivars had 84 alleles, 16 unique to this group. The smallest group of NM cultivars had an intermediate number of alleles (76), but the highest number (18) of specific alleles. The effective number of alleles was also higher in the NM group, with nearly seven more than either P or N, which indicates a more-even distribution of allele frequencies. Considering genetic distance values, NM were the most diverse with an average distance between entries of 0.53, compared with 0.39 for N and 0.41 for P. The NM group was also the most separate from the others, with average distances of 0.76 and 0.77 from the P and N groups, while P and N had an average distance of 0.47. Maximum distances were obtained when comparing the Spanish subgroup of NM (25 cvs) with P and N (0.87–0.88).

The values of H_e, H_0, and F indicated a different genetic structure in P and N compared to the NM cultivars. The observed heterozygosity was clearly lower in the NM group. Given that H_e values were similar in all groups, this resulted in a much higher value of *F* for the non-melting group (0.37) than for the P (0.03) and N (0.13) groups, which were closer to the genotype distribution expected for random mating (Table 3).

The expected and observed distributions of the number of pairs of cultivars with a given number (0 to 16) of SSR genotype differences can be found in Fig. 2. The mean values of the observed (10.31) and expected (10.36) distributions were similar but the variance of the observed distribution (4.97) was almost twice that of the expected (2.76). Discrepancies were particularly clear for the extreme values of the distributions: the sum of the expected proportions of individuals with 0–2 differences was close to zero (0.0003%), while we found at total of 79 from the 22,336 possible pairs (0.4%) that fell into this category. The same occurred at the other extreme: we found 148 pairs of cultivars different at 15 or 16 loci, about four times more than the expected (44). These two distributions were significantly different when compared with a χ^2 test (χ^2 = 11,299; *df* = 11; classes 0–4 and 15–16 were pooled for this test).

We modified two aspects of our data that could alter the correspondence between expected and observed values: (1) all cultivars but one that had the same SSR genotype were excluded, and of those cultivars considered to be sports from the pedigree data we only kept one, reducing the total number of cultivars to 192, and (2) we selected only loci that were placed at distances ≥25 cM (12 SSRs were retained). As a result, the observed and expected distributions were slightly more similar, but still significantly different [χ^2 = 8,285; *df* = 11 for (1)] and [χ^2 = 4,263; *df* = 9 for (1) + (2)].

Fig. 1 UPGMA dendrogram of 212 *P. persica* cultivars based on their variation at 16 SSR loci. ● Peach (melting flush); ○ Nectarine (melting); ▲ Peach (non-melting); △ Nectarine (non-melting)

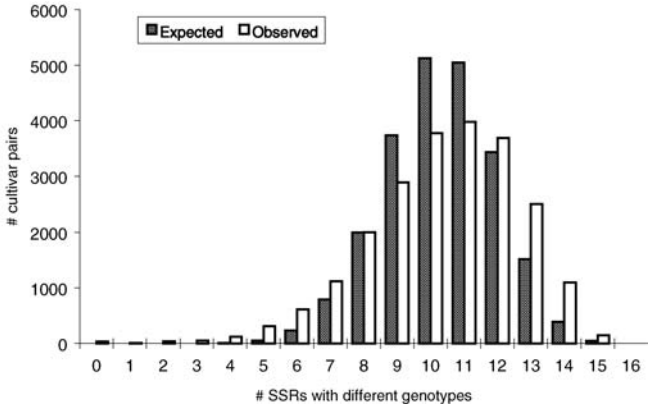

Fig. 2 Distribution of the expected and observed numbers of pairs of peach cultivars having a given number of genotype differences at 16 SSR loci using the 212 cultivars analyzed

SSR fingerprints and pedigree information

The yellow peaches 'Maycrest', 'Queencrest', 'Early Maycrest', 'Springold', Spring Lady', 'Springbelle' and 'Queen Lady' were the most numerous group of entries with identical SSR patterns. 'Queen Crest' and 'Early Maycrest' are sports of 'Maycrest', which in turn is a mutation from 'Springcrest'. 'Starcrest', another member of the 'Springcrest' mutant family, differed from the rest at two loci (BPPCT006 and pchgms2). 'Springold' and 'Springcrest' are full sibs, which may explain their SSR similarity. We do not have pedigree information on 'Spring Lady' and 'Springbelle'. Given that the two cultivars were released in 1981 and 1985, respectively, later than 'Springcrest' (1969) and 'Maycrest' (1977), it is possible that they are sports from 'Springcrest' or one of its mutants. Indeed, in a more-detailed analysis of 'Queencrest' and 'Spring Lady' with 24 SSRs (Aranzana et al. 2002a) no difference was found between these two cultivars. Using a further 26 SSRs (Testolin et al. 2000) it was not possible to discriminate between 'Springbelle' and 'Springcrest'. 'Queen Lady' is a seedling from the cross 'July Lady' × '59-125', with an unknown relationship to the 'Springcrest' group pedigree. In this case the data available on their pedigree does not justify their SSR identity.

Yellow flesh peach cultivars 'Lisbeth', 'June Lady' and 'Redtop', had the same SSR genotype. 'Lisbeth' and 'June Lady' are sibs from 'Gemfree' × 'Fortyniner'. 'Redtop' is less related, but shares a common ancestor with 'Gemfree' ('July Elberta').

Another group of cultivars that could not be separated by their SSR genotype included the yellow flesh peaches 'Elegant Lady', 'Rome Star' and 'Red Coast'. 'Elegant Lady' was released in California in 1979, and 'Red Coast' and 'Rome Star' were obtained in Italy in 1993. There are no data on the 'Red Coast' pedigree, and 'Rome Star' and 'Elegant Lady' are not closely related (see Table 1). The pedigree information available cannot explain the high SSR similarity of these three cultivars, but 'Elegant Lady' and 'Rome Star' were also found to have an identical genotype at 23 additional SSRs by Testolin et al. (2000) and 130 RAPD primers by Vinatzer et al. (1999). This strongly suggests that 'Rome Star' is either the same genotype or a mutation of 'Elegant Lady'.

Three of the seven groups of two varieties with the identical SSR fingerprint include known sports: 'Silver King' and 'Maybelle', both mutations of 'Armking' but differing from it at one SSR locus (BPPCT008), 'Fidelia' and its mutant 'Fidelia Ruth' and 'O'Henry' and 'John Henry', the latter a mutation of the former. 'Summer Lady', a mutant of 'O' Henry', differed in two SSRs (CPPCT030 and pchgms6). Two more pairs with the same SSR fingerprint, 'Peret Marino' and 'Auberge Marino', and of 'Escarolita' and 'Escarolita Ferran', are probably synonymous non-melting peaches collected in Spain. For the two remaining pairs – all nectarine cultivars: 'Fireking' and 'Fire Gold', both with an unknown pedigree, and 'Fantasia' and 'Sweet Red', of known but not directly related parents – we could not establish a clear cause for their SSR identity.

The use of a set of codominant markers of known inheritance allowed us to analyze how SSR data matched with known pedigrees. We found a total of 38 cases where SSR data from at least one of the parents were available (see Table 1). While SSR data from open-pollinated seedlings were always consistent with their pedigree (15 cases), we found a higher level of disagreement when the pedigree information was more precise: in 11 out of 23 cases studied, we found discrepancies between expected and observed SSR data. In five of them, the disagreement occurred at only one locus, and in four cases 'Stark Redgold' was involved as one of the parents of the cross.

Discussion

SSR polymorphism

The average number of alleles per locus was 7.3, which was higher than the 4.5 previously observed by Testolin et al. (2000) in a set of 50 peach cultivars with 26 microsatellites, the 3.0 observed by Sosinski et al. (2000) in 28 cultivars with eight polymorphic SSRs and the 3.2 detected by 24 SSRs in 25 genotypes by Aranzana et al. (2002a). The high number of alleles can be explained by the use of a larger set of cultivars and because nine of the SSRs were selected by their high level of polymorphism in previous observations (Aranzana et al. 2002a; Dirlewanger et al. 2002).

Cultivar identification

Of the cultivars analyzed, 87% had a unique SSR fingerprint, which confirms the high efficiency of these markers in a species with low variability such as the peach

(Byrne 1990). Many of the cultivars that had the same SSR genotype were known sports. If we remove them from the analysis, the proportion of cultivars individually distinguished increased to 95%. The origin of some of the cultivars that belong to groups with the same SSR genotype is unclear, and we cannot discard the fact that they may be unidentified sports or duplications from others of the same group. In other cases, however, the pedigree indicates an independent origin as a result of sexual reproduction.

The expected and observed distributions of the number of loci that differed between pairs of cultivars had similar mean values but gross departures from variance. While the expected values of the most extreme cases like 0–2 or 15–16 were close to zero, the observed proportions were much higher according to the chi-squared goodness-of-fit tests. Three possible causes of this disagreement are proposed. First, the presence of sports would increase the number of genotypes included in the 0 or low classes of the distribution, so each group of sports should be considered as a single individual. Second, the expected distribution was calculated assuming that the information provided by each locus is independent. Non-independence between linked loci (i.e. linkage disequilibrium) may be important in the set of SSRs and cultivars employed. Some of the markers used were tightly linked, such as CPPCT022 and pchgms6 (1 cM), CPPCT029 and PS9f8 (2 cM) or pchgms2 and CPPCT005 (3 cM), and we found highly significant linkage disequilibrium between them (data not shown). Indeed, most of the peach cultivars analyzed come from intercrossing a limited number of genotypes for a few generations (Hesse 1975; Scorza et al. 1985) and so the maintenance of linkage disequilibrium is a logical consequence. These results tell us that selection of loci from separate regions of the genome is important for increasing their efficiency in peach cultivar identification. Using markers from the "genotyping set" of SSRs located in 24 regions with full coverage of the *Prunus* genome proposed by Aranzana et al. (2002b), seems an appropriate solution for further studies. Five of the markers used here (BPPCT006, BPPCT007, BPPCT008, CPPCT002 and CPPCT022) belong to this "genotyping set".

A third level of departure between observed and expected data comes from the existence of subpopulation structures among the collection of cultivars sampled. UPGMA analysis showed the clustering of nectarine, peach and non-melting flesh cultivars, but it is likely that each *P. persica* breeding program constitutes a separate mating unit. This factor would seem an important one, given that consideration of the other two was not sufficient to give an agreement between the observed and expected values of the distribution of the SSR differences between pairs of genotypes.

In all, these results indicate that the quantitative measurements of the uniqueness of a cultivar or the power of discrimination produced by a set of loci (such as the probability of confusion, or the product of the genotype frequencies for all loci analyzed in the cultivar of interest) are underestimations of their actual values, and should be corrected. One way to do this would be to estimate the probability of two cultivars having an identical fingerprint by chance, or of having one or a few SSR differences, using the observed distribution instead of on the basis of theoretical approaches. For that, it would be necessary to use a set of well-characterized markers (single-locus markers of inheritance and map position known) previously studied in a large set of cultivars of the species of interest.

SSR mutations and pedigree inferences

The comparison between known peach sports has provided some insight into the level of mutation of the microsatellite loci studied. Fourteen of the cultivars used belonged to three mutation groups (we have added 'Spring Lady' and 'Springbelle' to the 'Maycrest' group based on the evidence provided in this paper). 'Starcrest' and 'Summer Lady' differed in two loci from the rest of their groups, and 'Armking' by one locus. All SSRs involved were different, and the mutant genotypes had a new allele with ±2 bp in three cases and the loss of one allele in the other two (presumably by the mutation towards a null allele, although our data cannot discard the mutation towards the size of the non-mutated allele). Assuming that the declared origin as mutations of this group of cultivars was correct, we found a total of five mutations out of 224 cases (16 loci in 14 cultivars), i.e. 1.1% of the observed alleles had a mutation. An additional SSR differed between 'Armking' and 'Maybelle', one between 'Maycrest' and 'Springcrest', and two between 'Maycrest' and 'Queencrest' (Testolin et al. 2000), providing more support for a high rate of SSR mutation among peach sports.

These results point to the need of additional research aiming at a precise characterization of the SSR mutation phenomenon. The overall mutation rate, the variation of this rate according to the SSR used and the cultivar genotype, the nature of the observed mutations, the strategy to estimate such mutation rates in species that are generally bred through sexual reproduction and vegetatively propagated, and the appropriateness of SSR mutants to identify sport cultivars, are some of the topics that need to be addressed. This information must be incorporated into the cultivar identification procedure to make the SSR test of identity a robust and reliable one. While the fact that a known cultivar and a problem sample have the same genotype for a set of SSRs similar to the one used here is strong evidence of their identity, the presence of one or a few discrepancies between them may not demonstrate that they are different. Only with a reliable estimate of the mutation rate of the SSRs used, and the time of separation between the two genotypes compared, can the expected number of mutation events be calculated in terms of probability. Given that the time factor is likely to be important, and that usually the cultivars that require protection are of recent creation, it seems reason-

able that the effect of SSR mutations will be lower in this case than when comparing a broad spectrum of cultivars obtained over a long period of time. In this respect, the data presented here would tend to overestimate the mutation rate.

Codominance makes SSRs an excellent marker for pedigree analysis. We have examined 38 cases and found discrepancies with pedigree data in 11 of them. These results indicate that there may be errors in our data, in the pedigree record, or in both. In five cases there was only one locus with unexpected alleles, indicating that some of the discrepancies may also have been caused by SSR mutations.

The genetic structure of the collection of *P. persica* cultivars

The UPGMA analysis allowed us to establish a division of the cultivars analyzed into the P, N and NM groups. This classification, which coincides with the major commercial division of *P. persica* (peaches, nectarines and canning peaches), suggests that crosses between genotypes of different groups have been used less frequently in peach breeding than crosses between members of the same group. In addition, the NM cultivars were more genetically distant and internally variable than the P and N groups. This agrees with previous analysis with isozymes (Messeguer et al. 1987) and SSRs (Aranzana et al. 2002a), suggesting that new variability useful for peach and nectarine breeding may be found in this group of cultivars.

When the population parameters were calculated for the NM, N and P groups, we found that the group of non-melting peaches, and more specifically the group including the Spanish cultivars, was much more homozygous than the P and N groups. These results may be the consequence of the different breeding methods used to obtain them. The P, N and the non-melting cultivars of the USA and Italian programs are the result of modern breeding strategies, often based on the selection of individuals from the progeny of a cross and where selfing is only used occasionally [80% of a sample of 600 cultivars of known pedigree surveyed from Okie's (1998) book]. In this case the organization of the variability should essentially maintain a random-mating-like structure as found in this research. On the other hand, Spanish cultivars, and in general traditional cultivars of the Old World, are individuals selected from populations that were seed-propagated for a long period of time (Badenes et al. 1998). Given that the mating system of peach is predominantly selfing (Miller et al. 1989), this would lead towards homozygosity as the SSR analysis has confirmed.

Acknowledgements This work was supported financially in part by funds from INIA ("Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria") SC97-097and RTAOl-l18-C2-2 projects, and by the contract between "Selección Plantas Sevilla S.L." and IRTA. We thank Ignasi Batlle, Maria Luisa Badenes and Jean-Clément Marcaillou for supplying some of the cultivars used, and Bryon Sosinski, Albert Abbott, Amy Iezzoni and Graham King for providing information about unpublished SSR markers. The M.J.A. scholarship was provided by the "Centre de Referència en Biotecnologia" (CeRBa) of the Catalan Government.

References

Aranzana MJ, Garcia-Mas J, Carbó J, Arús P (2002a) Development and variability of microsatellite markers in peach. Plant Breed 121:87–92

Aranzana MJ, Pineda A, Cosson P, Dirlewanger E, Ascasibar J, Cipriani G, Ryder CD, Testolin R, Abbott A, King GJ, Iezzoni AF, Arús P (2002b) A set of simple-sequence repeat (SSR) markers covering the *Prunus* genome. Theor Appl Genet (in press)

Arulsekar S, Parfitt DE, Kester DE (1986) Comparison of isozyme variability in peach and almond cultivars. J Hered 77:272–274

Badenes ML, Werner DJ, Martínez-Calvo J, Llácer G (1998) A description of peach native populations from Spain. Fruit Variet J 52:80–86

Byrne DH (1990) Isozyme variability in four diploid stone fruits compared with other woody perennial plants. J Hered 81:68–71

Cipriani G, Lot G, Huang W-G, Marrazzo MT, Peterlunger E, Testolin R (1999) AC/GT and AG/CT microsatellite repeats in peach [*Prunus persica* (L) Batsch]: isolation, characterisation and cross-species amplification in *Prunus*. Theor Appl Genet 99:65–72

Dirlewanger E, Cosson P, Tavaud M, Aranzana MJ, Poizat C, Zanetto A, Arús P, Laigret F (2002) Development of microsatellite markers in peach [*Prunus persica* (L.) Batsch] and their use in genetic diversity analysis in peach and sweet cherry. Theor Appl Genet 105:127–138

Downey SL, Iezzoni AF (2000) Polymorphic DNA markers in black cherry (*Prunus serotina*) are identified using sequences from sweet cherry, peach, and sour cherry. J Am Soc Hort Sci 125:76–80

Fideghelli C, Della Strada G, Grassi F, Morico G, Monet R (1997) The peach industry in the world: present situation and trend. Acta Hort 465:29–40

Hesse CO (1975) Peaches. In: Janick J, Moore JN (eds) Advances in fruit breeding. Purdue University Press, West Lafayette, Indiana, USA, pp 285–335

Joobeur T, Periam N, de Vicente MC, King GJ, Arús P (2000) Development of a second generation linkage map for almond using RAPD and SSR markers. Genome 43:649–655

Kloosterman AD, Budowle B, Daselaar P (1993) PCR-amplification and detection of the human DIS80 VNTR locus. Amplification conditions, population genetics and application in forensic analysis. Int J Leg Med 105:257–264

Kumar S, Tamura K, Nei M (1993) MEGA: molecular evolutionary genetics analysis software, version 1.0. The Pennsylvania State University, University Park, Pennsylvania

Messeguer R, Arús P, Carrera M (1987) Identification of peach cultivars with pollen isozymes. Sci Hort 31:107–117

Miller PJ, Parfitt DE, Weinbaum SA (1989) Outcrossing in peach. HortScience 24:359–360

Morgante M, Olivieri AM (1993) PCR-amplified microsatellites as markers in plant genetics. Plant J 3:175–182

Nei M (1972) Genetic distance between populations. Am Nat 106:283–292

Okie WR (1998) Handbook of peach and nectarine varieties. Agricultural Research Service

Rohlf FJ (1994) NTSYS-pc version 2.1. Exeter Software, Setauket, New York

Scorza R, Mehlenbacher SA, Lightner GW (1985) Inbreeding and coancestry of freestone peach cultivars of the Eastern United States and implications for peach germplasm improvement. J Am Soc Hort Sci 110:547–552

Scorza R, Sherman WB, Lightner GW (1988) Inbreeding and coancestry of low chill short fruit development period freestone

peaches and nectarines produced by the University of Florida breeding program. Fruit Variet J 42:79–85

Sosinski B, Gannavarapu M, Hager LD, Beck E, King GJ, Ryder CD, Rajapakse S, Baird WV, Ballard RE, Abbott AG (2000) Characterization of microsatellite markers in peach [*Prunus persica* (L.) Batsch]. Theor Appl Genet 101:421–428

Testolin R, Marrazzo T, Cipriani G, Quarta R,Verde I, Dettori M, Pancaldi M, Sansavini S (2000) Microsatellite DNA in peach [*Prunus persica* (L.) Batsch] and its use in fingerprinting and testing the genetic origin of cultivars. Genome 43:512–520

Vinatzer B, Pancaldi M, Sansavini S (1999) Potenzialita e limiti del "fingerprinting" nell'identificazione varietale del pesco. Riv Frutticoltura Ortofloricoltura 61:97–101

Viruel MA, Messeguer R, de Vicente MC, Garcia-Mas J, Puigdomènech P, Vargas F, Arús P (1995) A linkage map with RFLP and isozyme markers for almond. Theor Appl Genet 91:964–971

Warburton ML, Bliss FA (1996) Genetic diversity in peach [*Prunus persica* (L.) Batsch] revealed by randomly amplified polymorphic DNA (RAPD) markers and compared to inbreeding coefficients. J Am Soc Hort Sci 121:1012–1019

Wright S (1951) The genetical structure of populations. Ann Eugen 15:323–354